

On-line monitoring of solutes in dialysate using absorption of ultraviolet radiation: Technique description

I. FRIDOLIN¹, M. MAGNUSSON², L.-G. LINDBERG¹

¹ Department of Biomedical Engineering, Linköping University, University Hospital - Sweden

² Department of Nephrology, Linköping University, University Hospital - Sweden

ABSTRACT: Purpose: *The aim of this work was to describe a new optical method for monitoring solutes in a spent dialysate using absorption of UV radiation.*

Method: *The method utilises UV-absorbance determined in the spent dialysate using a spectrophotometrical set-up. Measurements were performed both on collected dialysate samples and on-line. During on-line monitoring, a spectrophotometer was connected to the fluid outlet of the dialysis machine, with all spent dialysate passing through a specially-designed cuvette for optical single-wavelength measurements. The concentrations of several substances of various molecular sizes, electrical charge, transport mechanism, etc. were determined in the dialysate and in the blood using standard laboratory techniques. The correlation coefficient between UV-absorbance of the spent dialysate and concentration of the substances in the spent dialysate and in the blood was calculated from data based on the collected samples.*

Results: *The obtained on-line UV-absorbance curve demonstrates the possibility to follow a single hemodialysis session continuously and to monitor deviations in the dialysator performance using UV-absorbance. The experimental results indicate a very good correlation between UV-absorbance and several small waste solutes removed such as urea, creatinine and uric acid in the spent dialysate and in the blood for every individual treatment at a fixed wavelength of 285 nm. Moreover, a good correlation between the UV-absorbance and substances like potassium, phosphate and β_2 -microglobulin was obtained. The lowest correlation was achieved for sodium, calcium, glucose, vitamin B₁₂ and albumin.*

Conclusions: *A technique for on-line monitoring of solutes in the spent dialysate utilising the UV-absorbance was developed. On-line monitoring during a single hemodialysis session exploiting UV-absorbance represents a possibility to follow a single hemodialysis session continuously and monitor deviations in dialysis efficiency (e.g. changes in blood flow and clearance). The UV-absorbance correlates well to the concentration of several solutes known to accumulate in dialysis patients indicating that the technique can be used to estimate the removal of retained substances. (Int J Artif Organs 2002; 25: 748-61)*

KEY WORDS: *Hemodialysis, Dialysis monitoring, Spectrophotometry, Ultraviolet, Absorption, Spent dialysate, Dialysis dose, Dialysis adequacy, Dialysis efficiency, Urea, Creatinine, Uric acid*

INTRODUCTION

A large number of patients with end-stage renal disease (ESRD) are treated by hemodialysis (1) and this number is

increasing every year (2). Despite being a routinely used treatment in clinical praxis, dialysis has still remained a complex therapy with side effects, requires a major time commitment by the patient and is associated with a

complex medical and dietary regimen (3). Moreover, patients undergoing hemodialysis suffer from several complications such as hypotension and dizziness due to hypovolemia, etc. (4). Because dialysis is a time-consuming and expensive treatment (5, 6), there is great interest in shortening the duration of the procedure from the patient's perspective and reducing the costs of treatment and hospitalisation from the economical viewpoint (7). Methods and remedies that allow monitoring of hemodialysis patients might enable early recognition of inadequate dialysis or cardiovascular instability in which action needs to be taken (8).

In 1981 the National Cooperative Dialysis Study (NCDS) reported a connection between high levels of blood urea nitrogen (BUN) and morbidity of hemodialysis patients (9). The results indicated a linkage between certain dialysis parameters (e.g. treatment length, BUN level) and the appearance of uremic manifestations such as gastrointestinal toxicity with anorexia, congestive heart failure, sudden death, to mention a few complications. Furthermore, a number of studies have demonstrated that prolonged and frequent dialysis improves survival, nutritional status and reduces acute and long-term complications (10). These types of treatments achieve higher solute clearance for different molecular weight solutes preventing high peak concentrations and normalise the fluid balance through slow but persistent ultrafiltration enabling adequate control of hypertension. However, several questions as to how to define and determine the quality and adequacy of the dialysis are still under debate (11-13).

The most important functions controlled in dialysis therapy are: 1) body water content; 2) acid-base balance; 3) balance of electrolytes and 4) concentration of several toxic solutes in the body (14). By definition, continuous, on-line monitoring of toxic solutes, responsible for uremic toxicity, seems to be one paramount feature of a dialysis monitor. To date, a long list of potential uremic toxins has been identified as possibly responsible for multifactorial and cumulative cause of uremic toxicity (15-17).

In clinical practice, urea and creatinine, two non-toxic substances, are used as markers for renal function and uremia. Over twenty years ago urea kinetic modelling was introduced as a method to individualise hemodialysis therapy (18). It has also been shown in large, mainly retrospective studies that the dialysis efficacy measured as urea reduction rate (URR) and/or Kt/V , correlates to patient outcome (19-21). The Kt/V is a dimensionless ratio

where K is the dialyser blood urea clearance in ml/min, t is the dialysis session length in min and V is the distribution volume of urea in the body in ml. Urea kinetic modelling including calculation of URR and Kt/V is in clinical practice usually based on blood-based samples. At the same time, direct dialysate quantification (DDQ), where all spent dialysate is collected and the total removed amount of urea from a patient is captured, is widely accepted as a reference method for evaluating the efficiency of dialysis (22). However, DDQ remains quite cumbersome, even though only a fraction of the total volume is collected.

