Peculiar character of dialyzate ultraviolet extinction spectra as an indicator of nucleic acid metabolism in humans

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Abstract. UV extinction spectra of effluent dialyzate in the process of hemodialysis and peritoneal dialysis are studied. It is found that they have distinctive individual features unique to each patient. The shape of the spectrum remains virtually invariable for a given subject during the whole period of the study (more than a year) and does not depend on the treatment modality or other factors like sex, age, the stage of chronic renal failure, and concomitant illnesses. Absorption bands in two spectral regions—255 to 265 nm and 288 to 298 nm—account for the unique character of dialyzate spectra. Spectra classification based on the ratio of integral extinctions in these regions are introduced. It is assumed that the shape of the spectrum depends on the relative contents of plasma components. Statistical analysis of individual features of spectra for the groups of patients undergoing hemodialysis (78 people) and peritoneal dialysis (42 people) is carried out. It is revealed that the distribution of patients by the ratio of integral extinctions in the specified spectral regions adheres to the normal law. Presumably individual features of spectra could be related to uric acid and a biochemically unidentified substance with an absorption peak near 260 nm. © 2005 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.1953268]

1 Introduction

On-line monitoring of extracorporal detoxication processes, including hemodialysis (HD) and continuous ambulatory peritoneal dialysis (CAPD), is vital for practical medicine. Today HD and CAPD are the only ways to support the life of patients with terminal stage of chronic renal failure (CRF). Despite an obvious progress in HD equipment technology, there are no effective and economically justified methods to control HD sessions on-line, which is key to achieve the prescribed level of dialysis adequacy. Devices containing urease-based urea sensors like Baxter Biostat 1000 ® (Tampa Bay, FL) or Ureascan P2 Hospal ® (Stockholm, Sweeden) are not widely used in clinical practice, as the cost of disposable materials is comparable to the cost of dialysis itself.[1–5]

Several attempts to develop methods for on-line hemodialysis monitoring that are based on UV and IR absorption spectrometry have been made.[6–11] We have been carrying out the research for several years and have developed equipment and techniques to monitor body fluids using UV extinction spectra.[12–15] Realizing such monitoring was proved in papers.[12,15,16] The study of many hemodialysis and peritoneal dialysis sessions has shown that there is a certain correlation between low molecular weight components, including creatinine, uric acid and urea, and concentration changes in effluent dialyzate, and corresponding UV extinction spectra changes in the 200 to 400-nm spectral region.

It is necessary to mention that the ultraviolet spectra of body fluids do not have narrow isolated absorption bands that could be linked to a certain chromophore. Furthermore, component concentrations are rather high, which lead to a significant deviation from the classical linear Beer's law.[17] Therefore, standard methods of spectrophotometric analysis based on the measurement of the extinction factor at one to two wavelengths and subsequent calculation of the concentration with the help of calibrations curves or tables is not applicable to biological fluids. For each given fluid, it is necessary to develop a complex nontrivial technique, taking into account fluid characteristics and using the entire absorption curve $k(\lambda)$ in λ_1 to λ_2 informative spectral range.

While solving the problem, we found that dialyzate extinction spectra obtained from different patients are quite similar but at the same time have distinctive individual features, which should be taken into account in the algorithm of spectrophotometric analysis. In the work, the results of the studies of the individual features of dialyzate extinction spectra obtained from a large group of patients suffering from CRF are given, quantitative parameters describing the shape of spectra

