Talanta 128 (2014) 38–43

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Multicommutated flow analysis system for determination of total protein in cerebrospinal fluid

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ARTICLE INFO

Article history:
Received 15 January 2014
Received in revised form
7 April 2014
Accepted 15 April 2014
Available online 29 April 2014

Keywords:
Cerebrospinal fluid
Protein determination
Multicommutation
Optoelectronic detector

ABSTRACT

A fully mechanized, computer-controlled, multicommutated flow analysis (MCFA) system dedicated for total protein determination in cerebrospinal fluid samples has been developed. For the protein determination the Exton method has been applied. Dedicated turbidimetric and nephelometric flow-through detectors operating according to paired-emitter detector diode principle have been fabricated by integration of two or three respective light emitting diodes. The developed MCFA system is characterized by robust, compact design and low consumption of the sample (72 μL). The limits of detection for turbidimetric and nephelometric detection mode are 65 mg L^{-1} and 9 mg L^{-1}, respectively. For turbidimetric measurements the range of linear response offered by the MCFA system is 72–900 mg L^{-1}, whereas in the case of nephelometric detection 18–500 mg L^{-1} linear range is obtained. The throughput of the MCFA system is over 30 injection per hour. The analytical system was optimized with bovine serum albumin standards and successfully validated with real samples of human cerebrospinal fluid.

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1. Introduction

Cerebrospinal fluid (CSF) surrounds brain and spinal cord and is situated between arachnoid and pia maters. The main functions of CSF are: protection against mechanical damage of the brain, transport of nutrients and excretion of toxic and waste substances [1–3]. CSF is selectively secreted mainly by choroid plexus but it is also formed by intrathecal synthesis of lining cells of brain and spinal cord. It is worth to emphasize that CSF is not a product of ultrafiltration of plasma (through blood–CSF barrier) but rather selective secretion of plasma components. CSF reflects, approximately, the composition of blood plasma but concentrations of particular metabolites and proteins can be elevated in comparison with concentration of plasma particles. The reason of specific composition of CSF is active transport from blood and secretion from the brain.

The analysis of CSF is very important for diagnostic assessment of clinical pathology. CSF total protein determination is requested in the case of infection, neoplasm, hemorrhage, inflammatory processes, polyneuritis, degeneration of the central nervous system or psychiatric illness [1–4]. Total protein determination and protein fractions analysis in CSF samples are crucial indicators of changes of permeability of the blood–brain barrier. Increased total protein concentration is observed in the examples of bacterial and viral infections of meningitis or neoplastic occlusion [4–6]. Total protein level in CSF varies also in the dependence of the age of patient [7]. In the literature, several methods for protein determination can be found including photometric [7–10] and light scattering methods [10,11] for total protein and immunochemical methods [6–12] as well as electrophoresis [13] and spectroscopic methods [13,14] for quantitative protein fractions analysis. In each method, the problem of the amount of sample appears. In normal conditions, the total volume of CSF in adult human body is approximately 150 mL. These limitations are the reason of hampering chemical analysis of cerebrospinal fluid [1,2].

The aim of this contribution is to develop the multicommutated flow analysis system (MCFA) for total protein determination that requires small amounts of CSF. The system is based on newly developed optoelectronic detectors for turbidimetric and nephelometric measurements [15]. The analytical method applied in this work for total protein determination is based on conventional Exton protocol [16,17] recommended for the needs of routine clinical analytics.

