

An In-Depth Analysis of Peritoneal Dialysate Effluent Composition with a Deep-UV-LED-Based Affordable Optical Chromatographic Sensor [†]

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Abstract: It was shown earlier that the use of fast protein and metabolites liquid chromatography (FPMLC) and low-cost deep UV–LED-based optical chromatographic sensors with PD-10 desalting columns as a separation element can facilitate the monitoring of patients on chronic peritoneal dialysis (PD). Previously, we established that the first peak in the FPMLC chromatograms of effluent dialysate is mainly responsible for proteins and could be used for the assessment of peritoneal protein loss in patients on PD, while the origin and clinical significance of the other two peaks still remain unclear. Optical absorption and fluorescence spectroscopy in the UV and visible regions of 240...720 nm were used for the analysis of PD effluent chromatographic fractions collected from a drainpipe of the sensor with photometric detection at 280 nm; chromatograms of twenty dialysate samples were processed. The absorption and fluorescence spectra of the first fraction demonstrated peaks at 270 nm and 330 nm, respectively, which is typical for proteins. The absorption spectra of the third fraction revealed the characteristic maxima of creatinine and uric acid, while the fluorescence spectra showed the characteristic peak of indoxyl sulfate 375 nm at 270 nm excitation. The second fraction had a single, extremely wide absorption band, strong fluorescence was observed at 440–450 nm while excited at 370 nm. Such spectral characteristics are typical for advanced glycation end products (AGE). Thus, it was demonstrated that deep UV–LED-based affordable chromatographic sensors could provide significantly more information about the composition of PD effluent dialysate than just the total protein concentration, including the contents of clinically significant metabolites, e.g., indoxyl sulfate and AGE. Moreover, the introduction of optical fluorescence detection could significantly improve the capabilities of such devices.

Keywords: optical chemical sensor; fast protein and metabolites liquid chromatography; deep UV LED; peritoneal dialysis; absorption spectroscopy; fluorescence spectroscopy; fractionation

1. Introduction

One of the modalities of renal replacement therapy (RRT) received by patients with end-stage kidney disease (ESKD) is chronic peritoneal dialysis (PD). This treatment has

several advantages over the more widely used hemodialysis (HD), including the possibility of undergoing the procedure at home without medical assistance, better tolerability of the therapy, and less dependence on sophisticated equipment [1,2]. Like the other more common RRT modalities, chronic PD has specific side effects, e.g., significant peritoneal protein loss [3–5] and the considerable risk of infections such as dialysis peritonitis [6,7]. To prevent serious complications associated with PD, regular control of patients' health status, including laboratory tests of the peritoneal dialysis effluent, is required. Conventional clinical laboratory methods are not designed for peritoneal dialysate and are not suitable for point-of-care testing (POCT), which can be especially important for outpatients living in rural or remote areas.

A more viable approach to this problem is the use of compact and affordable sensors designed in the POCT paradigm. Microfluidic platforms and other types of POCT instruments for rapid urine testing, which can determine the concentration of proteins, glucose, and other substances [8–10], have been actively developed in recent decades; however, to the best of our knowledge, there are no reports about such devices specifically designed for the analysis of peritoneal dialysis effluent, despite the fact that urine is quite similar (but not identical) in composition to peritoneal dialysate.

We demonstrated earlier that an affordable, compact, multiple-use, reagentless optical sensor based on the fast protein and metabolites liquid chromatography (FPMLC) technique can efficiently facilitate both screening proteinuria [11] and monitoring peritoneal protein loss in patients on chronic PD [12], serving as a possible alternative to conventional clinical laboratory methods. FPMLC chromatograms of urine and peritoneal dialysate have three combined peaks corresponding to the groups of metabolites and other components with different molecular weights (Figure 1). Previously, we established a high correlation ($R^2 > 0.95$) between the area of the first peak and the total protein concentration determined by gold-standard biochemical methods; it was shown that the sensor can determine the protein concentration in urine [11], peritoneal dialysis effluent [12] and peritoneal protein loss [13] with an accuracy of 10–15%. Detailed information about the sensor as applied to quantitative protein determination in urine and effluent dialysate, e.g., the limit of detection, selectivity, accuracy, and reproducibility, can be found in our earlier works [11–13].