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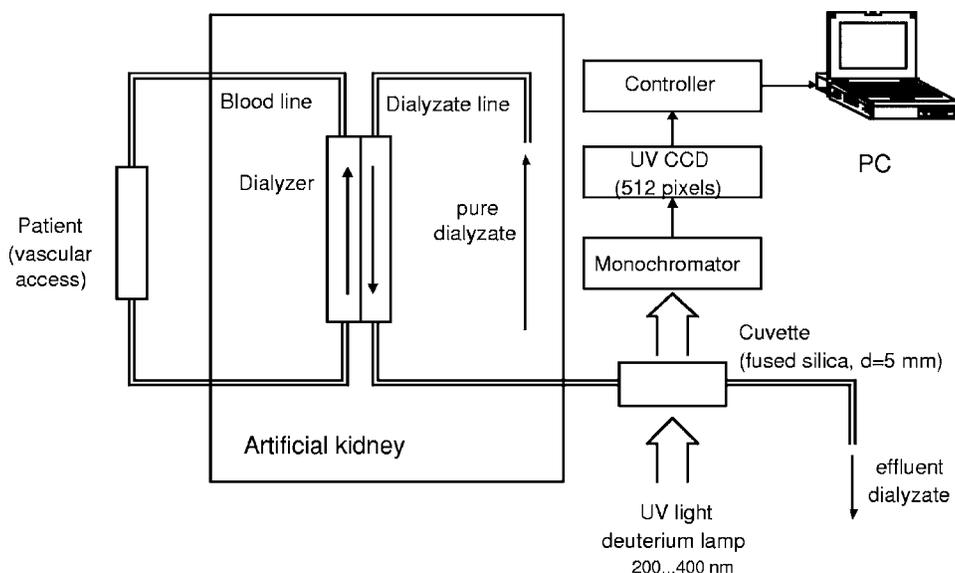
Fig. 1 System of hemodialysis monitoring using UV extinction spectra.

in the informative wavelength region are determined, statistical analysis of the spectral curve shape is carried out, classification of spectra is offered, and a possible correlation between the shape of the spectrum and blood plasma individual features is considered.

2 Instrumentation

The novel system for monitoring hemodialysis sessions shown in Fig. 1 was used to register dialyzate extinction spectra.[14] The core of the system is a multichannel spectrophotometer constructed according to Rowland's scheme with a concave diffraction grating. The registration is carried out with an UV sensitive CCD linear sensor containing 512 pixels; the signal from the sensor processed by the controller is directed to a PC. Minimal time of registration of one spectrum is 8 ms; the signal to noise ratio is $\geq 10^2$. The source of UV light was a deuterium-neon lamp. The error of measurements is no more than $\pm 5\%$.

The spectrophotometer is equipped with a flow-through fused silica cuvette with optical length $d=5$ mm. The cuvette is connected to the effluent line of a dialysis machine. It makes automatic spectra registration in the course of a HD session possible. Such connection completely excludes any influence of the equipment on the functioning of the dialysis machine and is absolutely safe for the patient.

Specially designed software provides the standard set of functions: choosing a registration mode, compensation of a dark signal, direct calculation of a transmission spectrum using previously saved 100% transmission reference [Eq. (1), as well as fully automatic on-line spectrum registration and processing for the determination of dialyzate composition changes during HD sessions. Spectral transmissions are calculated as follows:

$$T^n = \frac{(S^n - S_d^n)}{(S_0^n - S_d^n)} \cdot 100\% \; ; \quad k^n = \ln(100\% / T^n), \qquad (1)$$

where S^n is the signal corresponding to effluent dialyzate, S_0^n is the signal corresponding to 100% transmission reference, S_d^n is the dark signal, and n is the pixel number in the CCD sensor. The pixel number is subsequently translated into the wavelength using the built-in calibration.

3 Results and Discussion

3.1 Study of UV Dialyzate Fluid Extinction Spectra in the Process of HD and CAPD

The research revealed that the shape of dialyzate transmission spectra in the informative UV region has clear individual character, i.e., it greatly varies from one patient to another. At the same time, the shape of the spectrum for a certain patient remains virtually invariable in the course of the HD session, whereas the overall absorption level is decreasing. Moreover, prolonged observations of the group of patients have shown that for each given patient the shape of the spectra, measured during several sessions some months or weeks apart, is almost unchanged.

