TOTAL REMOVED URIC ACID DURING DIALYSIS ESTIMATED BY ON - LINE ULTRA VIOLET ABSORBANCE IN THE SPENT DIALYSATE

F. Uhlin*, L-G. Lindberg**, I. Fridolin***

* Department of Nephrology, University Hospital, Linköping, S-581 85 Linköping, Sweden
** Department of Biomedical Engineering, Linköping University, University Hospital, S-581 85 Linköping, Sweden
*** Biomedical Engineering Centre, Tallinn University of Technology, EST-19086 Tallinn, Estonia

F. Uhlin: fredrik.uhlin@lio.se, L.-G. Lindberg: larli@imt.liu.se, I. Fridolin: ivo@cb.ttu.ee

Abstract: The aim of this study was to compare the total removed uric acid obtained from the on-line UV-absorbance measurements in the spent dialysate (*TRua*) and the total dialysate collection (*TDC*) as reference method.

Six uremic patients, two females and four males, on chronic thrice-weekly hemodialysis were included in the study.

All spent dialysate during dialysis was collected in a tank that gave the *TDC* value.

A double-beam spectrophotometer was used for the determination of UV-absorbance.

Regression line from the first in week sessions was assessed to transform UV-absorbance into uric acid concentration. This relationship was used for the subsequent treatments' *TRua* calculations for each patient (method *UV1*). Also, *TRua* based on the transformation using the regression line from the total material was calculated (method *UV2*). *TRua* from the three methods was finally compared.

TRua obtained using *TDC* and two different transformations for UV-absorbance (mean ± *SD*) in μmol were: 5277 ± 897 from *TDC* (*N* = 23), 5150 ± 882 from UV1 (*N* = 23), and 5422 ± 1112 from UV2 (*N* = 23) transformation. None of the mean *TRua* values were significantly different (*P* > 0.05).
The results show the possibility to estimate total removed uric acid by using UV-absorbance. Still a larger material is needed to draw more general conclusions.

Introduction

Uric acid is a water–soluble compound (molecular weight of 168.1) that is the final metabolite of purine in humans. Evaluations of uric acid in blood are common in patients with kidney diseases as well as in those treated with dialysis. Urea, creatinine and uric acid have for a long time been known to accumulate in the bodies of haemodialysis (HD) patients. Uric acid is removed from plasma in a similar manner as urea during dialysis treatment [1] but so far has not been investigated concerning patient outcome, compared to urea. Uric acid is mostly associated with gout, but studies have found that uric acid affects biological systems [2], [3] and could also cause higher mortality in dialysis patients [3].

Earlier a good correlation between ultra-violet (UV)-absorbance in dialysate and the concentration of several solutes both in the spent dialysate and in the blood of the dialysis patients has been shown, indicating that the technique can be used to estimate the removal of retained substances [4]. Also, the possibility to estimate dialysis dose (urea-*Kt/V*) [5] and total removed urea by UV-absorbance [6] has been presented.

The aim of this study was to compare the total removed uric acid (*TRua*) obtained from the on-line UV-absorbance measurements in the spent dialysate using two different transformations to calculate the total removed uric acid from the on-line UV-absorbance and the total dialysate collection (*TDC*) as reference method.

Materials and Methods

This study was performed after approval of the protocol by the Ethics Committee, at the Department of Nephrology, University Hospital of Linköping, Sweden. An informed consent was obtained from all participating patients.

Six uremic patients, two females and four males, mean age 54.5 ± 23.5 years, on chronic thrice-weekly haemodialysis were included in the study. The patients were monitored during four dialysis treatments each with duration from 240 to 270 minutes (totally 24 haemodialysis sessions). The studied treatments were not consecutive but were performed within three weeks for each patient. An althane dialyser was used with the effective membrane area of 1.8 m2 (AF180, Ahltin Medical, Ronneby, Sweden). The dialysate flow was 500 mL/min and the blood flow was 300 mL/min except in one session (250 mL/min) due to temporary access (needle) problems. Two types of machines were used, AK 200 (Gambro Lundia AB, Sweden) and Fresenius 4008H (Fresenius Medical Care, Germany).