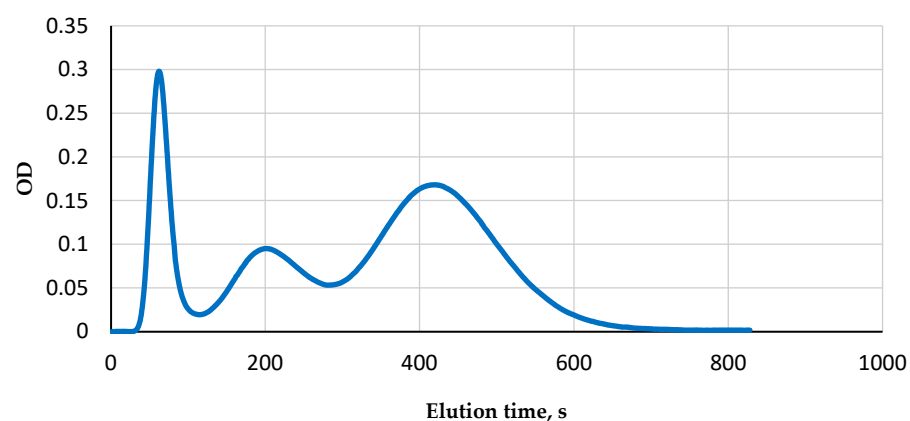


Figure 1. An example of an effluent peritoneal dialysate chromatogram.

The diagnostic value of the FPLMC sensor would be substantially expanded if other clinically significant substances in effluent dialysate, e.g., indoxyl sulphate [14] and advanced glycation end products [15,16], could be determined from chromatographic data simultaneously with the total protein. By now, the exact origin and possible clinical significance of the second and third chromatographic peaks (Figure 1), which are generally associated with middle and low molecular-weight metabolites, still remain unclear [13].

The main objectives of the current research are as follows:

- To perform the manual collection of three FPMLC chromatographic fractions separated with a PD-10 chromatographic column in the sensor in real samples of peritoneal dialysate effluent taken from a substantial group of chronic PD patients;
- To qualitatively analyze the dialysate fractions by UV–Vis absorption and fluorescence spectroscopy;
- To identify spectroscopically clinically significant metabolites that presumably contribute to the FPMLC chromatographic peaks of dialysate.

This study is essentially aimed at finding ways to further develop the existing FPLMC sensor to expand its functionality, e.g., by incorporating a fluorescent optical detector.

2. Materials and Methods

Dialysate samples. The residual samples of effluent peritoneal dialysate taken from 20 ambulatory peritoneal dialysis patients during regular hospital visits were provided by the Mariinsky City Hospital (Saint Peterburg, Russia). The study was coordinated and approved by the Institute of Experimental Medicine (Saint Peterburg, Russia). Permission from the Institute Ethics Committee, confirming that it meets ethical standards, was obtained, and patients' personal information was not disclosed by the hospital.

The chromatographic optical sensor. Detailed descriptions of the FPLMC technology, optical sensor, and analytical protocol are provided in previous studies [12,13]. The optical sensor employs PD-10 gel desalting columns (Code No. 17-0851-01) from GE Healthcare® Bio-Sciences AB (Uppsala, Sweden) for the separation of metabolites and other components in peritoneal dialysate samples, and a deep UV–LED (285 nm) and visible-blind UV photodiode as a photometric detection system. FPLMC chromatograms were processed according to the gravity protocol, with direct detection of the eluate UV absorption in a quartz flow-through cuvette. The sensor is PC controlled. The schematic diagram of the sensor is shown in Figure 2.

