Multimode Monitoring of AGE Excretion in Hemodialysis

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INTRODUCTION AND MOTIVATION

Advanced glycation end products (AGE) belong to the factors of high mortality among end stage renal disease patients (ESRD Pts.). Monitoring and reliable data acquisition on excretion of AGE in hemodialysis (HD) remain as challenges. No on-line monitoring data existed earlier for AGE. Main **aim** of the present study was to realise on-line optical dual-wavelenth monitoring, analyse AGE HD kinetics data with a two compartment model and compare optical data with immunoassay results.

METHODS AND PATIENTS

Characteristic absorption of AGE is in the region 360-370 nm [1-5] and it has low absorption coefficients k ≈ 0.1 cm^{-1} (Fig. 1A, B).
A special long path length cuvette (50 mm) was designed (AS Ldiamon, Tartu) and used in this study for monitoring at 365 nm (Fig. 2). For monitoring at 285 nm a cuvette with 8 mm path length was applied. The sensors based on the 8 mm cuvettes for 285 nm monitoring of AS Ldiamon production are being used in the B|Braun Adimea HD machines.
Original algorithm for spectral data processing has been applied [6]. Details of the data processing as well as overview of the monitoring process are given in the Supplement to this report. Additionally to the combination (285 + 360) nm another version of monitoring at (287 + 262) nm has been used to get refined kinetics patterns for uric acid separately (see [6]).
On-line monitoring was performed at the Tartu University Hospital (Fresenius 5008 machines; 9 Pts., 25 sessions, 285 + 360 nm) and Mariinsky Hospital in S.-Petersburg (B|Braun machines, 35 Pts. 210 sessions, 287 + 262 nm). On-line monitoring was supplemented by biochemlab assays of urea, creatinine, uric acid and by measurements in off-line mode of characteristic AGE absorption (365 nm) and fluorescence (430 nm) in the same effluent dialysate samples taken in numbers ≤45 per session. A polyclonal AGE Competitive ELISA kit (Biolabs, INC, catalog nr. STA-817) was used for immune assay in the off-line samples.

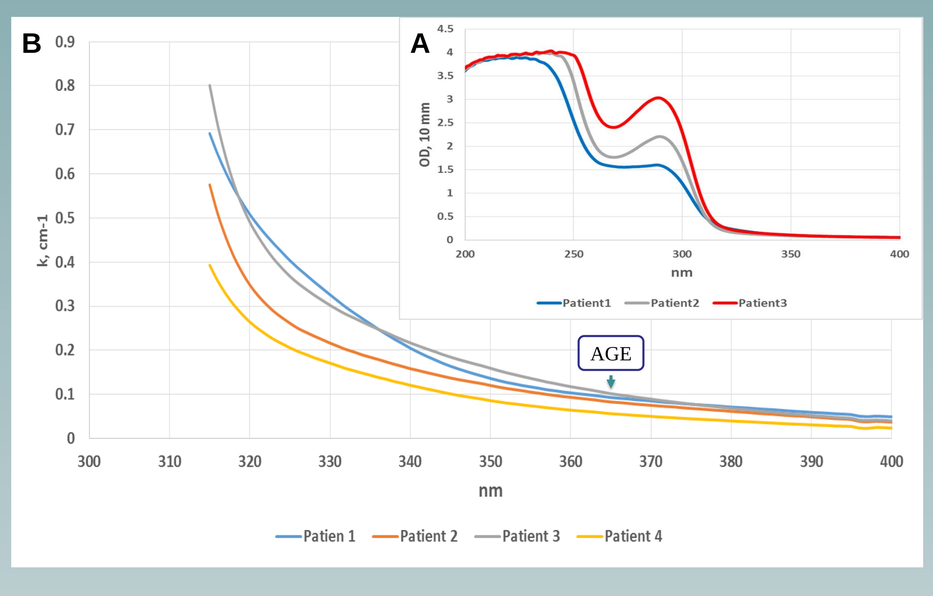


Fig 1.
A. Absorption spectra of hemodialysates with the selective band at 290 nm which is induced mostly by uric acid extinction.

B. Absorption continuum of hemodialysates in the range λ ≥ 315 nm. According to [1-5] absorption near 365 nm belong to AGE. Two absorptions at 285 nm and 365 nm have been used in this study.