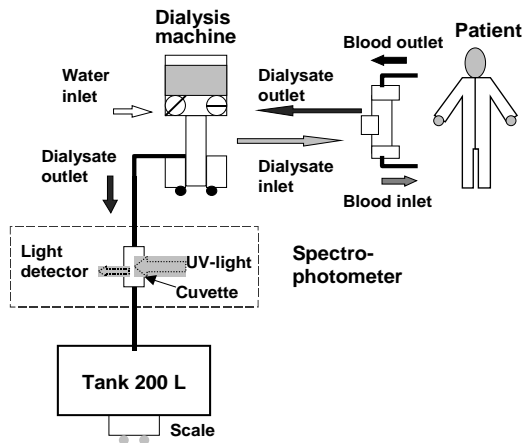The schematic clinical set-up of the experiments is shown in Figure 1.

Figure 1: Schematic clinical set-up of the experiments.

All spent dialysate during dialysis was collected in a tank equipped with a scale. Multiplying the weight with the uric acid concentration of the tank after the end of treatment (*Dtotal*) gave the *TDC* value. Dialysate samples were taken at discrete times for chemical uric acid analysis. Dialysate samples were taken before dialysis (pure dialysate), used as the reference solution, when the dialysis machine was prepared for starting and the conductivity was stable, and after 5, 15, 30, 60, 90, 120, 180, 240 minutes (270 minutes if treatment was longer than 240 minutes). If a periodical self-test or alarm occurred during a time-tabled sampling, the sample was instead taken after 1 to 3 minutes depending on whether the UV-absorbance monitoring curve had been stabilized.

A double-beam spectrophotometer (UVIKON 943, Kontron, Italy) was used for the determination of UV-absorbance. The absorbance A [a.u.] of a solution, obtained by the spectrophotometer using the pure dialysate as the reference solution, was determined as:

$$A = \log \frac{I_r}{I_{r+s}} \tag{1}$$

where I_r is the intensity of transmitted light through the reference solution (pure dialysate) and I_{r+s} is the summated intensity of transmitted light through the reference solution containing the solutions under study (pure dialysate + waste products from the blood). 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 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 sampling

frequency was set at two samples per minute and the wavelength was 285 nm.

In order to transform UV-absorbance (dimensionless) to dialysate uric acid value in μmol/L, a good correlation must exist between the two variables [4]. Regression line for the collected dialysate samples and corresponding UV-absorbance values from the first in week sessions was assessed to transform UV-absorbance into uric acid concentration (Figure 2). Figure 2 shows that a good linear relationship exists between UV-absorbance and dialysate uric acid concentration ($R = 0.999$). $N = 8$ is the number of collected dialysate samples. The obtained relationship from the first in week session dialysis for each patient was used for the subsequent treatments when calculating *TRua* and this transformation was marked as *UV1*. Similar high correlation between UV-absorbance and dialysate was obtained for all first-in-week sessions.
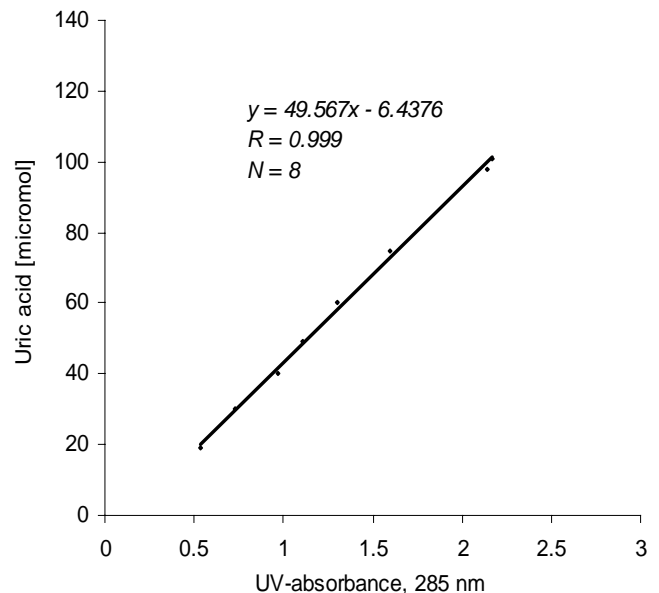

Figure 2: An example of the regression line between uric acid concentration in dialysate and UV-absorbance during a particular first-in-week dialysis session ($R = 0.999$, $N = 8$).