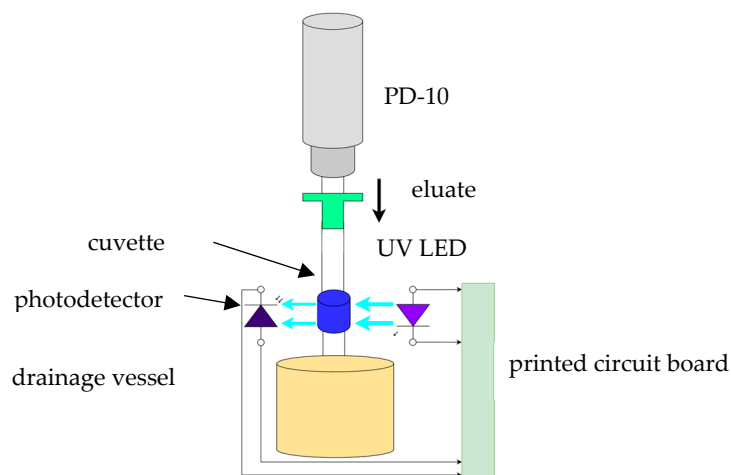


Figure 2. Schematic diagram of the sensor.

Analytical protocol. Briefly, the processing of a chromatogram is performed as follows. After equilibration of the column with 25 mL of TRIS buffer (pH = 8.0), a sample of peritoneal dialysate (500 µL) is introduced into the column. Within 20–40 s, as soon as the dialysate is absorbed by the gel, 25 mL of buffer is introduced for a second time, and the processing of the chromatogram is initiated. The whole measurement procedure takes up to 15 min; the optical density of the eluate can be viewed in real time on the computer screen.

Fractionation. Three chromatographic fractions responsible for the presence of the substances of different molecular weights in the effluent were manually collected from the drainage pipe of the sensor. Each fraction corresponds to a chromatographic peak in the order shown in Figure 1. To provide better separation, the first fraction was collected starting from the initial increase in optical density and up to the maximum of the first

chromatographic peak. After the local minimum, the second fraction was collected up to the maximum of the second peak. The third fraction was collected after the third maximum down to the optical density level of 10%, relative to the maximum of the third peak.

Spectroscopy. Absorption spectra of the fractionated dialysate samples were recorded with a UV–Vis CCD spectrophotometer with a deuterium lamp as a UV source (the working spectral range is 200–400 nm, the spectral resolution is 1 nm). Fluorescence spectra were recorded using the CM 2203 SOLAR spectrofluorometer (JSC “SOLAR”, Minsk, Belarus) with a pulsed xenon lamp as a light source. Quartz cuvettes with an optical length of 10 mm were used to analyze the absorption and fluorescence spectra. The excitation wavelengths were 250 nm, 270 nm, 310 nm, 330 nm, 370 nm. The fractionated samples were diluted with TRIS buffer at a ratio of 1:2 to increase the volume.

3. Results

We consistently analyzed the UV–Vis absorption and fluorescence spectra of three dialysate fractionations, separated from real effluent dialysate samples according to the protocol described above, to make grounded suggestions about the origin of the chromatographic peaks strongly based on the experimental data and to find possible solutions to determine additional clinically significant components of peritoneal dialysate effluent using the FPMLC sensor that was developed by Ldiamon AS (Tartu, Estonia).

Absorption and fluorescence spectra of the first fraction of peritoneal dialysate are shown in Figure 3 (three typical spectra were randomly selected from a set of twenty). A characteristic UV absorption band of proteins with a maximum at 285 nm due to radicals of sulfur-containing and aromatic amino acids, i.e., tryptophan, tyrosine, and phenylalanine [17], is clearly noticeable in the diagram. Taking into account that albumin with a characteristic absorption peak at 280 nm [18,19] is the most abundant protein in the peritoneal dialysate effluent [20], these data confirm our previous suggestion regarding the origin of the first chromatographic peak. Moreover, in Figure 3b, we can easily recognize the characteristic fluorescence peaks of tyrosine ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 202/304$ nm, $\lambda_{\text{ex}}/\lambda_{\text{em}} = 220/304$ nm and 275/304 nm) and tryptophan ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 230/348$ nm and 280/348 nm) contributing to the intrinsic fluorescence of proteins [21].

