Proceedings of the 28th IEEE
EMBS Annual International Conference
New York City, USA, Aug 30-Sept 3, 2006

Optical dialysis adequacy sensor: contribution of chromophores to the ultra violet absorbance in the spent dialysate

Kai Lauri, Risto Tanner, Merike Luman, Jana Jerotskaja, and Ivo Fridolin, *Member, IEEE*

***Abstract*— Several on-line methods have been developed to standardize the assessment of dialysis adequacy. Earlier studies have demonstrated that on-line monitoring of total ultra violet (UV) absorbance in spent dialysate can be utilized to follow continuously a single hemodialysis session. The aim of this study was to investigate the contribution of different compounds, acting as chromophores, to the UV-absorbance in the spent dialysate in order to explain origin of the cumulative and integrated UV-absorbance measured by the optical dialysis adequacy sensor.**

Four uremic patients, during 12 hemodialysis treatments, were followed by the optical dialysis adequacy sensor using the wavelength of 280 nm. The dialysate samples were taken and analyzed using reversed phase high performance liquid chromatography (HPLC). The total number of detected peaks from the HPLC gradient separation profiles measured at the wavelength 280 nm for the samples collected 10 min after the start of hemodialysis (Mean ± SD) was 38 ± 6. The relative contribution from the area of 10 main peaks to the total area of all detected peaks in percentage was 91.01 ± 2.52 %.

The optical dialysis adequacy sensor provides continuous, on-line hemodialysis measurements and may immediately identify and alert to any deviations in the dialysis. Our study indicates that there exists a number of prevalent compounds that are the main cause of the cumulative and integrated UV-absorbance.

I. Introduction

DIALYSIS is the most common method used to treat advanced and permanent kidney failure. Many studies have demonstrated a relationship between dialysis dose, estimated from the removal of a small molecule weight solute urea, and morbidity and mortality in hemodialysis patients [1]-[3]. To maintain and achieve pre-set treatment dose an on-line monitoring system has been suggested as a more accurate method [3]-[6]. Recently the spectrophotometrical sensors for on-line monitoring of total ultra-violet (UV) absorbance or urea in the spent dialysate have been presented, aiming to follow continuously a single hemodialysis session [7]-[9]. At the same time, a need arises for techniques, which can offer a tool to monitor separately several compounds retained in uremic patients and with potential clinical significance, because urea, the traditional marker for dialysis quality, should not be the only solute used to model the dialysis therapy [10]. Uremic toxicity or the uremic syndrome is described as a clinical picture resulted by the impaired renal elimination and accumulation of many compounds, so called uremic toxins, in the body [11]. To date, a long list of possible uremic toxins has been identified as believed to be responsible for multifactorial and cumulative cause of uremic toxicity [12], [13], [14]. However, only a few of the uremic retention solutes fully conform to a true definition of uremic toxins [11]. The methods, contributing to the identification, characterisation, and evaluation of uremic retention solutes, could be assessed in order to ensure dialysis adequacy and quality. Earlier a good correlation between the UV-absorbance measured by the optical dialysis adequacy sensor in dialysate and the concentration of several solutes both in the spent dialysate and in the blood of the dialysis patients have been shown [7], [15]. This indicates that the technique can be used to estimate the removal of retained substances.

Still a deeper understanding about the signal content of the optical dialysis adequacy sensor should be valuable to utilize the technique and create prerequisites for further development. For this purpose reversed phase high performance liquid chromatography (HPLC) is recommended as a sensitive, accurate and reproducible tool applicable for separation of different compounds in aqueous samples [16].

The aim of this study was to investigate the contribution of different compounds, acting as chromophores, to the UV-absorbance in the spent dialysate in order to explain origin of the cumulative and integrated UV-absorbance measured by the optical dialysis adequacy sensor.

II. Materials and Methods

A. Clinical study

This study was performed after approval of the protocol by the Tallinn Medical Research Ethics Committee at the National Institute for Health Development, Estonia. An informed consent was obtained from all participating patients.

Four uremic patients, one female and three males, mean age 66.5 ± 3.7 years, on chronic thrice-weekly

Manuscript received April 3, 2006. This work was supported in part by the Estonian Science Foundation Grant No 5871 and in part by the NATO Reintegration Grant EAP.RIG 981201.