Some characteristic types of transmission curves have been specified; they are referentially called A, B, and C types (see Fig. 2). On A spectra, the distinguished minimum in transmission is close to 265 nm; on C spectra the monotonous growth of transmission with increase of wavelength is observed; and on B spectra in the 265- to 285-nm range transmission weakly depends on wavelength. Figure 2 shows dialyzate UV transmission spectra of various types at different moments during dialysis sessions. The patients on HD were treated with a Fresenius 4000 - 8F machine, dialyzer F60 (Bad Homburg, Germany); for CAPD, the Stay-Safe set from Baxter (Tampa Bay, FL) was used. The time when the sample was taken is
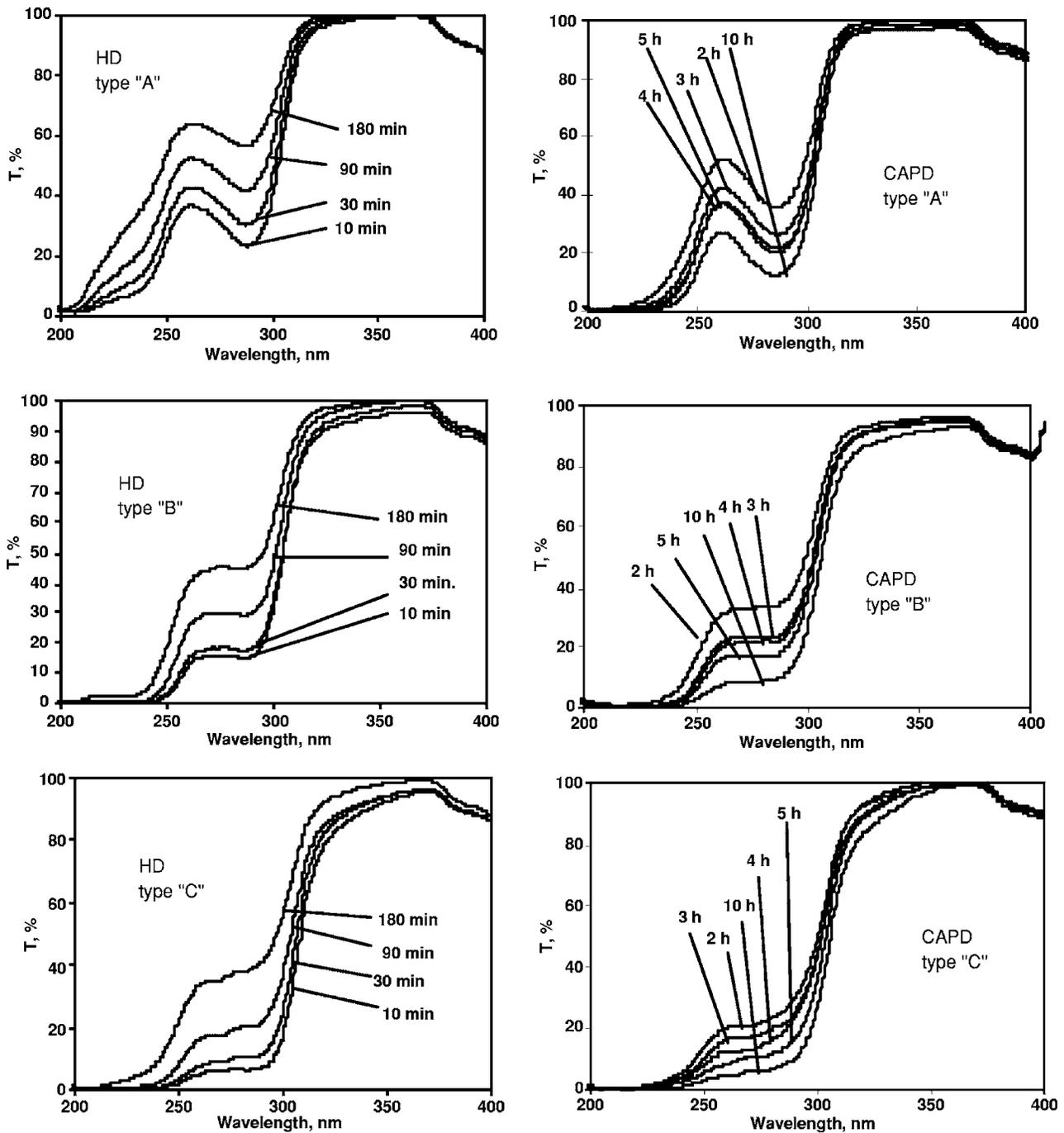
Fig. 2 Dialyzate transmission spectra of different types for HD (on the left) and CAPD (on the right).