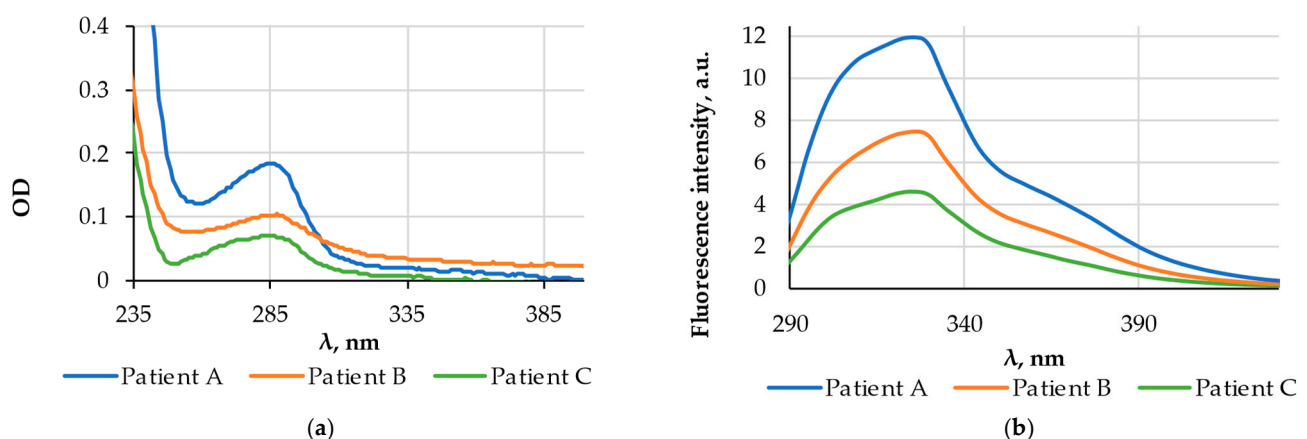


Figure 3. The absorption (a); and the fluorescence ($\lambda_{\text{ex}} = 270$ nm) spectra (b) of the first fraction of the peritoneal dialysis effluent for three samples.

Absorption and fluorescence spectra of the second fraction are presented in Figure 4. An extremely wide and strong absorption band with a maximum at 240 nm is clearly seen in the diagram; the UV absorption gradually decreases into a longer wavelength area of the spectra up to 400 nm. According to [22], this absorption band could be associated with advanced glycation end products (AGE), which are considered an important biomarker associated with the progression of chronic kidney disease and the complications of dialysis therapy [23–28]. Strong fluorescence at $\lambda_{\text{ex}}/\lambda_{\text{em}} = 370/440$ –450 nm (Figure 4b), typical for

AGE, further strengthens our suggestion [29]. The second weaker absorption maximum observed in a wavelength range of 260–270 nm (Figure 4a), which is overlapped by the stronger maximum at 240 nm, could be associated with free nucleotides [30].