Also *TRua* based on the transformation using the regression line from the total material was calculated, the transformation marked as UV2 (Figure 3).

When calculating the total removed uric acid *TRua* in μmol/L during a haemodialysis session with the dialysis session length *T* in minutes, the time integral can be generally used:

$$TRua = \int_0^T ua(t)[Qd(t) + UF(t)]dt \tag{2}$$

where *ua(t)* is the uric acid concentration in the spent dialysate in μmol/L, *Qd(t)* is the dialysate flow rate in L/min, and *UF(t)* is the ultra filtration rate in

L/min at the time moment *t* during a particular hemodialysis session, respectively.
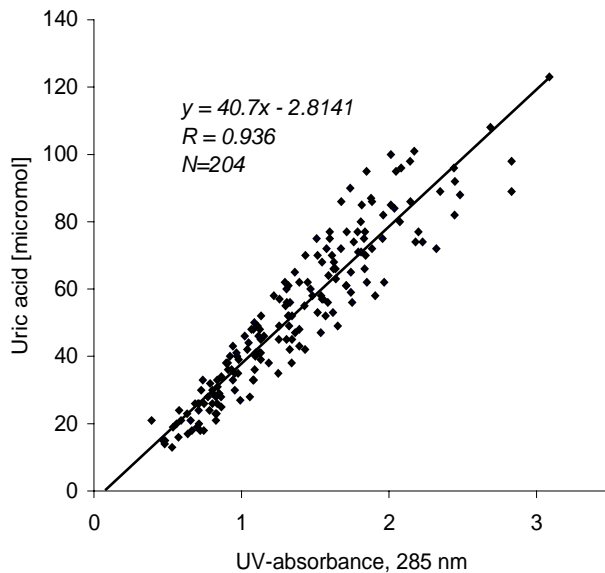

Figure 3: The regression line between uric acid concentration in dialysate and UV-absorbance from the total material (*R* = 0.936, *N* = 204, totally 23 haemodialysis sessions).

Assuming that the dialysate flow rate, *Qd(t)*, is constant and the total ultra filtrated volume *UF* in L is known, the following equation can be utilised:

$$TRua = Mean(ua) * (Qd * T + UF) \quad (3)$$

where *Mean*(*ua*) is the mean uric acid concentration in the spent dialysate of the particular hemodialysis session. For the *TRua* calculations *Mean*(*ua*) = *Dtotal* was utilized.

In a similar way, *TRua* may be calculated from the on-line UV-absorbance as:

$$TRua = (Slope * MeanA + Intercept) * (Qd * T + UF) \quad (4)$$

where the *MeanA* is the mean of all UV-absorbance values from the start to the end of the dialysis. The regression line between the UV-absorbance and *Dua* from one on-line measurement gives the Slope and the Intercept (Figures 2 and 3) inserted into equation 4 when calculating *TRua* during the following sessions.

TRua from *TDC* (reference) was calculated as uric acid concentration *Dtotal* [μmol/L]* collected weight [kg], assuming that 1kg =1L of the dialysate.

TRua from the three methods (*TDC*, *UV1* and *UV2*) was finally compared.

Results are expressed as mean ± *SD*. Samples taken at times coinciding with the self-test of the dialysis machine were excluded. In addition one session was excluded due to technical failure of the spectrophotometer. Student's t-test (two-tailed) and Levene Test of Homogeneity of Variances were used to compare means for different methods and *SD* values respectively. *P* < 0.05 was considered significant.