given on the diagrams (10, 30, 90, and 180 min in the course of a HD session; and for CAPD, the duration of a peritoneal exchange was 2, 3, 4, 5, and 10 h).

The individual character and the temporal stability of the spectrum shape can be caused by at least two different factors. First, it is possible to assume that UV absorbing components are present in patient's blood in constant proportions that do not change for a long time. Second, various HD modes and types of dialyzers with a set of parameters (blood flow, dialyzate flow, properties of semipermeable membrane, dialyzate concentrate) that are most suitable for a particular patient are

used. It can also influence the effluent dialyzate composition and, hence, spectrum shape. The most of these factors can easily be excluded by comparing the shape of dialyzate transmission curves of the patients undergoing different treatment modalities—HD and CAPD.

In this connection, the spectra of dialyzate obtained from two groups of patients suffering CRF of various stages were studied. The first group (78 people) consisted of patients on HD, second group (42 people) included patients who received CAPD. The measurements were carried out in City Hospital 12, Hemocorrection Center, Saint Petersburg, Russia.

Table 1 Experimental distribution of HD and CAPD patients by F_1/F_2 parameter.

F_1/F_2		0.5 to 0.7	0.7 to 0.9	0.9 to 1.1	1.1 to 1.3	1.3 to 1.5	1.5 to 1.7	Mean ± SD
Number of patients	HD	1	6	20	28	19	3	1.18±0.20
	CAPD	1	9	17	9	4	1	1.01±0.19

The technique of the experiment was as follows.
• Samples of hemodialyzate were taken from 78 patients.
• Hemodialyzate samples were collected from dialysis machine effluent lines.
• Time during a HD session when the sample was taken was voluntary.
• Samples of peritoneal dialyzate were taken from 42 patients.
• Peritoneal dialyzate samples were taken simultaneously with the peritoneal equilibration test.
• Duration of peritoneal exchanges was 2 h.
• To reduce the error of measurements caused be strong absorption in the working spectral region, peritoneal dialyzate samples were previously diluted with distilled water in proportion 1:3.
• Dialyzate transmission spectra were measured in the 200- to 400-nm region with 0.7-nm resolution.
• Fused silica cuvettes with the optical length $d=5$ mm were used.
• Distilled water was used as a reference.
• Extinction spectra were calculated.

The research has confirmed that the spectrum shape and overall transmission level are not the same for each patient, i.e., they have individual character. On the other hand, no considerable differences between HD and CAPD groups have been revealed at this stage. Hence, the processes of UV absorbing uremic toxin elimination by HD (artificial membranes) and CAPD (natural membrane) are virtually identical. Thus, the individual features of dialyzate spectra are not related to the properties of dialysis membranes, but reflect the peculiarities of plasma composition.

It is known that dialysis is carried out to remove from the patient's body low molecular weight uremic toxins—urea, creatinine, uric acid and a number of other substances—whose molecular weight does not exceed 500 Da. The rate of the process depends on the concentration gradient in the dialyzer, component's molecular weight, blood flow velocity and dialyzate flow velocity, parameters of dialysis membrane, and a number of other, less important factors. The shape of the transmission spectrum depends on the position of absorption bands and concentration of UV absorbing substances, which are contained in human blood and dialyzate. Only a few substances with high specific extinction in the working spectral region are informative, i.e., could make a substantial contribution to the overall absorption and, hence, influence the shape of the spectrum.