In order to automate the urea sampling procedure, the urea monitors, that permit the calculation of urea kinetic parameters from multiple measurements throughout the session, are currently being developed to measure urea in both the dialysate and in the blood (23). Dialysate-based methods of dialysis-dose quantification might offer advantages compared to blood-based methods. There is, for example, no need for repeated blood samples, no problems with biocompatibility and less cumbersome DDQ can be applied. The majority of these methods are based on measuring the result of the action of the enzyme urease on urea. The breakdown products from this reaction are measured by an ammonium ion (22, 24) or with a conductivity sensor (25, 26). The technique is independent of recirculation but requires disposable supplies that can be cost-ineffective in the long run and has a relatively complex measurement procedure under well-controlled conditions, e.g. stable temperature and pH. Several devices measuring urea in the spent dialysate are available on the market: e.g. Biostat 1000 Urea Monitor (Baxter Healthcare, Dirfield, Ill, USA) (27), Biotrack, Biocare Corp., Taiwan (28), and DQM200 urea monitor (Gambro AB, Sweden) (29). Common problems with these existing urea-specific sensors are that they monitor more or less intermittently, perform side-stream measurements (a sample is drawn from the mainstream dialysate flow), use chemicals and are developed to measure only urea.

Furthermore, for estimation of urea clearance there are methods based on the determination of ionic dialysance (30, 31). However, the precision of the methods depends on certain assumptions, e.g. ionic dialysance is an accurate estimate for urea clearance and patient urea distribution volume is known to calculate Kt/V (32). Those assumptions can be influenced by several parameters, such as dialyser membrane type, recirculation, etc (33,

34). At least two commercial products are available on the market, the "Diascan module" (COT, Hospal, Meyzeu, France) and OCM (Fresenius Medical Care, Germany), which are incorporated into the dialysis machines because of the necessity to synchronise the ionic dialysance measurement module and the dialysis machine.

In order to define the adequacy of the dialysis therapy and increase its efficiency it seems important to: 1) immediately identify and be alert for any deviations in dialysis treatment due to changes in dialysate side (dialysate flow), in dialyser performance (clearance), in vascular access (blood flow) or increased AV-fistula recirculation; 2) monitor early symptoms of cardiovascular instability; 3) ensure dialysis quality by evaluating the delivery of the prescribed treatment dose; 4) enable an individual approach to follow and plan each dialysis treatment giving feed-back to nursing staff after every intervention. The dialysis monitors that are available today still do not fulfil all the requirements stated above. A method that is more cost-efficient, continuously estimates the removal of molecules other than urea (potential toxins) and at the same time offers a possibility for an early detection of declining treatment efficacy, due to access failure or increasing AV-fistula recirculation, would be preferable.

UV-absorption may be used to monitor concentration variations of retained solutes in dialysate reflecting clearance status during dialysis. For this purpose one wants to know how the UV-absorbing substances or a combination of these in the spent dialysate are correlated to the substances used by physicians as markers to assess the quality of dialysis.

This enables an estimate of the dialysis dose quantification based on the UV-absorbing compound(s) versus other traditional measures. However, nobody knows exactly which substance(s) is (are) measured by the UV technique in the spent dialysate. As a first step this study is focused on: 1) finding out how the UV-absorbance is correlated to the substances with various molecular sizes in the spent dialysate, among them the solutes used by physicians as markers to assess the quality of dialysis today; 2) to assess how the UV method fulfils requirements for a more universal dialysis monitor using some illustrating examples.

The aim of this study was to design and assess a new method for continuous on-line monitoring of solutes/toxins in the dialysate, using UV-absorption phenomena.

METHODS

The method is based on UV-absorption phenomena where the UV-absorbance values are determined in the spent dialysate, using a spectrophotometrical set-up connected to the tubing guiding the spent dialysate. The absorbance A [a.u.] of a solution, obtained by a spectrophotometer using a reference solution, is given by

$$A = \log \frac{I_0}{I_{r+s}} - \log \frac{I_0}{I_r} = \log \frac{I_r}{I_{r+s}} = \epsilon Cd \quad [1]$$

where I_0 is the intensity of incident light from the light source (Fig. 1), I_r is the intensity of transmitted light through the reference solution (e.g. pure dialysate) and I_{r+s} is the summation of intensity of transmitted light through the reference solution mixed with the solution under study (e.g. pure dialysate + waste products from the blood). The units of intensity depend on how it is measured: the intensity has the units of W if measured as the radiant power, or W/m^2 if measured as the energy fluence rate, sometimes named as the optical intensity. The absorbance can be quoted in the arbitrary units [a.u.], but the units are, by convention, never expressed. ϵ [$cm^{-1} (mol/L)^{-1}$] and C [mol/L] are the extinction coefficient and the molar concentration of the absorbing solute under study respectively and d is the finite depth of the solution in cm.

Experimental set-up

The schematic clinical set-up of the experiments is shown in Figure 1.

Optics module: For the determination of UV-absorbance a double-beam spectrophotometer (UVIKON 943, Kontron, Italy) with an accuracy of $\pm 1\%$ was used. The most essential parts of the optics module incorporated into the spectrophotometer are: 1) a light source, 2) a monochromator to resolve the source of radiation into component "monochromatic" elements, and 3) a light detector to detect the radiation after passing the optical cuvette containing the solution under study. A double-beam spectrophotometer also has a similar optical configuration for the reference solution that is not shown in the figure. During the on-line experiments, the spectrophotometer was connected to the fluid outlet of the dialysis machine with all spent dialysate passing through the specially designed optical cuvette. The sampling

frequency was set at two samples per minute. Samples were also taken at pre-determined times during dialysis for spectrophotometric analysis afterwards. During the discrete sampling experiments a standard cuvette was used. The results from measurements preferably using the wavelength 285 nm are presented in this paper.

Signal processing module: The obtained UV-absorbance values were processed and presented on the computer screen by a PC incorporated in the spectrophotometer using Kontron's software (UVIKON 943, Kontron, Italy, version 7.0 for Windows). The final data processing was performed in EXCEL (version 7.0 for Windows).

Substances and the transport in the dialyser

To get manifold information about the relationship UV-absorbance vs. solutes in both blood and dialysate, the concentration of several substances of various molecular weight (MW), electrical charge, transport mechanism, etc. were determined. Summarised information about the substances analysed is presented in Table 1.