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http://dx.doi.org/10.1016/j.talanta.2014.04.047
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2. Experimental

Lyophilized bovine serum albumin (BSA), sulphosalicylic acid (SSA) and other reagents of analytical grade, were obtained from POCh (Poland). For all experiments doubly distilled water was used throughout. Samples of cerebrospinal fluid with known levels of total protein in pathological and physiological ranges were obtained from the Central Clinical Laboratory of Medical University of Warsaw. The samples were analyzed in clinical settings using a routine Exton precipitation method with turbidimetric detection [16,17]. These reference determinations were performed using quartz cuvettes from Optiglass Ltd. (England) and spectrophotometer (Helios Delta, Thermo Scientific, USA)

Light emitting diodes (LEDs) of 565 nm, 600 nm, 630 nm and 650 nm wavelengths used in these investigations were obtained from Optosupply (Hong Kong). These LEDs have common shape, 5 mm diameter, transparent lens, and 20 nm declared full width at half-maximum. For turbidimetric measurements a 565 nm LED-emitter and a 600 nm LED-detector were applied. In the nephelometric configuration two 630 nm LED-emitters and 650 nm LED-detector were used. The optical flow-through cell of 60 μL internal volume integrated with respective LEDs made of PEEK (PolyEther Ether Ketone) material that is resistant on acidic solutions. The cross-section of the detector is shown in Fig. 1. The detailed video tutorial illustrating step-by-step the procedure of mechanical fabrication of very similar compact flow-through detector for fluorimetric measurements is shown elsewhere [18] as a Supplementary material.

LEDs used as light sources were powered with stable currents generated by homemade low-voltage circuit based on L272 chip containing two independent operational amplifiers. All electronic components and solderless board were obtained from TME (Poland). The electromotive force generated by illuminated LED-detectors (treated as an analytical signal [19]) was measured, transduced and recorded using a Voltcraft multimeter (model VC820, Germany) connected with data storage PC via RS232 interface.

The MCFA manifold has been arranged using microsolenoid pumps (indicated stroke volume of 12 μL, product no. 120SP1210-4TE) and three-way microsolenoid valves (product no. 100T3MP12-62-5) purchased from Bio-Chem Fluidics (Boonton, USA) and PTFE Microbore tubing (ID 0.8 mm) obtained from Cole-Palmer (USA). The microsolenoid devices were controlled by PC-programmed KSP Measuring System (Poland).

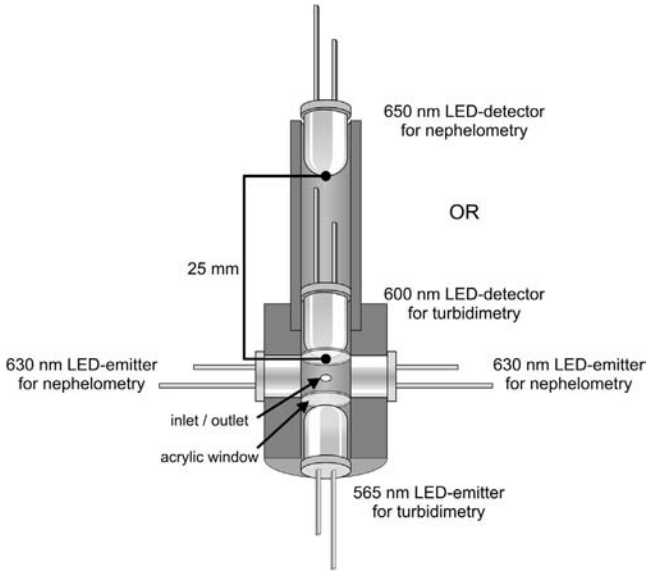

Fig. 1. The cross-section of flow-through cell integrated with LEDs.

3. Results and discussion

3.1. MCFA manifold

The Exton method for total protein determination, recommended for analysis of biomedical fluids, is based on measurements of light scattering by samples after treatment with concentrated sulphosalicylic acid (SSA) that causes all proteins precipitation. Although paired light emitting diodes are mainly used for photometric measurements, it has been shown recently that such optoelectronic devices are also applied for turbidimetric [15,20] as well as nephelometric [15] detection. Such devices constructed in the compact flow-through detector format are useful for measurements in conventional FIA systems [15] as well as SIA manifolds [20]. Both, turbidimetric and nephelometric, optoelectronic detectors have been successfully applied for determination of total protein level in urine samples [15]. In this contribution the MCFA system based on turbidimetric and nephelometric flow-through detectors dedicated for analysis of CSF is presented. The MCFA manifold is shown in Fig. 2. The manifold of developed flow system fulfills three requirements, especially important in the case of CSF analysis: (i) low amount of sample consumption, (ii) reproducible sampling of samples of different viscosities and (iii) possibility of differential measurements necessary for elimination of effects from own turbidity of samples.