The fact that the shape of the spectrum in the course of a session remains constant shows that the concentrations of such substances in patients' blood decrease proportionally. It has been partly proved earlier by experimental estimations of variations of uremic toxin concentrations (urea, creatinine, uric acid, and others) in plasma and dialyzate in the course of a dialysis session using rigorous biochemical methods including HPLC.[18] Toward the end of a session, concentrations of removing substances decrease 3 to 5 times, which accounts for the lowering of an overall absorption level.

If there was only one informative component, dialyzate transmission spectra taken from many patients would be identical in shape. The differences that can be seen in Fig. 2 could be explained if we assume that there are at least two informative components. The ratio of concentrations of these components in plasma is individual and determines the shape of the spectrum. As a dialysis membrane does not influence the spectrum shape, this phenomenon may be related to age, sex, stage of CRF, dialysis dose (KT/V parameter), or accompanying diseases. Also, the genetic origin cannot be disregarded. To exclude some of the previously stated reasons, it is
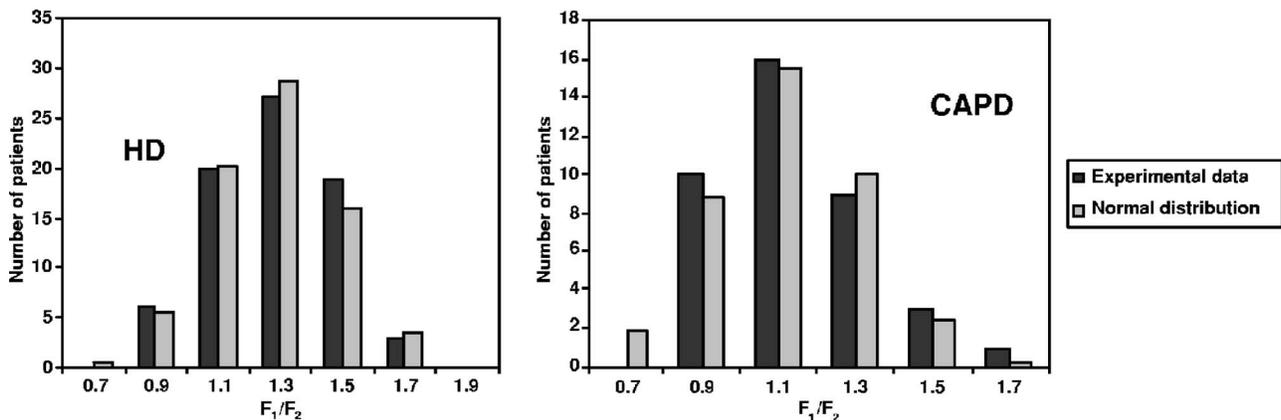
Fig. 3 Histograms of the patient distribution by F_1/F_2 parameter for HD (on the left) and CAPD (on the right) groups based on experimental data and corresponding Gaussian distribution.

Table 2 Variations of F_1/F_2 parameter for HD patients in the course of prolonged observations.

Patient M		Patient E	
Date of HD session	F_1/F_2	Date of HD session	F_1/F_2
04.10.2000	1.3	04.10.1996	1.03
18.10.2000	1.25	05.11.1996	0.99
25.10.2000	1.26	14.01.1997	1.05
22.11.2000	1.25		
Mean ± SD	1.27±0.02	Mean ± SD	1.02±0.03

necessary to carry out the statistical analysis of the spectrum shape probability distribution for a large group of patients.