The dialyser is usually operated as a combined diffusive and convective transport device where the total clearance K_{Tot} is defined as

$$K_{Tot} = K_{Diff} + K_{Conv} = \frac{Q_{Bi} * (C_{Bi} - C_{Bo})}{C_{Bi}} + Q_F * \frac{C_{Bo}}{C_{Bi}} \quad [2]$$

where K_{Diff} and K_{Conv} are the diffusive and convective clearances in ml/min, Q_{Bi} is the rate of blood flow into the dialyser in ml/min, C_{Bi} and C_{Bo} are the blood inlet and outlet substance concentrations in mmol/L and Q_F is the ultrafiltration rate in ml/min (35).

For smaller substances the main transport mechanism is diffusion (36), and K_{Tot} can be simplified as

$$K_{Tot} = K_{Diff} = \frac{Q_{Bi} * (C_{Bi} - C_{Bo})}{C_{Bi}} = \frac{Q_{Di} C_{Do}}{C_{Bi}} \quad [3]$$

where Q_{Di} is the rate of dialysate flow into the dialyser in ml/min and C_{Do} is the dialysate outlet substance concentration in mmol/L. Rearranging Equation 3 to obtain C_{Bi} we get

$$C_{Bi} = \frac{Q_{Di}}{K_{Tot}} * C_{Do} \quad [4]$$

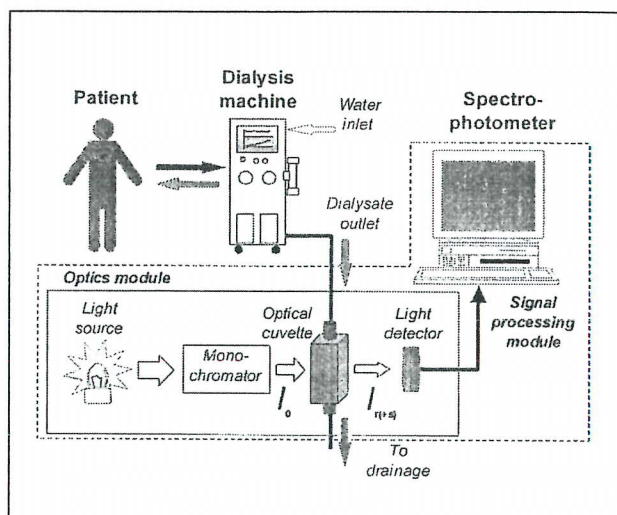


Fig. 1 - Schematic view of the clinical set-up (on-line). I_0 is the intensity of incident light from the light source, $I_{(rs)}$ represents either intensity of transmitted light through the reference solution (e.g. pure dialysate) I_r or summated intensity of transmitted light through the reference solution mixed with the solution under study (e.g. pure dialysate + waste products from the blood) I_{rs} respectively.

This dialysate-side mass balance indicates that C_{Do} is linearly related to C_{Bi} with the proportionality constant Q_{Di}/K_{Tot} (37) assuming that the parameters Q_{Di} and K_{Tot} are known. Thus measuring C_{Do} the value of C_{Bi} can be predicted. Similarly it can be shown that a linear dependence also exists for larger molecules, whose transport mechanisms are both diffusion and convection or solely convection in the dialyser. Generally this means that for any substance, whose transport in the dialyser can be described using Equation 2, the prediction of C_{Bi} can be made by measuring C_{Do} if Q_{Bi} , Q_{Di} , Q_F and K_{Tot} are known. This is valid even if C_{Di} is not zero and the clearance is replaced by the dialysance D . However problems arise because of the probability of protein-membrane interaction, Donnan effects, and other phenomena unique to physiologic solutions (36). This was the reason for calculating the correlation between UV-absorbance and substances both in the dialysate and in the blood. In this way it can be detected if a particular substance that is not behaving according to Equation 2 still contributes to UV-absorbance. In the last case a very good correlation may exist between UV-absorbance and the particular substance in the dialysate but the same good correlation may not necessarily be obtained for the blood.

To summarise, a good correlation between UV-absorbance and a certain substance both in the dialysate and in the blood indicates that UV-absorbance has a linear relationship with the substance both in the dialysate and in the blood. This linear relationship allows calculating the values of C_{Bi} and C_{Do} for the given substance from the measured UV-absorbance values and also indicates the presence of a linear dependence between C_{Bi} and C_{Do} . After that the transport of the substance in the dialyser can be described using Equation 2.

Material

After approval of the protocol by the local Ethics Committee a total of 13 hemodialysis patients were studied in 2 separate studies at the Department of Nephrology, University Hospital, Linköping. The treatment times ranged from 240 to 300 minutes and the dialysate flow was fixed at 500 ml/min. For every separate study a more detailed description about patient data and technical parameters is given in Table II.

Study 1: Six patients on chronic thrice-weekly hemodialysis were followed during a total of 44 sessions

(Tab. II). The type of dialyser was high-flux polysulfone dialysers of 1.0 m² in 22 sessions and low-flux polysulfone dialysers of 1.3 m² in 22 sessions (F50 and F6 respectively, from Fresenius Medical Care, Germany). The dialysis machine AK200 (Gambro Lundia AB, Sweden) was used for this study. All patients, except one, were treated with two-needle dialysis where the blood flow ranged from 250 to 350 ml/min. The single needle dialysis had the blood flow from 160 to 200 ml/min. Sodium, calcium, potassium in the spent dialysate and in the blood was measured by an electrolyte analyser (AVL Electrolyte Analyser 9140, AVL Scientific Corp., USA) with the accuracy of the CV ≤ 1.0% for sodium, potassium and SD ≤ 0.02 mmol/L for calcium. Glucose in the blood was measured by a HemoCue instrument (HemoCue B-Glucose, HemoCue AB, Sweden) with the calibrating accuracy of +/- 0.3 mmol/L.