The MCFA consists of two steam lines connected before the detector. The sample line (SL) contains the four-way cell of 8 μL volume playing the similar role as injection loop in conventional FIA systems. The sample aspiration is performed through the valve V2 using pump P3. This time valve V3 and pumps P1 and P2 are closed. After aspiration pump P3 and valve V2 are closed and the 8 μL segment of sample from the cell is pushing by pump P2 towards the detector. The detailed description of the module providing reproducible sampling independent of sample viscosity is given elsewhere [21]. The reagent line (RL) is equipped with pump P1 and additional valve V1 enabling performance of whole measurement cycle with or without presence of SSA. This way the MCFA system can be also easily applied for the detection of own turbidity of CSF samples caused by sporadic presence of cells, microorganisms or myelographic contrast media.

The detailed program controlling operation of the MCFA system is presented in Table 1. The first step is filling the manifold with water by pumps P1 and P2 while valve V1 is switched ON. This step plays the role of washing procedure in the case of continuous analysis of consecutive samples. Then, injection of the sample takes place. The sequence starts when segment of the sample is aspirated through valve V2 by single impulse of pump P3. For process of aspiration it is required that valve V3 has to be switched ON, otherwise segments from waste would be aspirated to the manifold as well and the volume of sample injected this way would not be known. The following step is pushing the sample segment towards detector by the single impulse of pump P1 while valves V2 and V3 are closed. At the same moment pump P2 transports segment of water. This sequence is repeated a few times to introduce known, intended volume of sample (multiplicity of 8 μL volume) into the manifold. Afterwards, segments of sample are mixed with water and pushed to the detector by pumps P1 and P2. As a result, peak for sample without SSA is recorded. A measurement of signal that refers to precipitation of total protein is a second part of the presented procedure. In this case, the whole sequence presented above is the same except that the valve V1 is switched OFF throughout this part of procedure. It provides the mixing of sample segments with SSA solution. As a result the precipitation takes place and the recorded peak is proportional to total protein level in sample. In the presented study the analytical signal for real sample of CSF is understood as a

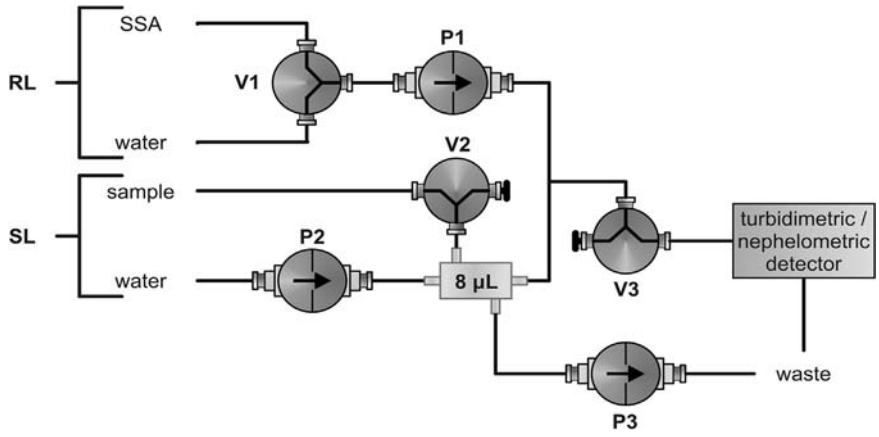

Fig. 2. The MCFA manifold: SL-sample line, RL – reagent line, P – microsolenoid pumps, V – microsolenoid valves.

Table 1
The program controlling the operation of MCFA system.