Kai Lauri is with the Centre of Biomedical Engineering, Tallinn University of Technology, 19086 Tallinn, Estonia (corresponding author, phone: +372 6202200; fax: +372 6202201; e-mail: kai.lauri@mail.ee).

Risto Tanner is with the Laboratory of Chemical Physics, National Institute of Chemical Physics and Biophysics, Akadeemia Rd. 23, 12618 Tallinn, Estonia (e-mail: risto@kbfi.ee).

Merike Luman is with the Department of Dialysis and Nephrology, North-Estonian Regional Hospital, J.Sütiste Rd 19, 13419 Tallinn, Estonia (e-mail: merike.luman@regionaalhaigla.ee).

Jana Jerotskaja and Ivo Fridolin are with the Centre of Biomedical Engineering, Tallinn University of Technology, 19086 Tallinn, Estonia (e-mail: jana@cb.ttu.ee and ivo@cb.ttu.ee).

hemodialysis were included in the study, using the clinical set-up of the experiments as described earlier [17]. Two different polysulphone dialysers with the effective membrane area of 1.8 m^2 were used: F8 HPS (N=9) and FX80 (N=3) (Fresenius Medical Care, Germany). The dialysate flow was 500 mL/min and the blood flow varied between 245 to 350 mL/min. The type of dialysis machine used was Fresenius 4008H (Fresenius Medical Care, Germany).

The optical dialysis adequacy sensor consisted of a spectrophotometer (HR2000, Ocean Optics, Inc., USA) that was used for the determination of UV-absorbance, and of a specially designed optical cuvette, connected to the fluid outlet of the dialysis machine with all spent dialysate passing through during the on-line experiments. The absorbance was measured in arbitrary units (a.u.). The sampling frequency was set at two samples per minute. The obtained UV-absorbance values were processed and presented on the computer screen by a PC incorporated in the spectrophotometer using Ocean Optics' software (OOIBase32, Ocean Optics, Inc., USA, version 2.0.2.2 for Windows). The results from measurements during 12 hemodialysis treatments using the wavelength of 280 nm are presented in this paper.

Two dialysate samples were taken during the dialysis: 1) in the beginning, 10 minutes after the start of the dialysis session; 2) immediately at the end of the treatment (210 or 240 minutes) (Fig. 1). Pure dialysate was collected before the start of a dialysis session, used as the reference solution, when the dialysis machine was prepared for starting and the conductivity was stable.

B. Reversed phase high performance liquid chromatography (HPLC) study

Samples of dialysate were acidified down to pH4 with formic acid and 100µL of each was used for chromatographic separation routinely. The HPLC system consisted of a quaternary gradient pump unit, a column oven, and a diode array spectrophotometric detector (DAD, all of Series 200 instruments from Perkin Elmer, Norwalk, CT, USA), a manual injector from Rheodyne (Rohnert Park, CA, USA), and a Zorbax C8 4.6 x 250 mm column from Du Pont Instruments (Wilmington, DE, USA) with security guard KJO-4282 from Phenomenex (Torrance, CA, USA). The eluent was mixed from 0.05 M formic acid adjusted to pH 4.0 with ammonium hydroxide (A), HPLC grade methanol (B) and HPLC-S grade acetonitrile (C), both from Rathburn (Walkerburn, Scotland), with six step gradient program as specified in Table I.

The total flow rate 1 mL/min was used continuously and the column temperature was adjusted to 30ºC. The UV absorbance was monitored at 280 nm with measurement interval of 880 ms, spectra registered between 200-400 nm with time interval of 1.76 s, and data processed respectively by means of Turbochrom WS and Turboscan 200 softwares from Perkin Elmer.

TABLE I
ELUTION PROGRAM USED FOR HPLC SEPARATION OF CONSTITUENTS IN THE DIALYSATE

Step	Time (min)	Buffer (A) %	Methanol (B) %	Aceto nitrile (C) %	Gradient
0	0	100	0	0	-
1	30	60	36	4	Linear
2	5	10	81	9	Linear
3	4	10	81	9	No grad.
4	1	10	0	90	Linear
5	6	10	0	90	No grad

III. RESULTS

Fig. 1 shows a typical on-line absorbance curve during a single hemodialysis treatment where UV-absorbance at the wavelength of 280 nm is plotted against the time. UV-absorbance drops and peaks during the dialysis correspond to the self-tests in the dialysis machine when the dialyser is in the by-pass mode.