Study 2: Ten patients on chronic thrice-weekly hemodialysis were followed during a total of 54 sessions (Tab. II). The type of dialysis membrane was cellulose diacetate of 1.8 m² (AF 180, Althin Medical AB, Sweden) for 47 sessions and high-flux polyarylethersulfone of 1.7 m² (Polyflux 17 S, Gambro AB, Sweden) for 7 sessions.

TABLE I - SUMMARY OF ALL OBSERVED SUBSTANCES

Category	Substance	MW [D]	Main transport mechanism
Small (MW < 300 D)	Sodium	22.99	Diffusion
	Potassium	39.10	
	Calcium	40.08	
	Urea	60.06	
	Phosphate	96.17	
	Creatinine	113.12	
	Uric acid	168.11	
Middle (MW = 500 - 5 000 D)	Glucose	180.16	Diffusion
	Vitamin B ₁₂	1355.39	
Low Molecular Weight (LMW) proteins (MW = 5 000- 50 000 D)	β ₂ -microglobulin	11818	Convection
	Albumin	68000	Diffusion
Large proteins (MW > 50 000 D)			Convection

TABLE II - PATIENT DATA AND THE TECHNICAL PARAMETERS OF THE HEMODIALYSIS SESSIONS

Study	No. of sessions	No. of patients	Sex (F+M)*	Age	Dialyser**	Machine
1	44	6	3+3	20-79	F50, F6HPS	AK200
2	54	10	4+6	21-81	AF-180, Polyflux 17 S	AK200, Fr4008H

* F - Female, M - Male

** More detailed data about the dialysers are given in the text

The blood flow ranged from 250 to 300 ml/min. Two types of dialysis machines were utilised: AK200 (Gambro Lundia AB, Sweden) and Fr4008H (Fresenius Medical Care, Germany). Phosphate, β_2 -microglobulin and albumin were analysed only during a subgroup of sessions (N = 14) and vitamin B₁₂ only during 5 sessions. For the determination of urea concentration in dialysate and for reference, the Urea Monitor 1000 from Baxter Healthcare Corp., USA was used with an accuracy of $\pm 5\%$ in this study.

For both studies samples of blood and spent dialysate were taken at discrete times for chemical and spectrophotometric analysis (Tab. III). The numbers for "Sampling time" correspond to minutes after the start of hemodialysis. Observe that the dialysate samples were taken at 270 and 300 minutes when the duration of the session was long enough. In accordance the blood samples were taken at $t_{\text{end}} = 270$ or 300 minutes for the longer sessions. The concentrations of substances such as urea, creatinine, uric acid, phosphate, albumin and β_2 -microglobulin were determined at the Clinical Chemistry Laboratory at Linköping University Hospital using standardised methods. The accuracy of the methods for determination of different solutes in dialysate and blood were: urea, uric acid $\pm 1\%$; phosphate, glucose, blood β_2 -microglobulin, albumin $\pm 2\%$; creatinine $\pm 5\%$ and vitamin B12 $\pm 10\%$.

Data analysis

Pearson's correlation coefficient between UV-absorbance on the spent dialysate and concentration of the substances in the spent dialysate and in the blood was calculated from data based on the collected samples. The samples taken at times coincident with the self-tests of the dialysis machine were rejected from the analysis. The correlation coefficient was not calculated when less than three data points were available for a single session

because when the concentrations in the spent dialysate were not measurable due to low levels (e.g. for vitamin B₁₂ and albumin). The median value of the correlation coefficient, r_{med} , between UV-absorbance and certain substances with different molecular weight in the spent dialysate and in the blood was obtained over all single dialysis correlation coefficients for a given substance. Non-outlier range, 25th and 75th percentile and outliers together with extremes were presented. The non-parametrical Wilcoxon Matched Pairs Test was used to compare groups of values. For the analysis, STATISTICA software (StatSoft version 6.0) was used.

RESULTS

Figure 2 represents a typical on-line absorbance curve during a single hemodialysis session (wavelength 285 nm and machine AK 200, Gambro Lundia AB, Sweden). 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.

Figure 3 shows the UV-absorbance response to changes in blood flow for different flow values at the wavelength 250 nm. A time period of 50 minutes taken from the 240 minute long dialysis session is represented in the figure. The blood flow was varied between 350 and 100 ml/min with an interval of 50 ml/min for 30 minutes. As seen from the figure a decrease in blood flow causes a lower UV-absorbance value in the spent dialysate.

Figure 4 shows the scatterplot between UV-absorbance and urea concentration in the spent dialysate during a single hemodialysis session from Study 2 (wavelength 285 nm, $r = 0.998$, number of samples N = 9).

Figure 5 shows the median value of the correlation coefficient r_{med} between UV-absorbance and certain substances with different molecular weight in the spent dialysate and in the blood. Wavelength was held constant

TABLE III - DISCRETE SAMPLING OF BLOOD AND SPENT DIALYSATE*

Substances	Sampling time [min] Dialysate	Sampling time [min] Blood
Sodium ¹ , calcium ¹ , potassium ¹ , phosphate ² , urea, creatinine, uric acid ² , glucose, vitamin B ₁₂ ² , β_2 -microglobulin ² , albumin ²	0, 2 ¹ , 4 ¹ , 5 ² , 10 ¹ , 15 ² , 20 ¹ , 30, 45 ¹ , 60, 90 ¹ , 120, 180, 210 ¹ , 240, 270, 300	0, 4 ¹ , 10 ¹ , 20 ¹ , 30 ¹ , 60, 120 ¹ , 180, 240 ¹ , t_{end}

* The index in superscript for the particular substance shows in which study the substance was analysed and the index in superscript for a sampling time shows in which study the sample was taken, if not the same for both studies