Step	V1	V2	V3	P1 (Hz)	P2 (Hz)	P3	Number of cycles	Cycle time (s)	Description
1	1	0	0	3.(3)	3.(3)	0	12	24	Flushing of the system with water
2	1	0.8 Hz	0.8 Hz	0.8	0.8	0.8 Hz	12	15.6	Injection of CSF sample
3	1	0	0	3.(3)	3.(3)	0	50	100	Background signal
4	0	0	0	3.(3)	3.(3)	0	8	16	Flushing of the system with water/SSA
5	0	0.8 Hz	0.8 Hz	0.8	0.8	0.8 Hz	12	15.6	Injection of CSF sample
6	0	0	0	3.(3)	3.(3)	0	50	100	Signal from precipitated proteins

difference between peaks for segment samples mixed with water and with SSA. For transparent samples this feature can be omitted and the whole procedure is reduced to measurements only with SSA.

3.2. Optimization of MCFA system

The optimization of presented MCFA system was performed using 50, 125 and 250 mg L^{-1} bovine serum albumin (BSA) standards prepared in distilled water and therefore only the second part of program shown in Table 1 was repeatedly executed. In the course of all measurements 20% (w/w SSA) was used. Three important parameters were checked: (i) current powering detectors, (ii) volume of sample and (iii) flow rate.

Fig. 3 shows selection of current powering LED emitters for turbidimetric and nephelometric detection The measurements were performed at constant injection volume of calibrant (144 µL) and flow rate (2 impulses/s). The recordings shown in Fig. 3 illustrate good stability of baselines as well as good reproducibility of recorded peaks at each tested current. In the case of turbidimetric detection, an increase of supplying current of LED emitter causes increase of sensitivity of the method as well as increase of a background signal, whose effects are opposite to each other. As a result, an increase of slope of calibration plots from 5 mA to 10 mA can be observed. Above current of 10 mA, the blank signal dominates thus the registered peak height signal for the same concentrations are lower. Therefore, an optimal current offering maximal sensitivity can be found (see the inset at the top of Fig. 3). As can be seen from the bottom inset of this figure, in the case of nephelometric measurements the increase of LEDs supplying current causes increase of sensitivity until the maximal intensity of emitted light (saturation of LED emitter) is reached. Further increase of current did not cause the increase of sensitivity but is dangerous for emitters (burnout). For further turbidimetric and nephelometric investigations, the LED supplying currents of 10 mA and 2 × 10 mA (in a nephelometric device two emitters are applied – Fig. 1) have been chosen, respectively.

The same measurements at selected current have been repeated at different flow rates and calibrant injection volumes. Flow rates were defined by number of impulses inducing pump per second (a single impulse causes pumping of 12 µL of solution). Effective injection volume, that reached the detection cell, was multiplicity of 8 µL (a single filling of cell in sample line). The obtained results, shown in Fig. 4, are common for measurements performed under conditions of flow analysis. The higher sensitivity is observed for larger injection volumes and slower flows. Therefore the selection of measurements parameters is the compromise between required sensitivity, flow throughput and volume of consumed sample. For further experiments 144 µL injection volume and 12 µL s^{-1} flow rate have been selected. Under such conditions the injection throughput of the MCFA system is 30 h^{-1}.

3.3. Analysis of human CSF samples

The optimized MCFA system has been calibrated in the wider range of concentration (up to 1 g L^{-1}) using BSA standards. The obtained calibration graphs for both detectors are shown in Fig. 5. The ranges of linear response for turbidimetric and nephelometric mode of measurements are 72–900 mg L^{-1} and 18–500 mg L^{-1}, respectively. The sensitivity offered by turbidimetry is 0.96 mV mg^{-1} L, whereas for nephelometry it is 0.44 mV mg^{-1} L. The values of detection limits (calculated for 3 standard deviations of signal for 10 injections of blank standard) for turbidimetric and nephelometric measurements are 65 mg L^{-1} and 9 mg L^{-1}, respectively. For turbidimetric mode