3.2 Statistical Analysis of Dialyzate Spectra Distribution Through the Shape of the Spectral Curve

To statistically process the results obtained on the previous stage, it is necessary to introduce a quantitative parameter describing the spectrum shape. It can be made in two ways. In the first way, such parameter was the ratio of integral extinctions F_1/F_2 in the $\Delta\lambda_1=255$- to 265-nm and $\Delta\lambda_2=288$- to 298-nm spectral regions.

$$F_1/F_2 = \frac{\Delta\lambda_2}{\Delta\lambda_1}\int_{\Delta\lambda_1} k(\lambda)d\lambda \bigg/ \int_{\Delta\lambda_2} k(\lambda)d\lambda. \qquad (2)$$

The F_1/F_2 ratio was calculated for dialyzate extinction spectra of each patient included in the studied group (77 for HD and 41 for CAPD). The shape of dialyzate spectra for two patients (one in each group) greatly differed from others and is considered separately. The parameter F_1/F_2 varied from 0.5 to 1.7. This range was divided into intervals with a 0.2 step. Then the number of patients for each interval was counted up (see Table 1) and the histograms for HD and CAPD groups were plotted (see Fig. 3).

Statistical analysis has shown that the distribution of patients by the F_1/F_2 parameter both for HD and CAPD can be mathematically described by the normal law (Gauss distribution):

$$p(F_1/F_2) = \frac{1}{\sqrt{2\pi}\sigma}\exp\left[-\frac{(F_1/F_2 - \overline{F_1/F_2})^2}{\sigma^2}\right]. \qquad (3)$$

As stated before, spectra of dialyzate can be visually classified into three groups A, B, or C by the shape of a transmission curve. In the terms of the F_1/F_2 parameter, each group corresponds to the specific range of F_1/F_2 values:

group A, range $(\overline{F_1/F_2}-3\sigma \ldots \overline{F_1/F_2}-\sigma)$, mean insight the group 0.78

group B, range $(\overline{F_1/F_2}-\sigma \ldots \overline{F_1/F_2}+\sigma)$, mean insight the group 1.18

group C, range $(\overline{F_1/F_2}+\sigma \ldots \overline{F_1/F_2}+3\sigma)$, mean insight the group 1.58.

Parameter F_1/F_2 is the ratio of integral extinction in two selected spectral regions, each of them embraces no less than 25 discrete wavelengths (polychromator channels). The error of F_1/F_2 determination for the given spectrum depends on the absorption level and the shape of the curve, and does not exceed ±0.06.

For the group of patients characterizing different spectrum types (12 HD patients and 8 CAPD patients), prolonged observations have been carried out to determine long-term F_1/F_2 variations. Table 2 shows F_1/F_2 for hemodialyzate samples obtained at the 60th minute of several HD sessions conducted at intervals in weeks or months (see Fig. 4). Table 3 shows the same data for CAPD sessions; duration of peritoneal exchanges is 2 h.

Summarizing the results of the experimental data analysis is as follows.

The shape of the spectrum remains virtually unchanged in the course of a HD or CAPD session for every given patient. Differences between the spectra of different patients surely exceed variations within a session for one patient and the error of measurement.

The shape of the spectrum remains almost constant for every given patient in the course of prolong observations dur-
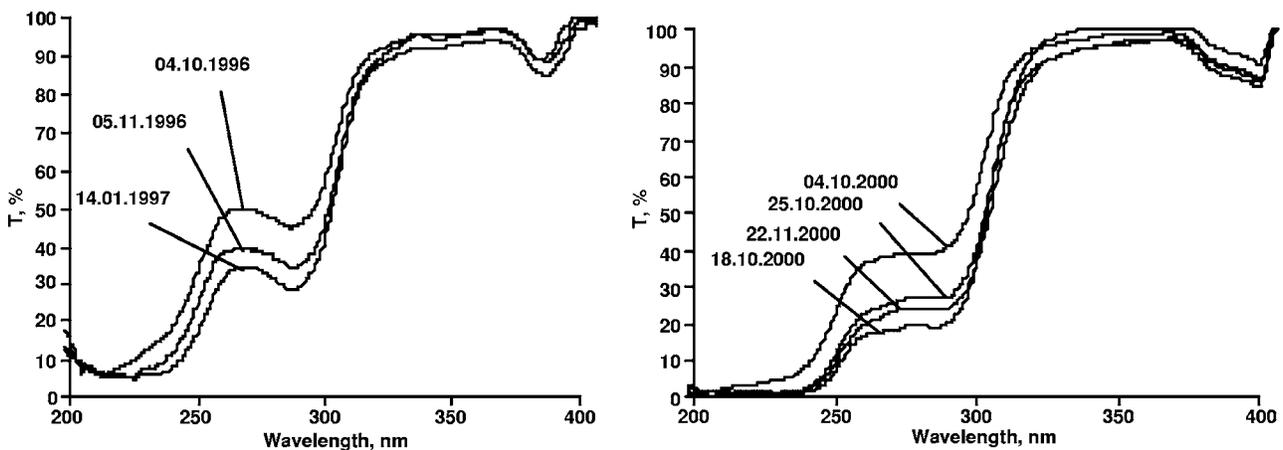
Fig. 4 Transmission spectra of dialyzate samples taken from two patients at the 60th minute of several HD sessions.