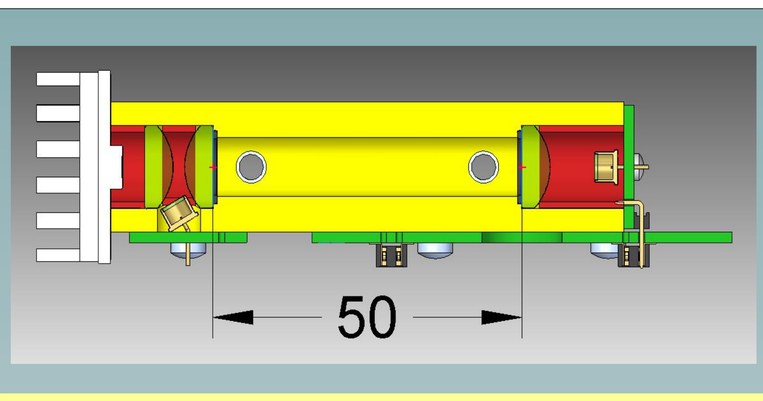


Fig. 2.
Long path length cuvette (50 mm) for monitoring of AGE absorption in effluent hemodialysate at the wavelength 365 nm

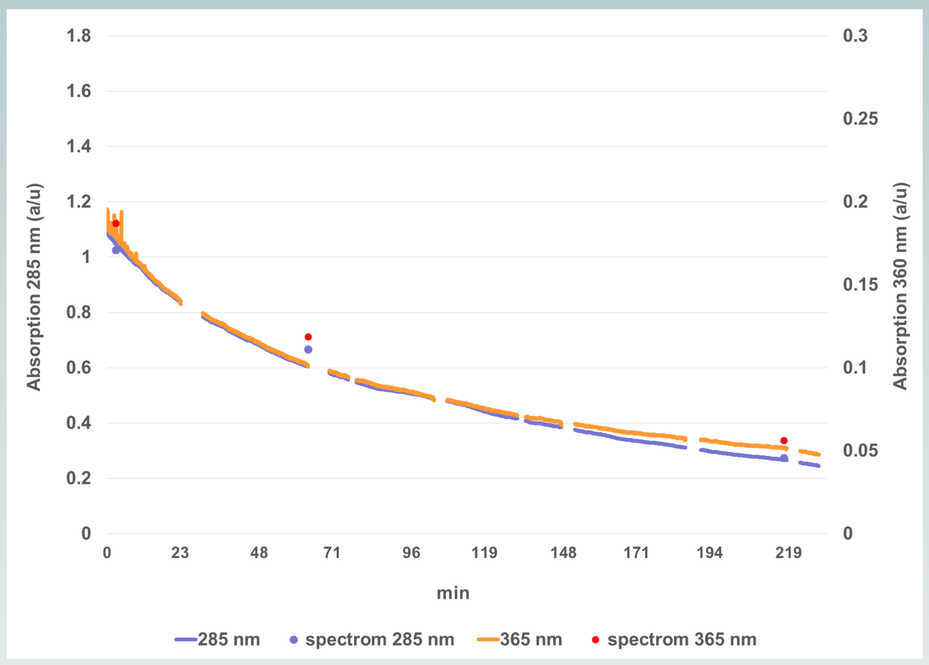


Fig 3.
Two kinetics measured on-line at 285 and 365 nm during a HD session. For comparison there are added off-line data measured with a laboratory spectrophotometer.