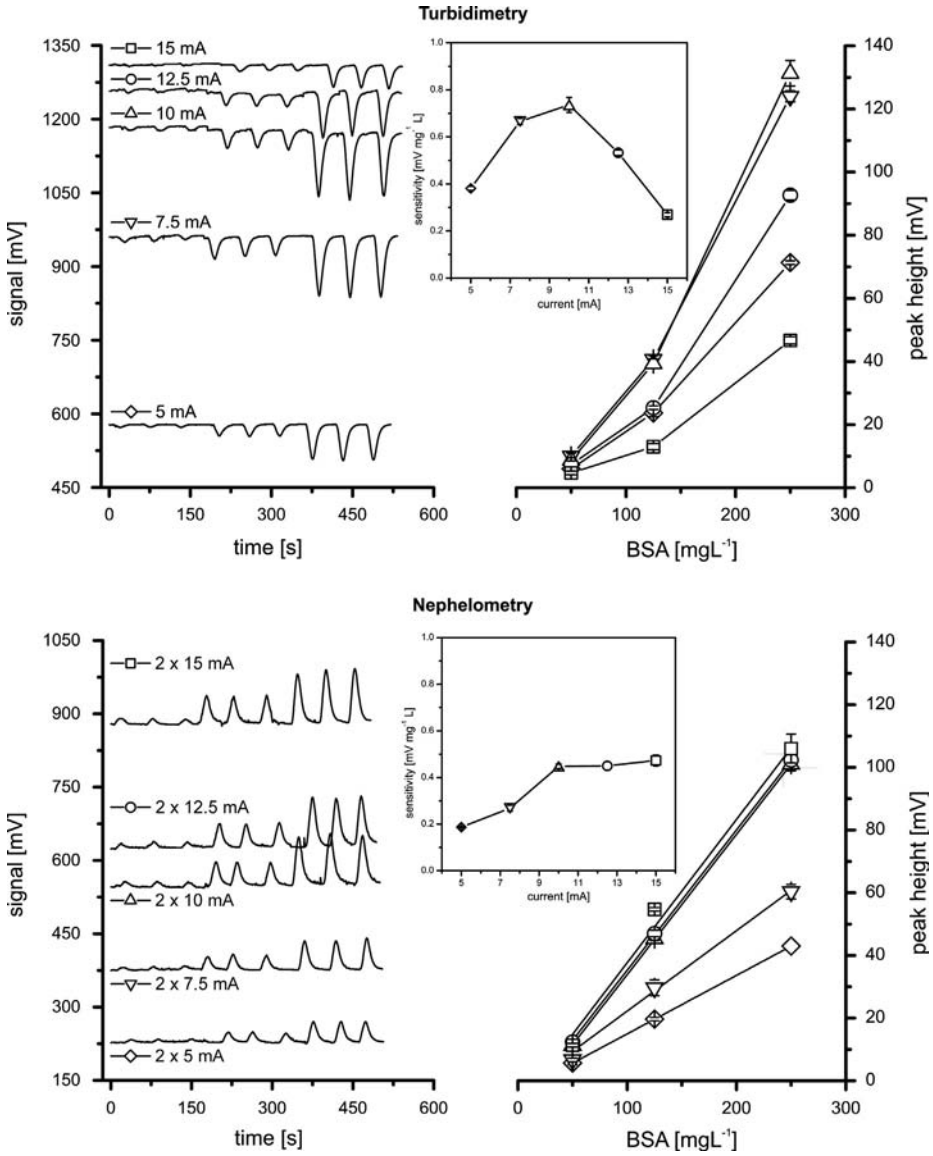

Fig. 3. Recordings from calibration (left) and corresponding calibration graphs (right) for MCFA system with turbidimetric (top) and nephelometric (bottom) detectors. The dependence between sensitivity and current powering the detector is shown in the corresponding insets.

of measurements the relative standard deviations ($n=5$) for 100 mg L^{-1} and 500 mg L^{-1} BSA standards are 3.0% and 2.6%, respectively. In the case of nephelometric mode of measurements these values are 2.8% and 1.0%, respectively.