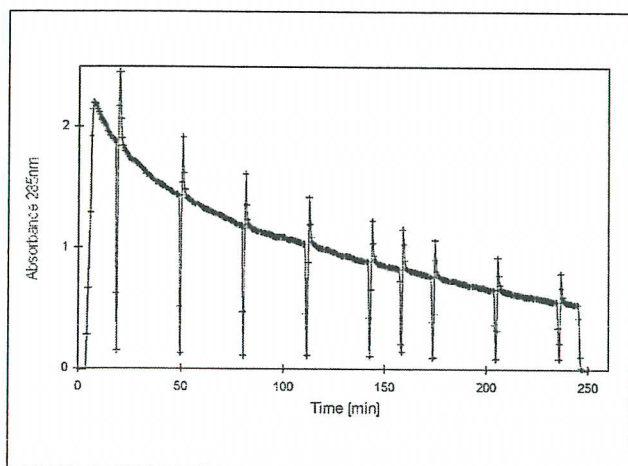


Fig. 2 - A typical on-line absorbance curve during a single hemodialysis treatment where UV-absorbance at the wavelength 285 nm is plotted against the time. (See text for more detailed description).

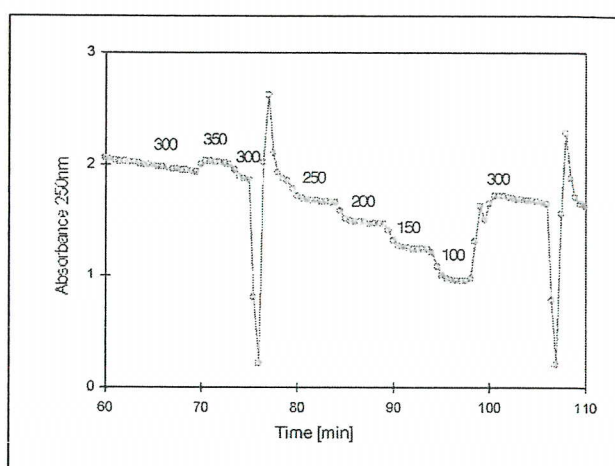


Fig. 3 - UV-absorbance response to changes in blood flow for different blood flow values at the wavelength 250 nm. The numbers above the absorbance curve correspond to blood flow in ml/min.

at 285 nm. Also non-outlier range, 25th and 75th percentile and outliers together with extremes are presented. The number of single dialyses included in the material, after rejecting unsuitable sessions, was as follows: N(urea, creatinine) = 98 (Study 1 and 2), N(glucose) = 84 (Study 1 and 2), N(uric acid) = 54 (Study 2), N(sodium, calcium) = 44 (Study 1), N(potassium) = 39 (Study 1), N(phosphate, dialysate β_2 -microglobulin, blood albumin) = 14 (Study 2), N(blood β_2 -microglobulin) = 13 (Study 2), N(dialysate albumin) = 8 (Study 2), N(blood vitamin B₁₂) = 5 (Study 2). The highest r_{med} value was obtained for urea ($r_{med} = 0.996$ both in the spent dialysate and in the blood), creatinine ($r_{med} = 0.997$ in the spent dialysate and $r_{med} = 0.998$ in the blood) and uric acid ($r_{med} = 0.994$ in the spent dialysate and $r_{med} = 0.999$ in the blood) both in the spent dialysate and in the blood. The median correlation value is somewhat lower and the non-outlier ranges higher for substances such as potassium ($r_{med} = 0.913$ in the spent dialysate and $r_{med} = 0.942$ in the blood), phosphate ($r_{med} = 0.965$ in the spent dialysate and $r_{med} = 0.978$ in the blood) and β_2 -microglobulin ($r_{med} = 0.948$ in the spent dialysate and $r_{med} = 0.988$ in the blood). The lowest correlation was achieved for sodium ($r_{med} = -0.621$ in the spent dialysate and $r_{med} = -0.554$ in the blood), calcium ($r_{med} = -0.504$ in the spent dialysate and $r_{med} = -0.666$ in the blood) and glucose ($r_{med} = 0.459$ in the spent dialysate and $r_{med} = -0.509$ in the blood). A relatively high correlation coefficient was obtained for albumin in the

spent dialysate ($r_{med} = 0.932$), whereas a slightly lower and negative one was obtained for albumin in the blood ($r_{med} = -0.805$) and ($r_{med} = -0.883$ in the blood). In the latter case the non-outlier range is extremely high. Comparing the correlation coefficient values for the spent dialysate and blood groups using the non-parametrical Wilcoxon Matched Pairs Test, the correlation coefficient was different for uric acid, glucose, β_2 -microglobulin and albumin ($P < 0.05$).

Figure 6 illustrates the time-dependent slope of the absorbance curve (285 nm) and the slope of urea concentrations in the spent dialysate, measured by the Urea Monitor 1000, after taking the natural logarithm of the collected values. The negative absorbance values seen in the figure are obtained when the natural logarithms are calculated on values between 0 and 1.

DISCUSSION

Figure 2 presents a typical on-line UV-absorbance curve in the flowing dialysate at a fixed wavelength (285 nm) versus time during a single hemodialysis session. The absorbance drops occur during the dialysis machine self tests when the dialysator is in by-pass mode (dialysate does not pass the dialysator). Figure 2 also demonstrates the possibility to follow a single hemodialysis session continuously and to monitor deviations in the dialysator

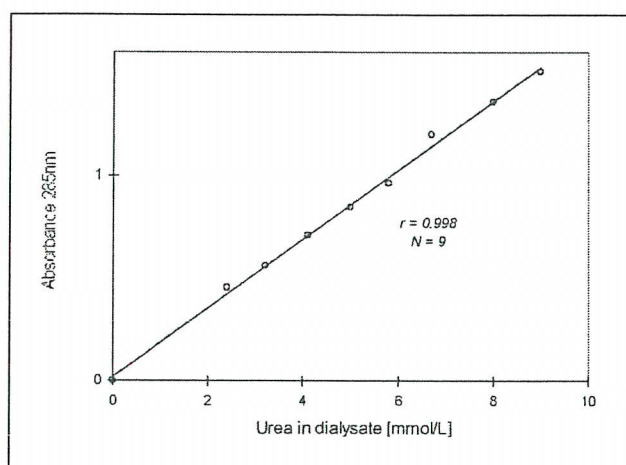


Fig. 4 - The scatterplot UV-absorbance (wavelength 285 nm) against the urea concentration in the spent dialysate ($N = 9$) for a single hemodialysis treatment. The Pearson's correlation coefficient r is also given.

performance using UV-absorbance. The sampling frequency was set at two per minute during this experiment to restrict the amount of collected data. Considering that all events during hemodialysis are relatively slow, the sampling frequency is practically not a limiting factor when using the UV method.

