Chapter (17)

Optical Monitoring of Dialysis Dose

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CHAPTER OUTLINES

- Background of optical dialysis dose monitoring
- Overview of optical principles in spectroscopy and biofluid optics
- Dialysis dose monitoring utilizing optical techniques
- Clinical parameters from optical dialysis dose monitoring
- Optical monitoring of uremic toxins beyond urea
- Future directions in optical monitoring of dialysis dose
- Conclusion

CHAPTER OBJECTIVES

- Describe the basic principles in spectroscopy and biofluid optics
- Present and compare optical methods for dialysis dose monitoring
- Describe how to calculate dialysis dose from UV absorbance measurements
- Describe how to calculate solute removal and nutrition parameters from UV-absorbance measurements
- Describe future directions in optical monitoring of dialysis dose

KEY TERMS

- Dialysis dose
- On-line monitoring
- Optics
- UV absorbance
- Near infrared spectroscopy (NIRS)
- Solute removal
- Nutrition assessment

ABSTRACT

Utilizing optics in hemodialysis estimating quality parameters for dialysis dose has been developed during the last ten years. In principle, two optical techniques have made progress toward clinical use, namely the ultraviolet (UV) absorbance- and the near infrared (NIR) techniques. Both methods have shown reliable results of estimating urea in the spent dialysate resulting in the possibility to calculate urea-based quality parameters of dialysis dose in terms of Kt/V and URR. Even nutrition parameters derived from optical urea estimations has been provided to be possible using the UV absorbance method. The UV absorbance method cannot measure urea

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as a single solute; instead the high correlation between urea concentration and UV absorbance in spent dialysate is utilized when estimating urea parameters. The NIR-method can measure urea directly using signal processing of the raw NIR spectra. Predicted urea concentrations from the NIR measurement show an excellent agreement to urea concentrations measured by the standard chemical assays. The UV-method has recently been commercialized as a monitoring tool for dialysis dose in terms of the urea-based parameters, Kt/V and URR. On-going research is now focusing to monitor even other waste solutes than urea in spent dialysate. This aims to move towards a more comprehensive picture of the dialysis clearance process that is strongly linked to morbidity and survival of the dialysis patients compared with current dialysis dose calculations.

17.1 BACKGROUND OF OPTICAL DIALYSIS DOSE MONITORING

Optical methods in hemodialysis dose monitoring have a young history, started for approximately thirty years ago with the development of the high performance liquid chromatography (HPLC) technique, which utilizes Ultraviolet/Visible (UV/Vis) spectroscopic data for analysis (Gordon et al. 1975; Asaba et al. 1979; Schoots et al. 1985; Grof and Menyhart 1982). HPLC were utilized for molecule separation and identification of contents in plasma, urine and also spent dialysate (Brunner and Mann, 1984; Mabuchi and Nakahashi 1987, Vanholder et al. 1992, 1994). The "era of uremic toxins search" was born.

Utilizing standard laboratory photometers as a measure of solute removal during hemodialysis was introduced by Boda and coworkers (Boda et al. 1977). They demonstrated an exponential decrease of UV absorption in spent dialysate at 210 nm. With the introduction of light-fiber optics in the mid-1980s and the monochromator-detector developments in early-1990s, Near Infrared spectroscopy (NIRS) became more powerful for scientific research. Still, one had to wait until the end of the 20th century, when both UV- and NIRS- techniques took steps forward in development as tools applied for hemodialysis dose monitoring.

Aside dialysate based measurements, early attempts by optical disposable blood ultrafiltrate based sensors to measure urea content in blood entering the dialyzer were made (Lindsay et al. 1993), (Smirthwaite et al. 1993). The sensors drew periodic ultrafiltrate samples across permeable capillaries through which arterial line blood flows. A urea reading was then derived from an optical density measurement of the plasma sample

after mixing with the reagents. Some small clinical studies to validate the monitors were also carried out (Lindsay et al. 1993; Smirthwaite et al. 1993). Typical disadvantages were related to blood handling (e.g. sterility), need for disposables, etc. Today, the most promising and clinically appropriate optical sensors monitoring dialysis dose are measuring elimination of uremic solutes in the spent dialysate. For this reason, this chapter focuses mostly on this kind of optical systems.