The final goal of this work was to developed the MCFA system for determination of protein level in CSF. Both developed MCFA systems were validated using human CSF samples with known concentration of proteins beforehand analyzed by clinical laboratory. The CSF samples were diluted twice at least before the analysis, thus maximal consumption of undiluted CSF sample for single injection was 72 μL. The samples with protein level that excess the upper limit of determination were diluted 4 or 8-fold with distilled water. Each sample was injected in triplicate. Additionally, each sample was injected in the absence of SSA (the first part of procedure given in Table 1) to detect its own turbidity. Twelve samples of real human CSF have been used for validation procedure. Only for two highly pathologic samples the effect from own turbidity (in both cases lower than 8% of turbidity after treatment with SSA) was not negligible. The results of analysis CSF samples with both, physiological and pathological levels of proteins are shown in Fig. 6 (in the inset data for physiological samples). For both modes of detection the results are fully compatible with those obtained in clinical laboratory setting using recommended equipment and protocol. For both modes of measurements the regression coefficients are better than 0.99. To compare accuracy of presented methods with reference method, the two-tail paired Student's *t*-tests (11° of freedom at a 95% confidence level) were used. The calculated *t*-values for turbidimetry and nephelometry were 1.974 and 1.976, respectively. They are lower than the tabulated value (2.201) confirming statistical agreement between results obtained by both developed systems and reference method.

4. Conclusion

In this contribution multicommutated flow analysis system developed for total protein determination in CSF samples has been presented. The system was integrated with highly economic and compact optoelectronic detectors designed for turbidimetric and nephelometric measurements. Both detection modes offered by

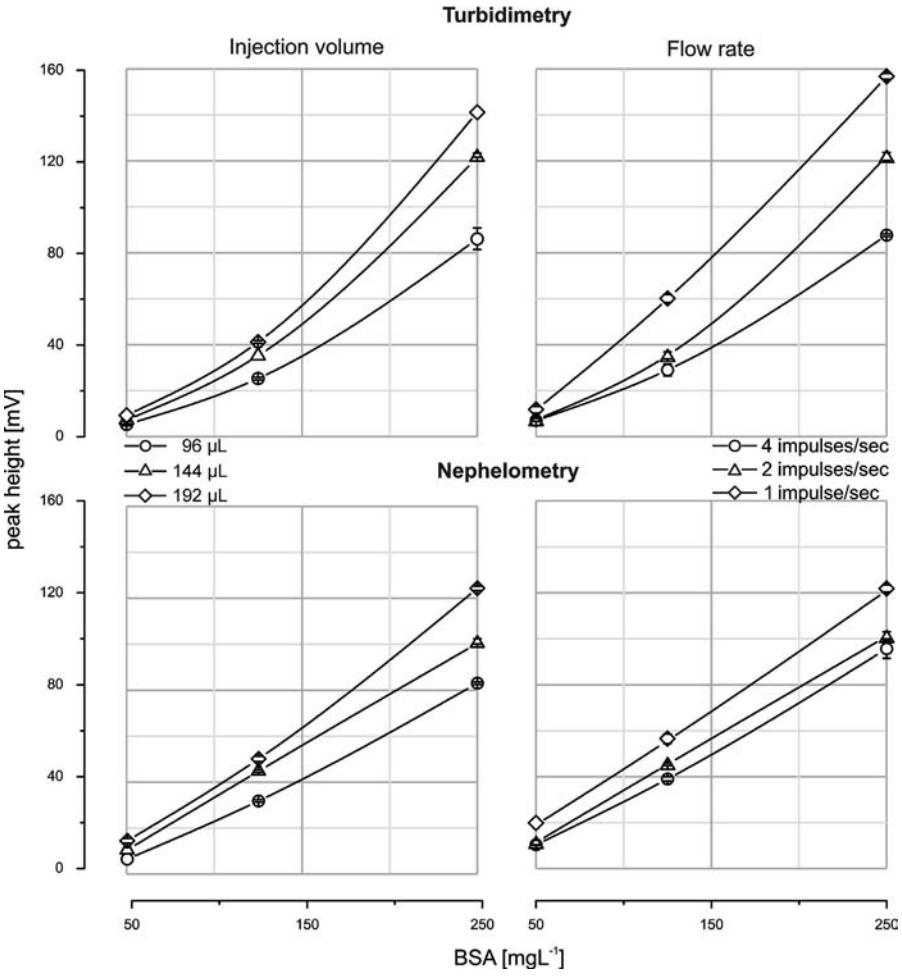

Fig. 4. The effects of injection volume (left) and flow rate (right) on analytical response for turbidimetric (top) and nephelometric (bottom) measurements.