Figure 3 demonstrates that the response of the absorbance measurements due to changes in blood flow makes it possible to detect changes in blood flow during a hemodialysis session. As seen, a decrease in the blood flow causes a reduced light absorbance in the spent dialysate. This is due to a decreased amount of substances in the dialysate outlet at a specific time interval when blood flow is decreased. This occurs because the solute removal rate, clearance, is proportional to the blood flow (Eq. 2) and as a result the amount of solutes in the spent dialysate follows the changes in blood flow. The UV method can also detect changes in dialysate flow because clearance is dependent on dialysate flow as well (Eq. 3). This illustrates how the new method can be used to detect variations in treatment efficiency.

Figure 4 shows an example of the scatterplot between UV-absorbance (wavelength 285 nm) and urea in the spent dialysate for a single hemodialysis treatment. The value of the linear correlation coefficient is very high ($r = 0.998$, number of samples $N = 9$) which means UV-absorbance follows linearly urea concentration in the spent dialysate.

Figure 5 presents the median value of the correlation coefficient between UV-absorbance and several substances with different molecular weight in the spent dialysate and in the blood, calculated over all single hemodialysis sessions at a fixed wavelength of 285 nm. Also non-outlier range, 25th and 75th percentile and outliers together with extremes are shown.

Small substances

The experimental results indicate a very good correlation between UV-absorbance and several removed small solutes (urea, creatinine, uric acid) in the spent dialysate and in the blood (Fig. 5). The median value of the correlation coefficient for those solutes is high ($r_{\text{med}} > 0.99$) and the non-outlier range is very small when calculated over all individual treatments at a fixed wavelength. The high correlation may be due to the UV-absorbing properties of these solutes as they contribute to the total UV-absorbance. On the other hand, the good correlation may be obtained even for solutes that are not highly UV-absorbing in the UV region. This happens if UV-absorbing solutes have a similar removal rate (clearance) as non-UV-absorbing solutes (like urea) at this wavelength. Creatinine and uric acid, known as UV-absorbing compounds (15) and acquiring a similar transport mechanism as urea (36-38), are potential solutes that contribute to the total UV-absorbance. The correlation coefficient values for the uric acid in the spent dialysate and in the blood showed a significant difference because of really high correlation for the material (Fig. 5) that made the statistical test sensitive even to small differences.

The correlation is somewhat lower and the non-outlier ranges larger for substances like potassium and phosphate. The presence of outliers and extreme values for potassium could not be fully explained. One reason that must be considered is the rejection of the potassium values in the spent dialysate due to the measurement error (low measurement limit) of the electrolyte analyser.

Phosphate is a small molecule that has a removal pattern similar to other solutes removed mainly by diffusion, e.g. urea and creatinine. At the same time phosphate is a charged molecule which can be influenced by the ratio of ionic concentrations in dialysate and blood relevant to the diffusive transport of ions (39). Moreover the distribution volume of phosphate is quite different compared to urea because of large amounts of phosphate in the bone tissue and being partly protein bound (40).

The lowest correlations were achieved for sodium, calcium, and glucose, indicating that they are probably not highly UV-absorbing. The poor correlation for these substances means that the removed UV-absorbing solutes do not behave as sodium, calcium and glucose do, which may have a reversed concentration gradient from blood to dialysate (35) but more likely as the small waste solutes urea, creatinine and uric acid, that have a concentration gradient from blood to dialysate.

Middle molecules

Despite vitamin B₁₂ being widely used for the characterisation of dialyser performance *in vitro*, the concentrations measured *in vivo* were not measurable in the spent dialysate due to its extensive plasma protein binding. This is the reason why the correlation was not calculated for vitamin B₁₂ in the spent dialysate. Accordingly, the vitamin B₁₂ concentration in the blood was almost constant as expected because it was not removed by the dialyser. Despite high negative median correlation value in the blood ($r_{\text{med}} = -0.883$) the large non-outlier range value indicates no systematically linear relationship between UV-absorbance and vitamin B₁₂ in the blood.

It is worth mentioning that to measure the concentration of the middle molecules (MM) in the spent dialysate can be a difficult task due to their low concentration (41). Moreover, early efforts at identifying MM failed because the analytical techniques available were not sophisticated enough. Later studies demonstrated that the middle molecular weight fractions isolated by gel filtration comprised many low-molecular-weight solutes, such as carbohydrates, amino acids, aromatic substances, and other UV-absorbing solutes, not exclusively representing MM (42). To underline this, the correlation of MM is marked by "?" in Figure 5. Even if more advanced methods are available today, the MM hypothesis will be hard to validate considering the difficulties in establishing the toxic effects of the readily measured and manipulated small solute urea (43).