17.2 OVERVIEW OF OPTICAL PRINCIPLES IN SPECTROSCOPY

Optical dialysis monitoring techniques utilize mostly phenomena described by the optics of biological fluids. The term biological fluids covers all kind of fluids produced by living organisms like blood, lymph, saliva, mucus, gastric juice, urine, aqueous humour, semen etc. Also spent dialysate can be classified as a biological fluid derived by filtering the compounds usually less in size than 50 000 Dalton from blood through a dialyzer membrane into the pure dialysate containing water and electrolytes.

From the viewpoint of optics, biological tissues and fluids can be divided into two large classes: (1) strongly scattering (opaque) tissues and fluids, such as skin, brain, vessel walls, eye sclera, blood, milk and lymph, and (2) weakly scattering (transparent) tissues and fluids such as cornea, crystalline lens, vitreous humour, aqueous humour of the front chamber of the eye (Tuchin 2000) and spent dialysate. For the second class, consisting of weakly scattering tissues and fluids, the Beer-Lambert law is often applicable (Regan and Parrish 1982).

In this section we will present a short description of the electromagnetic spectrum, some basic principles about photon propagation in biological fluids, Beer-Lambert law, examples how this could be utilized as a measurement and further most important components in an Ultra Violet (UV), Visible (Vis), Near Infrared (NIR) spectrophotometer.

17.2.1 Electromagnetic Radiation

Generally, electromagnetic (EM) radiation is classified by wavelength into radio wave, microwave, infrared, the visible region we perceive as light, ultraviolet, X-rays and gamma rays. The behaviour of EM radiation depends on its wavelength. Fig. 17.1 shows an illustration of the EM spectrum range. Infra-Red (IR) is per definition EM radiation with wavelength range between 760 nm-0.5 mm, Vis 390-770 nm and UV 100-400 nm. The

EM spectrum of UV light can be subdivided in a number of ways. The draft ISO standard on determining solar irradiances (ISO-DIS-21348; Van de Hulst 1980) describes the following ranges relevant to dialysis optical monitoring:

- Ultraviolet A, long wave (UVA) 400 nm–315 nm;
- Ultraviolet B or medium wave (UVB) 315 nm–280 nm;
- Ultraviolet C, short wave (UVC) 280 nm–100 nm.

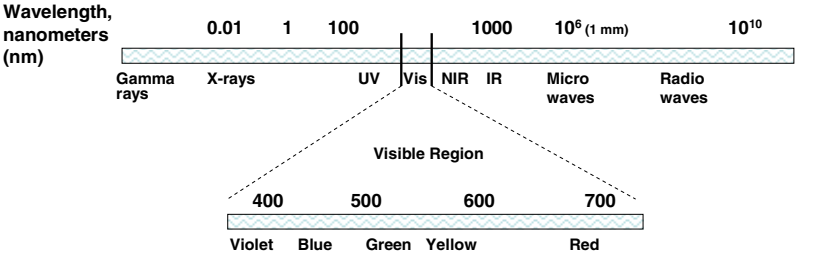


Fig. 17.1 Illustration of the electromagnetic spectrum range

The infrared (IR) part of the electromagnetic spectrum covers the range from roughly 300 GHz (1 mm) to 400 THz (750 nm). It can be divided into three parts:

- Far-infrared, from 300 GHz (1 mm) to 30 THz (10 μm). The lower part of this range may also be called microwaves. This radiation is typically absorbed by so-called rotational modes in gas-phase molecules, by molecular motions in liquids, and by phonons in solids.
- Mid-infrared, from 30 to 120 THz (10 to 2.5 μm). Hot objects (black-body radiators) can radiate strongly in this range. It is absorbed by molecular vibrations, where the different atoms in a molecule vibrate around their equilibrium positions. This range is sometimes called the fingerprint region since the mid-infrared absorption spectrum of a compound is very specific for that compound.
- Near-infrared, from 120 to 400 THz (750 nm to 2500 nm). Physical processes that are relevant for this range are similar to those for visible light.

In the context of NIR spectroscopy, wavelength is measured in "wave numbers", which have the units cm^{-1}, wave number = 1 / wavelength in centimeters.