The exact time when the dialysate samples were taken for the later analysis are also presented in Fig. 1.

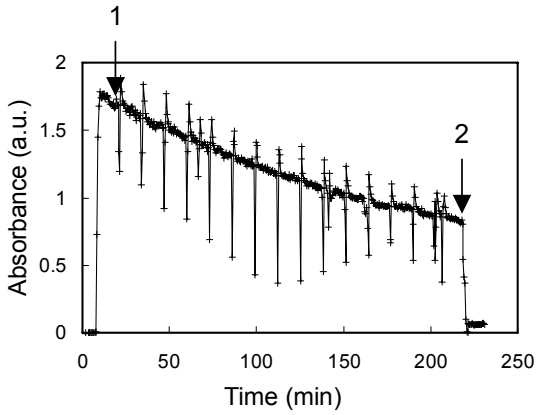

Fig. 1. Typical on-line absorbance curve during a single hemodialysis treatment where UV-absorbance at the wavelength 280 nm is plotted against the time. The time moments when the dialysate samples were taken for the later analysis: *1)* dialysate sample collected 10 min after start of hemodialysis, *2)* dialysate sample collected 240 min after start of hemodialysis (end of the hemodialysis).

Fig. 2 presents characteristic HPLC profiles of the spent dialysate collected 10 min after start of the hemodialysis 1), and at the end of the treatment 2) measured at the wavelength 280 nm. A number of higher prevalent peaks can be observed from the HPLC profiles indicating the presence of a compounds, acting as chromophores, to the UV-absorbance in the spent dialysate, which are the main cause of the cumulative and integrated UV-absorbance measured by the optical dialysis adequacy sensor.

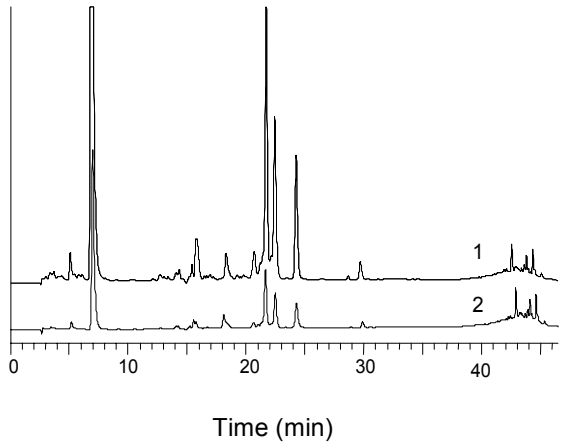

Fig. 2. Characteristic HPLC profiles of the spent dialysate monitored at the wavelength of 280 nm; *1)* dialysate sample collected 10 min after start of hemodialysis, *2)* dialysate sample collected 240 min after start of hemodialysis (end of hemodialysis).

The total number of detected peaks from the HPLC gradient separation profiles for the samples collected 10 min after the start of hemodialysis from each dialysis session are presented in Table II. The Mean ± SD of the total number of detected peaks was 38 ± 6.

TABLE II
TOTAL NO. OF DETECTED PEAKS AND AREA % OF 10 MAIN PEAKS (10 MIN DIALYSIS SAMPLE)

Dialysis no	Total No. of detected peaks(chromophores)	Area % of 10 main peaks
1	30	91.81
2	33	90.43
3	33	92.77
4	33	92.69
5	40	89.50
6	45	88.83
7	42	89.20
8	43	88.28
9	50	86.71
10	37	94.29
11	40	93.27
12	35	94.31
Mean ± SD	**38 ± 6**	**91.01 ± 2.52**

peaks to the total area of all detected peaks in percentage was calculated for each dialysis treatment (Area % of 10 main peaks). The mean value ± SD of "Area % of 10 main peaks" was 91.01 ± 2.52.