LMW proteins

The UV-absorbance is fairly highly correlated with the β_2 -microglobulin concentration ($r_{\text{med}} = 0.948$ in the spent dialysate and $r_{\text{med}} = 0.988$ in the blood) (Fig. 5). The correlation is however lower than for waste solutes of smaller molecular weight, indicating that the β_2 -

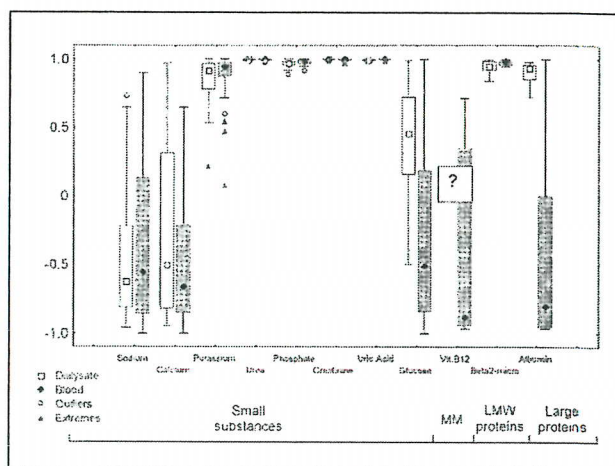


Fig. 5 - The median value of the linear correlation coefficient r_{med} between UV-absorbance and certain substances in the spent dialysate. Also non-outlier range (whisker plot), 25th and 75th percentile (lower and upper box value respectively) and outliers together with extremes are presented. The number of single dialyses, the statistical analysis is based on, was as follows: $N(\text{urea, creatinine}) = 98$ (Study 1 and 2), $N(\text{glucose}) = 84$ (Study 1 and 2), $N(\text{uric acid}) = 54$ (Study 2), $N(\text{sodium, calcium}) = 44$ (Study 1), $N(\text{potassium}) = 39$ (Study 1), $N(\text{phosphate, dialysate } \beta_2\text{-microglobulin, blood albumin}) = 14$ (Study 2), $N(\text{blood } \beta_2\text{-microglobulin}) = 13$ (Study 2), $N(\text{dialysate albumin}) = 8$ (Study 2), $N(\text{blood vitamin B12}) = 5$ (Study 2). The abbreviation "MM" denotes the middle molecules and the meaning of "?" for the correlation of MM is explained in the text.

microglobulin removal mechanisms are not exactly the same as for smaller substances. This is also confirmed by the significant ($P < 0.05$) difference between the spent dialysate and blood correlation coefficient groups for β_2 -microglobulin. Moreover, the kinetic behaviour of β_2 -microglobulin is not simple because its production may be increased due to dialyser bio-incompatibility (44) and elimination can vary with different types of dialysers (45).

The rather good correlation between UV-absorbance and β_2 -microglobulin in the spent dialysate and in the blood in this study reinforces the need to investigate the relationship between UV-absorbance and β_2 -microglobulin in more detail.

Large proteins

The albumin concentration in the spent dialysate was very low (< 0.06 g/L) due to the low permeability of the cellulose diacetate membrane (AF 180) and polyarylethersulfone membrane (Polyflux 17 S), for large proteins. Still, a relatively high median correlation

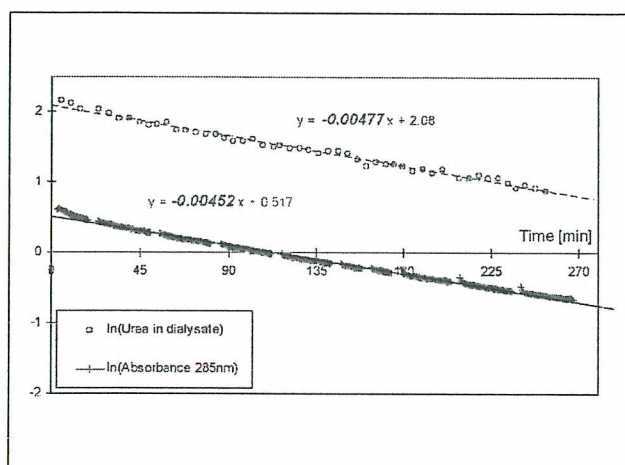


Fig. 6 - Determination of the slope from the logarithmic plot of the on-line absorbance values (wavelength 285 nm), and from the logarithmic plot of the urea in the spent dialysate measured by Urea Monitor 1000. A linear fitting curve presented as $y = \text{Slope} \cdot x + \text{Intercept}$, for every time-dependent plot.

coefficient ($r_{\text{med}} = 0.932$) was obtained for albumin in the spent dialysate (Fig. 5) after rejecting the sessions where the concentrations were not measurable. However, such small amounts removed by convective transport do not influence blood status and therefore the correlation coefficient for albumin in the blood was negative ($r_{\text{med}} = -0.805$) and the large non-outlier range indicates no systematically linear relationship between UV-absorbance and albumin in the blood. According to our experience from earlier studies the albumin level in the blood seems to correlate to the changes in the blood volume measured by Critline. The albumin level in the blood was approximately constant in patients who had a decrease in blood volume of less than 8% during the treatment and increased in patients, who had a decrease in the blood volume of more than 15% during the treatment. This means that the changes in albumin level in the blood occurred due to the decreasing blood volume (less blood plasma corresponds to the higher concentration) and not because a significant amount of albumin was removed from the patient by the dialyser.

As evident from Figure 5, a good correlation between UV-absorbance and a particular substance in the spent dialysate seems to depend on many factors besides molecular weight. A good correlation between UV-absorbance and a non-UV-absorbing solute can be achieved when the removal rate of the solute is similar to the UV-absorbing substances. For the substances with

higher molecular weights (LMW and large proteins) and with different main transport mechanisms compared to smaller waste solutes, the correlation coefficient is lower and probably they do not essentially influence the UV-absorbance in the spent dialysate.

Several studies, e.g. (15, 41, 46-52) employing various chromatographic techniques have demonstrated distinct and readily recognisable differences in UV-absorbance peaks between pre and post dialysis uremic serum, ultrafiltrate or dialysate containing UV-absorbing small constituents and MM. Also UV-transmittance of the spent dialysate has been measured at 254 nm, believed to be the best wavelength to monitor the efficacy of hemodialysis, enabling assessment of the elimination rate of hardly diffusible constituents, like MM (53). Moreover, it was concluded that the hardly diffusible constituents had a higher elimination rate compared to the removal rate of small, more readily diffusible components as was reported. Our study confirms that at least some small molecular weight substances correlate well with the UV-absorbance at 285 nm.

