Investigation of the elimination kinetics of low and middle molecular weight uremic markers during hemodialysis treatment with the optoelectronic multispectral sensor

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Abstract. The elimination kinetics of conventional low-molecular weight uremic markers and advanced glycation end products (AGE products), which are considered as a possible middle molecular weight marker of uraemia in hemodialysis (HD) patients, was investigated by direct ultraviolet (UV) absorption spectroscopy of waste dialysis fluid. Light emitting diodes (LED) were exploited as quasi-monochromatic light sources in the deep UV spectral region for measuring optical transmission at the wavelengths 285 nm (the maximum of uric acid UV absorption) and 365 nm (the maximum of AGE absorption band). Optical absorption of waste dialysate was monitored during multiple HD sessions for a group of patients; the double-pool model of elimination kinetics was used for the curve fitting and approximation. It was revealed that kinetic behaviour of AGE products is almost not different comparing with uric acid and other conventional clinically proved uremic markers.

1. Introduction
In worldwide clinical practice most of the patients suffering from chronic renal failure (CRF) are treated with hemodialysis (HD) using various types of dialysis machines. HD therapy partially replaces normal renal function and provides elimination of middle and low-molecular weight metabolic waste products, including creatinine, phosphates, urea, uric acid, peptides, advanced glycation end products (AGE products) and many other substances. These molecules are evacuated from blood to dialysate (specially prepared fluid with electrolyte composition, which is identical to plasma) via a semi-permeable membrane by diffusion and convections mechanisms, while larger proteins and blood cells cannot travel through the pores of the membrane and remain intact [1].

The fractional urea clearance (Kt/V) is recommended by KDOQI Clinical Practice Guideline for HD Adequacy as the most preferable laboratory parameter for estimation of HD efficiency (dialysis dose). According to multiple clinical studies there is strong a correlation between patient survival and Kt/V [2]. From the other side, this approach is often considered outdated and not fully informative by many researchers [3]; alternative uremic markers have been evaluated, such as β-2-microglobuline or Cystatin C [4].

Advanced glycation end products (AGE), which may be considered as a middle molecular weight uremic toxin, are often associated with higher risk of mortality for patients with CRF [5-7]. Existing biochemical methods for the determination of AGE concentration are too sophisticated for routine clinical use, while optical spectroscopic methods such fluorescence spectroscopy or absorption
spectroscopy in the UV and visible regions are suitable not only for the analysis of biological samples, but even for the online monitoring of AGE concentration in effluent dialysate [8].

The aim of this work is to experimentally investigate and compare the kinetics of elimination of uric acid and AGE products by UV absorption spectroscopy of effluent dialysate and assess the possibility of using AGE product for the evaluation of the dose of dialysis.

2. Experiment and results
An optoelectronic multispectral sensor based on UV LEDs was specially designed by Ldiamon AS (Tartu, Estonia) for the monitoring of water dialysate optical absorption at the wavelengths 285 nm (the maximum of uric acid UV absorption) and 365 nm (the maximum of AGE absorption band). The sensor was connected to the dialysis line of a machine after a dialyzer (Figure 1). Optical modules equipped with 285 nm and 365 nm deep UV LEDs are independent from each other because much longer flow-through quartz cuvette (50 mm optical length) are needed for reliable detecting of AGE products due to significantly weaker absorption of AGE compared to uric acid; two visible-blind photodetectors are used to avoid any unwanted influence of ambient light.

Reference signal (100% transmission) is recorded before the beginning of a HD treatment session when fresh dialysate is being circulated within the dialysis line of a machine. Device control and data acquisition were realized with HD Monitor 3.0 software; text files containing treatment parameters and dialysate transmittance at both analytical wavelengths, which is measured every 30 seconds, are recorded for each HD session.

Measurements with the spectral sensor were carried out during more than 70 HD treatment sessions for 20 patients suffering from CRF. Time dependences of effluent dialysate transmission were recorded for each session; the results for two sessions are presented in the figure 2. It can be seen from the figure 2 that effluent dialysate transmittance at 365 nm is higher than at 285 nm despite the fact that a longer cuvette was used, otherwise both curves are very similar.