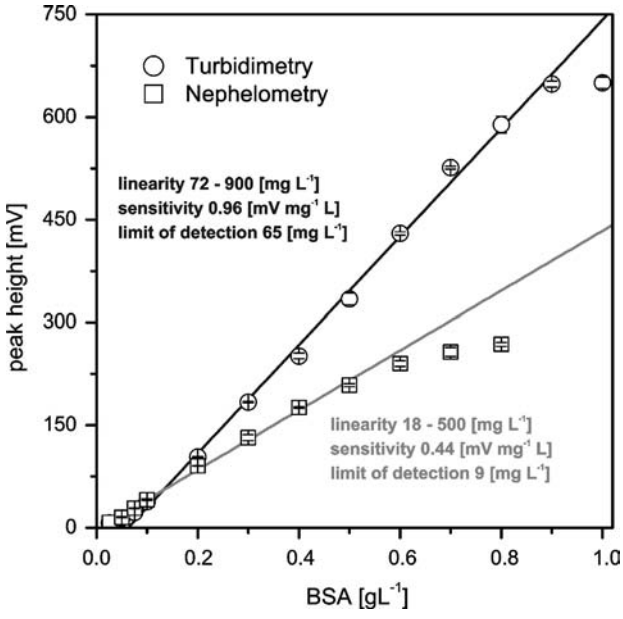

Fig. 5. Calibration graphs of MCFA system operating under optimized conditions.

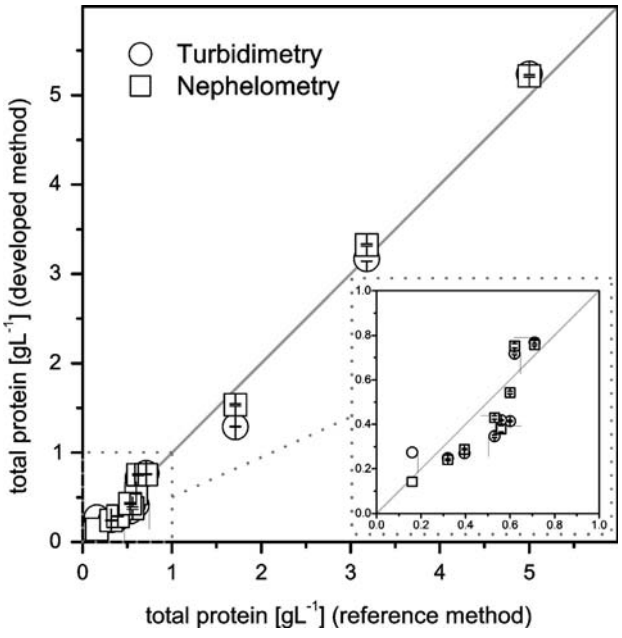

Fig. 6. Results of human CSF analysis.

optoelectronic device in the developed MCFA system are useful for CSF analysis, however at the same geometry of flow cell turbidimetric detection offers wider range of linear response and higher sensitivity. In the case of nephelometric mode of measurements lower detection limit is obtained allowing lower consumption (higher dilution) of CSF sample. The optimized flow analysis systems have been successfully validated with real human CSF samples.

Acknowledgments

Kamil Strzelak as a MISDoMP student acknowledges partial support from the EU through the European Social Fund, Contract number UDA-POKL.04.01.01-00-072/09-00. This research was granted by the Polish National Science Centre in the frame of Preludium Project (Grant NCN-2012/07/N/ST4/01848).

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