The high correlation coefficient for certain small substances in the spent dialysate and in the blood removed mainly by diffusion, means that the presence of those substances in the blood can be determined by measuring their appearance in the spent dialysate. This means, e.g. for urea, that if urea clearance and dialysate flow do not vary during dialysis, urea concentration in the spent dialysate is a fixed fraction of urea concentration in the blood and a spent dialysate urea concentration trace can be used to determine Kt/V for urea (23). However larger proteins, like albumin, which are removed mainly by convection and have quite different behaviour compared to the smaller substances, can be present in small amounts in the spent dialysate. Still, such amounts do not influence blood status and therefore the blood concentrations cannot be predicted solely by measuring larger proteins in the spent dialysate.

Figure 6 illustrates the slope of the absorbance curve (285 nm) and the urea concentration curve in the spent dialysate after taking a natural logarithm of the raw values. For stable treatments, Kt/V should be estimated from the slope of the logarithmic plot of the urea concentration (23, 29). Knowing the Kt/V value and dialysis duration t , the parameter Kt/V can be calculated. For example, if the corresponding slope of the absorbance $S_A = -0.00452$ and the slope for urea in the spent dialysate measured by the Urea Monitor 1000, $S_{UM} = -0.00477$ (Fig. 6), the Kt/V

values for a 270 minutes long dialysis can be obtained as $(Kt/V)_A = -S_A * t = 1.22$ for UV-absorbance and $(Kt/V)_{UM} = -S_{UM} * t = 1.29$ for urea in the spent dialysate measured by the Urea Monitor 1000, respectively.

As mentioned earlier, a good correlation between UV-absorbance and a particular solute may be achieved when the removal rate of a non-UV-absorbing solute is similar to UV-absorbing solutes during hemodialysis. This gives rise to a possibility to determine K/V and calculate Kt/V even when the technique does not measure urea. The same assumption is also valid for total removed urea (TRU) calculations to obtain urea generation rate (G) and protein catabolic rate (PCR).

In a similar manner as for urea, the Kt/V can be calculated for other solutes as well (54). This requires a good correlation between UV-absorbance and a particular solute and knowledge of the distribution volume and the intercompartmental equilibration rates for the solute. At present these requirements seem to be fulfilled for e.g. creatinine (36). It has been suggested that creatinine kinetic modelling can be used to determine the patient's lean body mass (LBM) since a linear relationship between LBM and creatinine generation has been demonstrated (55). Repeated measurements of the LBM could be an indicator of protein nutrition status and thus be used as an indicator of increased risk of morbidity and mortality. Uric acid, yet another small solute, is also removed from the blood by hemodialysis in a similar manner as urea and is associated with disturbances of calcitriol production and metabolism (38).

The chromatographic studies confirm the accumulation of many compounds that might be toxic or indirectly representative for overall toxicity and accumulation. The total UV-absorbance in serum, that can be obtained as the cumulative and integrated peak height of all UV-absorbing high performance liquid chromatogram (HPLC) peaks together, has been evaluated to obtain a dialysis ratio and an extraction value (56). This value has been estimated to give a good assessment of overall solute retention and its decrease might be a good parameter of overall solute elimination (15).

CONCLUSIONS

This paper describes a technique for monitoring the concentration of the solutes in the spent dialysate utilising UV-absorption phenomena.

The experimental results indicate a good correlation between UV-absorbance and certain removed solutes, such as urea, creatinine and uric acid, in the spent dialysate and lower correlation for other solutes such as potassium, phosphate and β_2 -microglobulin for every single dialysis session. The lowest correlation was achieved for sodium, calcium, glucose and albumin as discussed above in Discussion. This means that the UV-absorbance correlates well to several different solutes of molecular weights less than 200 D. Even if the technique does not solely detect urea or other single solutes, a close correlation, found between changes in UV-absorbance and the particular solute concentrations, should enable utilising UV-absorption to follow the solute removal for each dialysis. Moreover, a parameter based on total UV-absorbance and characterising overall retention of accumulated UV-absorbing solutes, may be an interesting alternative. The major advantages of the new technique are:

- provides continuous, on-line measurements of spent dialysate (while the other methods often measure intermittently);
- no need for repeated blood samples;
- no disposables or chemicals (compared with other existing methods);
- allows continuous measurements directly on the dialysate line thus providing the simplest plug/unplug possibility without disturbing the ongoing session in any way;
- may immediately identify and alert to any deviations in dialysis treatment due to flow changes both in the dialysate and blood side and in the dialyser performance.

We conclude that the new optical method, using UV-absorption, might be a helpful tool to provide continuous, on-line measurements of solutes in the spent dialysate. The results from measurements preferably using the wavelength 285 nm have been presented in this paper. The results also obtained for other wavelengths in the UV region are currently being prepared for publishing. The main attention was paid to the solutes in the dialysate and blood measured by the conventional standard methods in this work. Further studies are underway to investigate in more detail the relationship between different UV-wavelengths and solutes where more specific methods are required for concentration measurements. Additionally, research is currently being performed to study the technique's capability in evaluating the delivery of prescribed treatment dose based on traditional measures, e.g. Kt/V, URR, TRU. However, it should be kept in mind,

that adequate dialysis dose comprises several treatment-related variables nowadays, where "removed urea" is only one piece in the jigsaw (12). From this viewpoint, the UV-absorbance monitoring technique may become a more universal method to ensure the quality and adequacy of dialysis.

Address for correspondence:
I. Fridolin, M Sc.
Department of Biomedical Engineering
Linköping University
University Hospital
SE-581 85 Linköping, Sweden
e-mail: ivofr@imt.liu.se

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