Dialysate optical absorbance at the analytical wavelengths was estimated; normalization procedure was applied to the absorption coefficients in such a way that normalized values vary in the range from 0 to 1 (extreme absorbance at the start of a treatment was used as a denominator). Normalization was
useful for a direct comparison of AGE products elimination kinetics with the kinetics of uric acid, because the absolute values of absorbance at 365 nm is significantly lower than at 285 nm. The curves of normalized absorbance at 285 nm and 365 nm are very similar to each other, but for most of the sessions absorption at 365 nm decreases slower than absorption at 285 nm (figure 3).

Figure 2 (a, b). Optical transmittance of waste dialysate at 285 nm and 365 nm during HD treatment for the patients L. (a) and M. (b).

The kinetics of uremic markers elimination during hemodialysis can be analysed in the framework of double-pool kinetic model, which initially was suggested for urea, but with some limitations could be applied to other substances. This model describes both the transfer of uremic markers via a dialysis membrane and the intercompartment transfer from extracellular fluid to blood in the human body [9]:

\[ C(t) = C_1 e^{-\tau_1 t} + C_2 e^{-\tau_2 t} + C_3, \]

where \( \tau_1 \) – the time constant that characterizes the transfer of uremic markers via a dialysis membrane; \( \tau_2 \) – the time constant that characterizes the intercompartment transfer. According to Beer–Lambert law the concentration of a uremic marker is directly proportional to the absorption coefficient. Because of this fact we assumed that the double-pool model could be technically applied to the normalized optical absorbance at 285 nm and 365 nm directly.

Figure 3(a, b). Normalized optical absorbance of waste dialysate at 285 nm and 365 nm during HD treatment and approximation according to the double-pool kinetic model for the patients L. (a) and M. (b).

The curves of normalized optical absorbance at the wavelength 285 nm and 365 nm were approximated with exponential functions (1) using Mathcad software; the time constants \( \tau_1 \) and \( \tau_2 \) were estimated (table 1). The average values of the time constant of the “fast” exponent (responsible for a
dialyzer clearance) for the 285 nm curves (uric acid absorption) and the 365 nm curves (AGE products products) were 144 min and 138 min respectively; for the «slow» exponent (responsible for intercompartment transport) the average time constants were 15 min and 14 min respectively.

In case of the 365 nm absorption curves both “slow” and “fast” time constants are a little bit lower than for the 285 nm absorption curves, but the difference (Δτ₁=6 min and Δτ₂=1 min) does not exceed the standard deviations in the group. It is not clear yet in what extent this difference is clinically significant; additional researchers on larger groups of patients are necessary.

| # of patient | Time constants of the double-pool model at 285 nm | Time constants of the double-pool model at 365 nm |
|--------------|-----------------------------------------------|-----------------------------------------------|
|              | τ₁    | τ₂   | τ₁    | τ₂   |
| 1            | 169   | 21   | 129   | 11   |
| 2            | 146   | 11   | 155   | 9    |
| 3            | 120   | 16   | 119   | 20   |
| 4            | 132   | 16   | 111   | 13   |
| 5            | 204   | 13   | 261   | 21   |
| 6            | 137   | 11   | 121   | 9    |
| 7            | 151   | 29   | 134   | 27   |
| 8            | 214   | 45   | 215   | 37   |
| 9            | 118   | 7    | 113   | 10   |
| 10           | 131   | 19   | 135   | 17   |
| 11           | 124   | 15   | 101   | 12   |
| 12           | 120   | 8    | 123   | 9    |
| 13           | 99    | 2    | 92    | 2    |
| 14           | 193   | 11   | 155   | 11   |
| 15           | 110   | 3    | 110   | 6    |
| 16           | 129   | 14   | 137   | 12   |
| mean±SD      | 144±34| 15±10| 138±43| 14±9 |

3. Conclusion
The results of HD monitoring with the multispectral optoelectronic sensor based on deep UV LEDs reveal that kinetic behaviour of AGE products during HD treatment is almost not different comparing with uric acid and other conventional clinically validated uremic markers. It was proved that optical monitoring at 365 nm can be potentially used for assessing HD adequacy.

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