**Table 3** Variations of F_1/F_2 parameter for CAPD patients in the course of prolonged observations.

Patient B		Patient Se		Patient Sv	
Date of PET	F_1/F_2	Date of PET	F_1/F_2	Date of PET	F_1/F_2
06.04.2001	1.00	08.10.2001	1.08	19.04.2001	1.34
21.09.2001	1.03	19.04.2002	1.12	19.04.2002	1.22
11.04.2002	1.05	31.05.2002	1.14	31.05.2002	1.26
Mean ± SD	1.03±0.03	Mean ± SD	1.11±0.03	Mean ± SD	1.27±0.06

ing several HD or CAPD sessions some month or weeks apart. Differences between the spectra of different patients surely exceed long-term variations for a given patient.

Spectra of one patient on HD ($F_1/F_2=2.38$) and one patient receiving CAPD ($F_1/F_2=1.97$) do not fall into 3σ intervals and cardinally differ in shape from the spectra of other patients. There is a strong absorption in all working spectral ranges. Such spectra could not be described by the offered classification; a special group–conditional CC type—should be specified (see Fig. 5). Most likely there is one more informative component characterized by at least two absorption bands in the spectral regions shorter than 260 nm and larger than 280 nm, respectively.

In the second approach, the coefficient of similarity in relation to a reference spectrum was used as the parameter describing the shape of dialyzate spectra. It makes it possible to estimate spectrum shape variations in a wide wavelength range regardless of any absorption bands. To do this, a working spectrum $k(\lambda)$ and a reference spectrum $k_{ref}(\lambda)$ should be presented as an N-dimensional vector of absorption coefficients in λ_1 to λ_2 wavelength range.

$$S[k(\lambda),k_{ref}(\lambda)] = \frac{\sum_{n=N_1}^{N_2} k^n(\lambda)k_{ref}^n(\lambda)}{\left\{\sum_{n=N_1}^{N_2} [k^n(\lambda)]^2 \cdot \sum_{n=N_1}^{N_2} [k_{ref}^n(\lambda)]^2\right\}^{1/2}}.$$

$$\lambda \to n \quad \lambda_1 \to N_1 \quad \lambda_2 \to N_2. \tag{4}$$
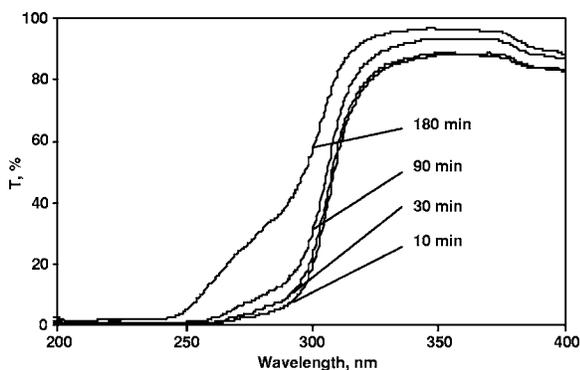
Fig. 5 Hemodialyzate transmission spectra of CC type.