Results

Figure 4 shows the mean values of *TRua* obtained using *TDC* and two different transformations for UV-absorbance.
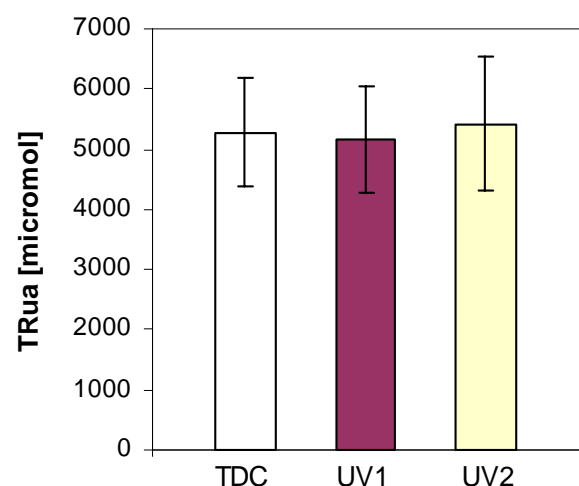

Figure 4: Mean values of *TRua* in μmol from *TDC* (*N* = 24), UV-absorbance for the first-in-week session *UV1* (*N* = 23) and UV-absorbance for the total material *UV2* (*N* = 23) transformation, respectively.

The mean ± SD in μmol was 5277 ± 897 from *TDC* (*N* = 23), 5150 ± 882 from *UV1* (N = 23), and 5422 ± 1112 from *UV2* (*N* = 23) transformation, respectively. None of the mean *TRua* values were significantly different (*P* > 0.05) when comparing the different methods.

Figure 5 shows the mean values of *TRua* for the follow up sessions performed more than 7 months after the first part of the study. The mean ± *SD* was 5932 ± 562 μmol from *TDC* (*N* = 6), 5901 ± 675 μmol from UV-absorbance for the first in week session calibration *TRua* 1 (*N* = 6) and 6082 ± 886 μmol from UV-absorbance for the total material calibration *TRua* 2 (N = 6) respectively. None of the mean *TRua* or *SD* values were significantly different (*P* > 0.05) compared different methods
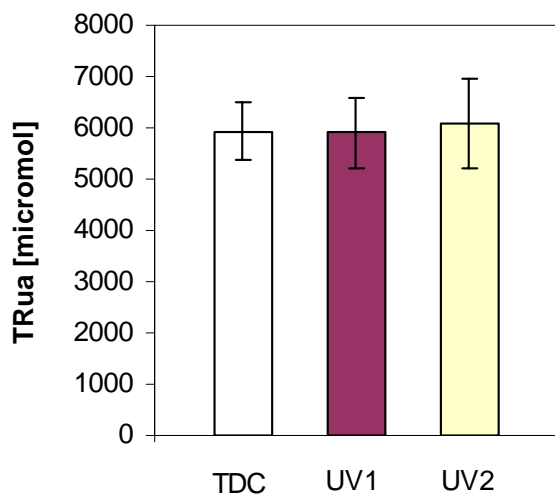
Figure 5: Mean values of *TRua* in µmol for the follow up sessions from *TDC* (*N* = 6), UV-absorbance for the first in week session *UV1* (*N* = 6) and UV-absorbance for the total material *UV* (*N* = 6) calibration respectively.

Discussion

The presented results show the possibility to estimate *TRua* by using UV-absorbance. The values of *TRua* obtained using the UV-absorbance measurements were in the same order as *TRua* calculated from *TDC* (reference method) (Figures 4 and 5). Also, the *SD* of the mean value for *TRua* was of the same magnitude for all methods.

Interestingly, after more than 7 months the mean values of *TRua* exhibited the same good agreement as above using the transformation *UV1* or *UV2*. This shows that the long-term patient calibration based on some dialysate samples taken during one session from which a regression line could be assessed for transformation could be an alternative to calculate total removed uric acid from the on-line UV-absorbance measurements in the spent dialysate.

The need of individual dialysate samples is a tedious and laborious procedure when estimating *TRua* with the UV-method. A general regression model based on the correlation between UV-absorbance and uric acid for all subjects should be preferred in the future.