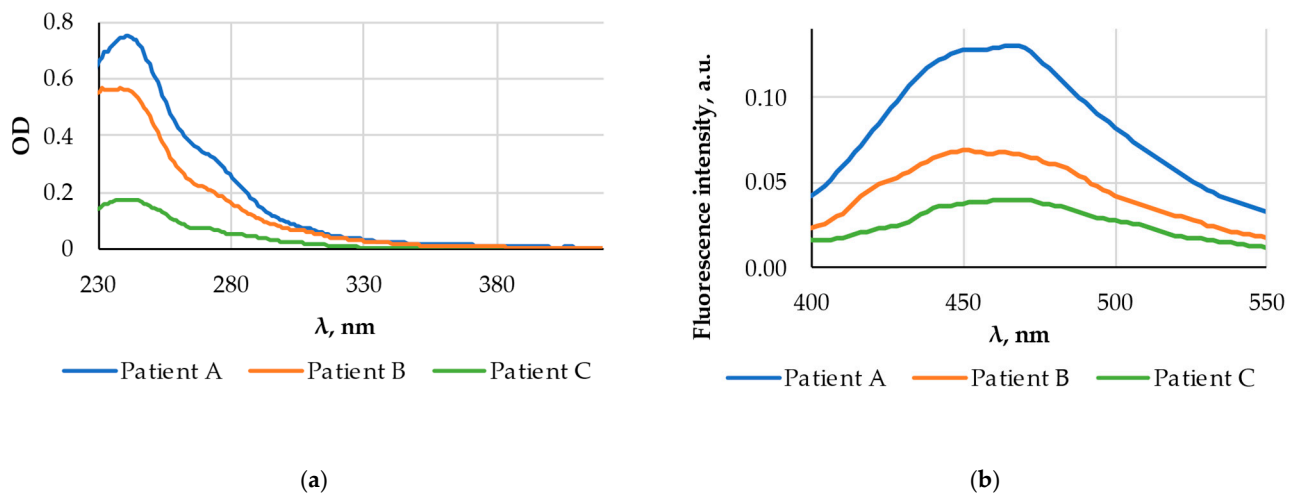


Figure 4. The absorption (a); and the fluorescence ($\lambda_{\text{ex}} = 370$ nm) spectra (b) of the second fraction of the peritoneal dialysis effluent for three samples.

The absorption spectra of the third fraction (Figure 5a) have two maxima at 240. . . 250 nm and 295. . . 296 nm. These peaks can be associated with creatinine and uric acid, with characteristic peaks at 234 nm and 233 nm/290 nm, respectively [31,32]. The fluorescence spectrum of the third fraction at $\lambda_{\text{ex}} = 270$ nm has a maximum at $\lambda_{\text{em}} = 375$ nm (Figure 5b), which is characteristic for indoxyl sulfate [33], which is related to the development of various medical conditions associated with ESKD and dialysis therapy [34–37].

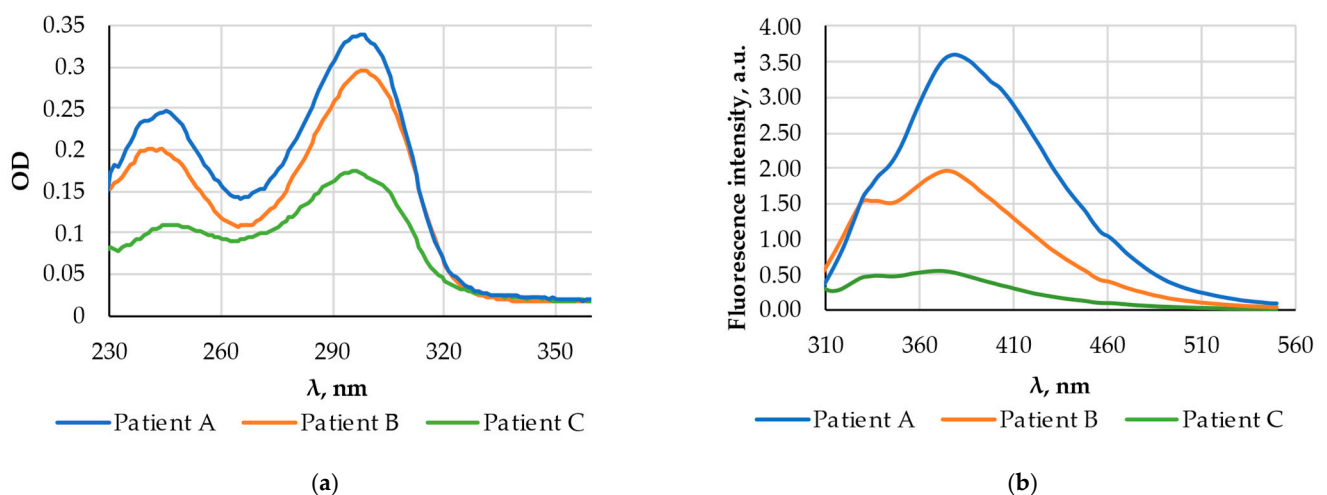


Figure 5. The absorption s (a); and fluorescence ($\lambda_{\text{ex}} = 270$ nm) spectra (b) of the third fraction of the peritoneal dialysis effluent for three samples.