IV. Discussion

Fig. 1 shows a typical on-line absorbance curve during a single hemodialysis treatment where UV-absorbance at the wavelength of 280 nm is plotted against the time. UV-absorbance is higher in the beginning of the treatment because of high concentration of the metabolic waste products in the body fluids. UV-absorbance decreases during the session when the waste products are removed. The figure demonstrates the possibility to follow a single hemodialysis session continuously and to monitor deviations in the dialysator performance using the optical dialysis adequacy sensor. The different clinical dialysis adequacy parameters used by physicians to estimate dialysis quality like Kt/V, URR, etc., can be calculated from the on-line curve [17] that makes the technique suitable for clinical routine application.

Characteristic HPLC profiles of the spent dialysate collected 10 min after the start of hemodialysis 1), and at the end of the treatment 2) measured at the wavelength of 280 nm presented in Fig. 2 demonstrate a number of higher prevalent peaks on the HPLC profiles. This indicates that there exists a group of compounds, acting as chromophores to the UV-absorbance in the spent dialysate and which are the main cause of the cumulative and integrated UV-absorbance. Similarity of the HPLC profiles for 10 min and the end dialysis sample indicates that composition of the spent dialysate (e.g. number and type of chromophores) does not change remarkably during the treatment. At the same time the peaks are lower for the end dialysis sample due to decreased amount of compounds transported from the blood into the dialysate at the end of the treatment.

Table II presents the total number of detected peaks from the HPLC gradient separation profiles for the samples collected 10 min after the start of hemodialysis from each dialysis session. The Mean ± SD of the total number of detected peaks 38 ± 6 demonstrates that the number of chromophores varies from treatment to treatment. The number of peaks detected in this work is still less compared to the number of peaks detected in serum in earlier published works [16, 18], partly because the dialyzer acts as a filter selecting out certain compounds that can pass from uremic serum into the dialysate and partly because the wavelength of 254 nm was used. Moreover, the mean value ± SD of "Area % of 10 main peaks" was 91.01 ± 2.52 (Table II) indicating that approximately 90 % of the cumulative and integrated UV-absorbance measured by the optical dialysis adequacy sensor originates from the 10 main peaks for a particular dialysis treatment.

To our knowledge, some early experiments with the gel and ion chromatography have been performed to study compounds in the spent dialysate at the wavelength of 280 nm [19, 20]. The present study adds new information about the origin of the signal measured by the optical dialysis adequacy sensor and composition of the spent dialysate.

V. Conclusion

The optical dialysis adequacy sensor provides continuous, on-line measurements with no need for repeated blood samples or disposables-chemicals and may immediately identify and alert to any deviations in dialysis treatment due to changes in the dialyser performance or the solute's removal from the blood.

This study investigated contribution of compounds to the UV-absorbance in the spent dialysate, acting as chromophores, in order to explain the origin of the cumulative and integrated UV-absorbance measured by the optical dialysis adequacy sensor when several solutes in the dialysate are measured simultaneously. This will be useful when studying the possibility of monitoring continuously and on-line the removal of solutes/toxins during hemodialysis and the effect of common medication that patients are treated with. Exact identification of compounds corresponding to particular peaks in the HPLC profiles of the spent dialysate will be issue of the next studies.

ACKNOWLEDGMENT

The authors wish to thank Galina Velikodneva for assistance during clinical experiments, Rain Kattai for skillful technical assistance and also those dialysis patients who so kindly participated in the experiments.

REFERENCES

[1] P. Keshaviah, A. J. Collins, J. Z. Ma, D. N. Churchill, and K. E. Thorpe, "Survival comparison between hemodialysis and peritoneal dialysis based on matched doses of delivered therapy", *Journal of the American Society of Nephrology*, 2002, vol. 13 Suppl. 1, pp. S48-52.

[2] F. K. Port, V. B. Ashby, R. K. Dhingra, E. C. Roys, and R. A. Wolfe, "Dialysis dose and body mass index are strongly associated with survival in hemodialysis patients"', *Journal of the American Society of Nephrology*, 2002, vol. 13, pp. 1061-1066.

[3] NKF-DOQI, "NKF-DOQI clinical practice guidelines for hemodialysis adequacy", *American Journal of Kidney Diseases*, 1997, vol. 30, pp. S1-S64.