A good correlation between UV-absorbance and uric acid could be explained by relatively high millimolar extinction coefficients with three distinct maxima around 202, 235 and 292 nm and two minima around 220 and 260 nm in the wavelength range from 200 to 380 nm [7]. The absorbance around 292 nm is characteristic for uric acid and is utilised for uric acid concentration determination by the enzymatic degradation method [8]. At the same time the millimolar extinction coefficients for urea have very low values compared to uric acid at the observed wavelengths [9], [7]. This confirms that the general correlation between

UV-absorbance and urea for the total material tends to be lower compared to uric acid [6].

A relatively dominant absorbance for uric acid and creatinine, compared to other compounds in different fluids (serum, spent dialysate, urine), is confirmed by several HPLC studies at the wavelength 254 nm [10], [11], [12], [13]. Uric acid, being a small solute, is also removed from the blood by haemodialysis in a similar manner as urea and is associated with disturbances of calcitriol production and metabolism [2]. Since the spent dialysate contains many UV-absorbing compounds, a summated effect of the compounds must be regarded. A relatively good correlation between UV-absorbance and a particular solute may be achieved when the removal rate of a non-absorbing solute, e.g. urea, is similar to UV-absorbing substances during haemodialysis. A similar relation between certain solutes has been confirmed using HPLC studies [1].

The fact that the UV-method has similar results as the standard methods at the chemical laboratory indicates that the removal rate of UV-absorbing solutes is comparable to a solute like uric acid during hemodialysis. This is also confirmed by a very good correlation between several small molecular weight waste products and the UV-absorbance [4] and similar concentration changes during dialysis for several azotemic markers (e.g. urea, creatinine, uric acid and pseudouridine) as reported earlier [14], [1]. The elimination of such a small molecular weight waste product as uric acid can therefore be assessed by the UV-technique. As a consequence, this makes it possible to determine uric acid concentration and calculate *TRua* even when the technique does not measure solely uric acid.

A clear advantage of the UV-method is the possibility to estimate uric acid concentration intermittently in the spent dialysate at a sampling rate that ensures that the measured values are less sensitive to measurement errors compared to manual dialysate sampling.

A relationship between Kt/V urea (or URR) and mortality and morbidity has been demonstrated in uremic patients [15-23],[24],[25] despite the fact that urea is considered to be atoxic [26],[27]. With the UV-method, it is possible to measure the elimination of other toxic or atoxic substances that may be significant in uremic patients. The next studies should investigate how the *TRua* relate to *Kt/V* and *PCR* and if there is some correlation between uric acid based parameters and clinical outcome in dialysis patients.

The UV technique could be a tool monitoring dialysis quality by evaluating the delivery of the prescribed treatment dose and immediately identifying and being alert for any deviations in dialysis treatment. This gives the possibility for an individual approach to follow and plan each dialysis treatment giving feedback to nursing staff during and after interventions.

Conclusions

The results show the possibility to estimate total removed uric acid by using UV-absorbance. The mean values of *TRua* obtained using the UV-absorbance measurements were very close to *TRua* calculated from *TDC* (reference method) (Figures 4 and 5). Even if the pure uric acid concentration is measured in the spent dialysate at the chemical laboratory, while the UV method measures all UV-absorbing compounds in the spent dialysate, the assumption that UV-absorbing solutes are removed in a similar manner compared with uric acid seems to be valid in this material. The best calibration method to calculate total removed uric acid from the on-line UV-absorbance measurements in the spent dialysate should be validated in the next studies where a larger material is included to draw more general conclusions. Moreover, the obtained uric acid based parameter should be related to urea quantification and clinical outcome in dialysis patients. Also the possibility of the total removal of other solutes, e.g. those presented by the EUTox group [28], [29], can be used as a measure of dialysis adequacy in the same manner as urea should be validated. This information will be useful as a source for analysing and revising hemodialysis quality and existing standards and methods to ensure treatment quality and patient welfare.

Acknowledgements

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