At first, similarity coefficients for all measured spectra in relation to the B type reference spectrum were calculated. It appeared that it is unpractical to use it directly, as its absolute value for all spectra is close to unity. More informative is the difference of similarity coefficients in the wavelength region 240 to 320 nm, calculated relatively to C and A type reference spectra, characterized by the greatest and the least F_1/F_2 values in the sample, respectively.

$$S_{c-a}[k(\lambda)] = S[k(\lambda),k_c(\lambda)] - S[k(\lambda),k_a(\lambda)]. \tag{5}$$

Then S_{c-a} values were calculated for patients from both groups. Statistical analysis (reference spectra were excluded from the sample) has shown that patients are also distributed by S_{c-a} parameter under the normal law: for the HD $\overline{S_{c-a}}$ =0.004 (sample $N=75$), and for CAPD $\overline{S_{c-a}}=0.025$ (sample $N=39$). Moreover, there is a strong correlation between F_1/F_2 and S_{c-a}: $r=0.94$ for CAPD and $r=0.97$ for HD.

Statistical analysis has shown that there is no correlation ($r<0.5$) between the shape of spectra (F_1/F_2 parameter) and patients' sex, age, stage of CRF, the time since the beginning of dialysis therapy, dialysis KT/V doze, azotemia severity, hemoglobin, or general protein levels. Therefore, determination of the diagnostic and prognostic importance of the spectrum shape is a subject of future research.

Thus, the research shows that in the 250- to 300-nm wavelength interval in dialyzate spectra there are indications of at least two informative components. Their relative concentrations determine the shape of the spectral curve. Analysis of the absorption spectra of known dialyzate constituents allows us to assume that the absorption band in the 280- to 300-nm spectral range can be attributed to uric acid. This substance freely penetrates through the dialysis membrane, and its presence in dialyzate has been confirmed by chromatographic measurements.[18,19]

The second absorption band in the 250- to 270-nm spectral range can be attributed to many low molecular weight substances. It is necessary to take into account that analyzed dialyzate samples were obtained from patients suffering CRF of various stages with different accompanying diseases, and many such substances are retained in plasma in concentrations much higher than normal ones. The study of plasma, urine, and dialysate by HPLC before and after HD sessions[18] shows that the following components have strong ultraviolet absorption at 254 nm: uric acid, hippuric acid, creatinine, hypoxanthine, xanthine, pseudouridine, uracyl, adenosine, and p-hydroxyhippuric acid. Considering the characteristic fea-

tures of the absorption spectra (positions of absorption minimum and maximum, curve shape) of these substances in the wavelength region 250 to 300 nm, it can be concluded that uracyl, pseudouridine, uridine, and adenosine match the spectral criteria for unknown components. Only adenosine takes part in the purine metabolic chain, which ends up with uric acid. But by now the second component is not reliably identified and also could be related to other metabolic chains.

4 Conclusion

Thus, the comparative analysis of hemodialyzate and peritoneal dialyzate UV extinction spectra shows that there are distinctive individual features unique to each patient. The shape of the spectrum remains virtually invariable for a given subject during the whole period of the study (more than a year), and does not depend on the treatment modality (HD or CAPD) or other factors like sex, age, the stage of chronic renal failure, or concomitant illnesses. It allows us to assume that the revealed spectra peculiarities are mainly caused by the differences of blood plasma composition and do not directly depend on dialysis membrane properties. The ratio of dialyzate integral extinction in two specified spectral regions could be used as a quantitative parameter, reflecting individual features of plasma composition. It has been revealed that for both groups (HD and CAPD treatment), patients statistically distributed by this parameter according to the normal law.

The research proves that in the spectral range of 240 to 320 nm in dialyzate transmission spectra, there are indications of at least two components. One of them is most likely uric acid. The other is not biochemically identified, but there are indirect evidences that it is one of the purines or pyrimidine derivatives, presumably adenosine. In any case, individual features of plasma need to be taken into account while conducting peritoneal or hemodialysis.

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