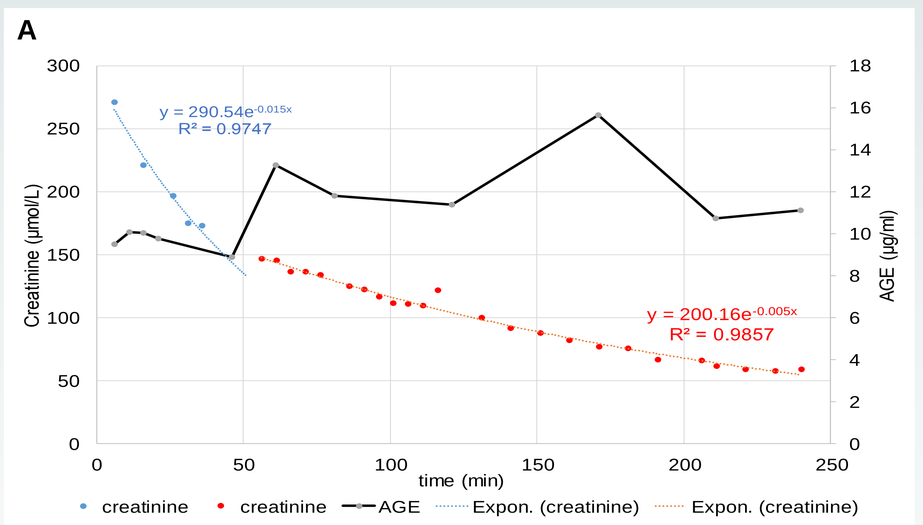
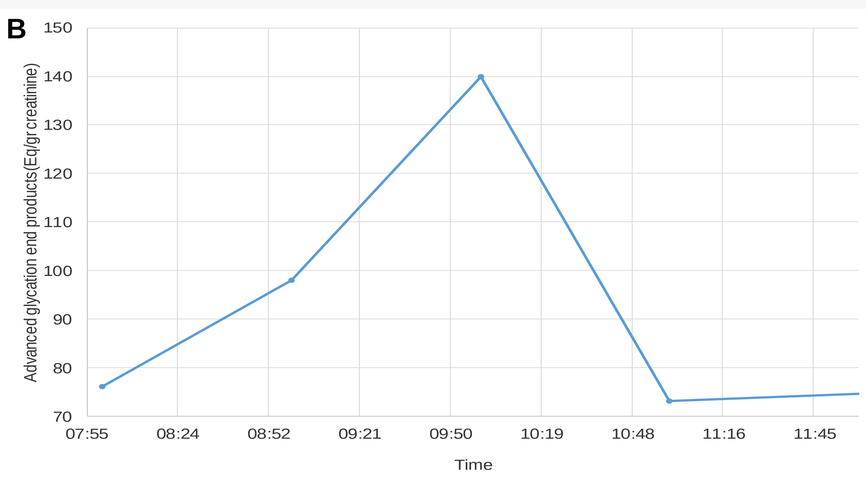


Fig 4. Comparison of temporal patterns obtained in off-line mode in hemodialysate (**A)** and healthy control's urine (**B).**

A. Creatinine and AGE concentration profiles in hemodialysate taken during a morning dialysis session. Creatinine was measured in the biochemlab and AGE determined by immunoassay with use of polyclonal ELISA kit.



B. AGE concentration profile in a healthy control's urine as determined for the morning time samples by immunoassay and related to urine creatinine. The data **B** are reproduced from [1].

RESULTS

In Fig.3 there are depicted two kinetics measured on-line at 285 and 365 nm during a HD session. The curves coincide quite well but the kinetics for AGE is somewhat slower. If the optical and biochemical data were combined we got the averaged values for reduction rates (RR) as it follows: $RR_{uric\ acid} \approx 1.25RR_{creatinine} \approx 1.45RR_{urea}$ and RR_{AGE}(optics, on-line) ≈ RR_{urea}(biochem, off-line). The results of processing in more details in terms of two-compartment model are given in the Supplement.
Striking irregularities have been observed in the HD kinetics of AGE determined by immunoassay (Fig.4A). These are in full contrast to optical data (Fig.3) and to the concentration profile of creatinine (Fig.4A). Additionally, we have $RR_{AGE\ immune} \approx 1$. The irregularities in the curve for AGE in Fig.4A resemble variations of AGEs concentration in on-spot urines taken from a healthy control (Fig. 4B). The irregularities and $RR_{AGE} \approx 1$ are caused most probably by intense generation or/and rebound of ELISA active AGEs during the pertinent 4 hours intervals.

CONCLUSIONS

A good concordance of on-line optical monitoring data for the "classical" monitoring at 285 nm and at 365 nm for the "new agent"- AGE has been observed.
Nevertheless, large difference between the data obtained by optical monitoring and immunoassay of AGE was encountered. Moreover, $RR_{AGE\ immune} \approx 1$ and this is the manifestation of no remarkable depletion of some kinds of AGE during HD sessions. The given fact demonstrates once again the problem of AGE excretion and the need for adequate on-line monitoring using all technical possibilities.
In further dialysis work more attention should be paid to direct optical monitoring of AGE in the range λ ≥ 315 nm. It is also not excluded that the approach of "characteristics optical absorption/ fluorescence" of AGE at 360-70/430-40 nm [1-5] should be revised and supplemented by the concept of fluorescent endogenous nanoparticles in biological fluids [7].

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