Table 1 shows all the fluorescent peaks detected during this study. Some peaks occur in two or three fractions, in which case the intensity is indicated in parentheses in comparison with the rest of the fractions as either high (H), or low (L). The wavelengths for each fraction are arranged in order of decreasing peak intensity.

Table 1. Excitation and emission wavelengths for all fractions.

Fraction	λ_{ex} , nm	250	270	310	330	370
1	λ_{em} , nm	330 (H)	330 (H)	-	440	440 (L)
2	λ_{em} , nm	310, 330 (L), 405	300	405	410	440 (H)
3	λ_{em} , nm	380, 335	375, 330 (L)	370–410	425	-

4. Conclusions

In this study, twenty residual samples of effluent peritoneal dialysate collected from a group of chronic PD outpatients during a regular hospital visit were manually fractionated using an optical chromatographic sensor based on the simplified FPMLC technique and consistently analyzed by UV–Vis spectrophotometry and fluorescence spectroscopy. It was further confirmed that the first peak in the FPMLC chromatograms is firmly associated with proteins (serum albumin being a major contributor) and could be used for qualitative protein determination in peritoneal dialysis effluent [12,13].

The UV–Vis absorption and fluorescence spectra of the second fraction suggest that the second peak in the FPMLC chromatograms is at least partially related to low molecular-weight AGE products (a significant absorption band at 240–400 nm coupled with intensive fluorescence at excitation/emission wavelengths $\lambda_{ex}/\lambda_{em} = 370/440$ –450 nm were observed) and free nucleotides (noticeable absorption at 260–270 nm). Probably, the third peak in the chromatograms is responsible for low molecular-weight substances, i.e., uric acid, creatinine and free indoxyl sulfate (intensive fluorescence at $\lambda_{ex} = 270$ nm, $\lambda_{em} = 375$ nm).

According to the experimental data, the autofluorescence of the second chromatographic fraction of dialysate at 440–450 nm and the third fraction of dialysate at 375 nm could be potentially used for the quantitative determination of AGE products and indoxyl sulphate, respectively. AGE products and indoxyl sulfate are associated with cardiovascular risk and other dialysis complications; therefore, their determination may be important for patients with chronic kidney disease on hemodialysis or peritoneal dialysis [15,16,23–28,34–40].

The optical chromatographic sensor with photometric detection used in this research can reliably determine the concentration of total protein in urine and dialysate with good accuracy and reproducibility and assess peritoneal protein loss [11–13]. The sensor can be upgraded by incorporating a fluorescence detecting module for the determination of AGE products and free indoxyl sulfate. This would significantly expand the capabilities of this sensor as a POCT device for ambulatory PD patients.

As other studies show [14–16], the rapid determination of indoxyl sulfate and AGE products by optical methods is feasible; however, the possibility of developing and implementing such a module and ensuring its accuracy is the subject of future research. At the current stage, which could be only considered as preliminary, only the qualitative determination of metabolites was performed, and perspectives on the future development of the sensor based on the FPMLC approach have been demonstrated.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Institute of Experimental Medicine (protocol #2/22 from 6 April 2022).

Informed Consent Statement: Patient consent was waived according to the Decision of the Council of the Eurasian Economic Commission No. 29 of 12 February 2016 “On Rules for Clinical and Clinical Laboratory Trials (Studies) of Medical Products”. The document states that written informed consent is not required when residual laboratory samples are used exclusively for in vitro research and testing of clinical laboratory equipment. Patients’ personal data or medical history were not disclosed by the hospital.

Data Availability Statement: Data are available on request due to the hospital’s ethical policy.

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Conflicts of Interest: Authors Artur Kuznetsov and Aleksandr Frorip were employed by the company AS Ldiamon, and author Vadim Korsakov was employed by the company Jeko Disain OÜ. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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