[4] G. Hernandez-Herrera, A. Martin-Malo, M. Rodriguez, and P. Aljama, "Assessment of the length of each hemodialysis session by on-line dialysate urea monitoring", 2001,*Nephron*, 89, pp. 37-42.

[5] S. H. Lambie and C. W. McIntyre, "Developments in online monitoring of haemodialysis patients: towards global assessment of dialysis adequacy," *Current Opinion in Nephrology & Hypertension*, 2003, vol. 12, pp. 633-638.

[6] F. Locatelli, U. Buoncristiani, B. Canaud, H. Khler, T. Petitclerc, and P. Zucchelli, "Haemodialysis with on-line monitoring equipment: tools or toys?", *Nephrology Dialysis Transplantation*, 2005, vol. 20, pp. 22-33.

[7] I. Fridolin, M. Magnusson, and L.-G. Lindberg, "On-line monitoring of solutes in dialysate using absorption of ultraviolet radiation: technique description", *The International Journal of Artificial Organs*, 2002, vol. 25, pp. 748-761.

[8] P. Jensen, J. Bak, S. Ladefoged, and S. Andersson-Engels, "Determination of urea, glucose, and phosphate in dialysate with Fourier transform infrared spectroscopy," *Spectrochim Acta A Mol Biomol Spectrosc*, 2004, vol. 60, pp. 899-905.

[9] J. T. Olesberg, M. A. Arnold, and M. J. Flanigan, "Online Measurement of Urea Concentration in Spent Dialysate during Hemodialysis", *Clin Chem*, 2004, vol. 50, pp. 175-81.

[10] F. A. Gotch, J. A. Sargent, and M. L.Keen, "Whither goest Kt/V?", *Kidney International*, 2000, vol. 58, Suppl. 76, pp. S3-S18.

[11] R. Vanholder, G. Glorieux, R. De Smet, and N. Lameire, "New insights in uremic toxins", 2003, *Kidney International*, Suppl. 84, pp. 6-10.

[12] S. Ringoir, (1997): 'An update on uremic toxins', *Kidney International*, vol. 62, pp. S2-4.

[13] [T. Niwa, (1997): 'Mass spectrometry in the search for uremic toxins', *Mass Spectrom Rev*, vol.. 16, pp. 307-32.

[14] R. Vanholder, R. De Smet, G. Glorieux, A. Argiles, U. Baurmeister, P. Brunet, W. Clark, G. Cohen, P. P. De Deyn, R. Deppisch, B. Descamps-Latscha, T. Henle, A. Jorres, H. D. Lemke, Z. A. Massy, J. Passlick-Deetjen, M. Rodriguez, B. Stegmayr, P. Stenvinkel, C. Tetta, C. Wanner, and W. Zidek, "Review on uremic toxins: classification, concentration, and interindividual variability", *Kidney International*, 2003, vol.. 63, pp. 1934-1943.

[15] I. Fridolin and L.-G. Lindberg, "On-line monitoring of solutes in dialysate using absorption of ultraviolet radiation - wavelength dependence", *Medical & Biological Engineering & Computing*, 2003, 41, pp. 263-270.

[16] R. Vanholder, R. De Smet, and N. H. Lameire, "Redesigning the map of uremic toxins," *Contr. Nephrol*., vol. 133, pp. 42-70, 2001

[17] F. Uhlin, I. Fridolin, L.-G. Lindberg, and M. Magnusson, "Estimation of delivered dialysis dose by on-line monitoring of the UV-absorbance in the spent dialysate", *American Journal of Kidney Diseases*, 2003, 41, pp. 1026-1036.

[18] R. C. Vanholder, R. V. De Smet, and S. M. Ringoir, "Assessment of urea and other uremic markers for quantification of dialysis efficacy," *Clin Chem*, , 1992, vol. 38, pp. 1429-1436.

[19] T. Abiko, I. Onodera, and H. Sekino, "Isolation, structure and biological activity of the Trp-containing pentapeptide from uremic fluid," *Biochem Biophys Res Commun*, vol. 89, pp. 813-821, 1979.

[20] K. Ehrlich, F. Holland, T. Turnham, and E. Klein, "Osmotic concentration of polypeptides from hemofiltrate of uremic patients," *Clin Nephrol*, 1980, vol. 14, pp. 31-5.