"Light" is usually defined as visible EM radiation of the entire EM spectrum where the human eye is sensitive (Sliney and Wolbarsht 1980; Judy 1995; Saleh and Teich 1991; Welch and van Gemert 1995). But the term light is often extended to adjacent wavelength ranges that the eye cannot detect (Welch and van Gemert 1995). Spectroscopy can detect a much wider region of the EM spectrum than the visible range. A common laboratory spectrophotometer can detect wavelengths from 200 nm to 2500 nm. Detailed information about the physical properties of objects e.g. gases and fluids can be obtained from this type of device.

17.2.2 Photon Propagation in Biological Fluids

Interaction between light/EM radiation and molecules in e.g. a biological fluid result in different phenomenon that could be utilized during measurements to identify molecules that are solved in the fluid and even possibilities to estimate the concentration. In this sub-section, some basic parts of those phenomenons will be described.

In principle, photons emerged from a source and propagating into a medium can be (see Fig. 17.2):

1) On the surface of the medium (van de Hulst 1980; Ishimaru 1991; Saleh and Teich 1991):
    - Reflected off the medium.
    - Penetrated (transmitted) into the medium.

2) Inside the medium:
    - Absorbed by the medium.
    - Scattered by the medium.
    - Internally reflected, becoming once again the subject of absorption.
    - Scattered in the medium (Welch et al. 1995).

3) Emerged from the medium:
    - Transmitted with no effect, unscattered (primary or direct) transmission (van de Hulst 1980).
    - Transmitted after attenuation (absorption and scattering) in the medium, diffuse transmission (van de Hulst 1980; Welch and van Gemert 1995).

- Reflected from the medium (backscattered after scattering in the medium), diffuse reflection (van de Hulst 1980).

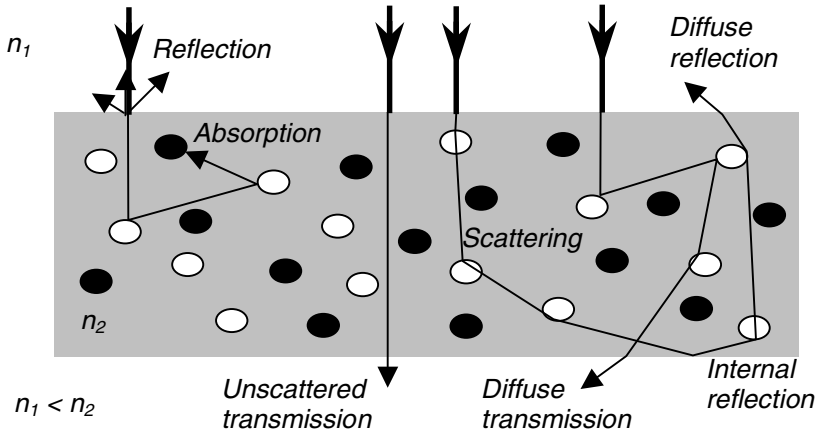


Fig. 17.2 Schematics of photon propagation into, inside and from a medium; ●- absorber, o – scatterer, n_1 and n_2 are the refractive indices of two different media

In tenuous media, like spent dialysate, all the incident light is either absorbed, transmitted or reflected, and scattering from other regions of the medium can be ignored (Welch et al. 1995). For this reason we will consider only the parameters absorbance, transmittance and neglect scattering in the following description. This is the reason why absorption spectroscopy in the spent dialysate can be successfully applied for dialysis monitoring. For the tense medium, like blood, scattering should be taken into account.

17.2.2.1 Photon Propagation into a Medium

At the boundary between two media of different refractive indices incident light is split into two – a refracted (or transmitted) and a reflected portion (Saleh and Teich 1991).

A. Refraction

Refraction occurs whenever a beam of light passes from one transmitting medium to another having a different refraction index (Sliney and Wolbarsht 1980; Saleh and Teich 1991). The index of refraction, n, of a

transparent medium is a direct measure of its optical density and is equal to the ratio of the speed of light in a vacuum, c, to the speed of light in the medium, v:

$$n = \frac{c}{v} \tag{17.1}$$

Refractive indexes are usually equal to or greater than 1: for air $n \approx 1$, for water $n \approx 1.33$ and for tissue, $n \approx 1.44$ (can vary from 1.33 to about 1.55) (Bolin et al. 1989; Duck 1990; Welch et al. 1995; Tuchin 2000).

Snell's law or the law of refraction relates the angle of incidence θ_1 (angle between the incident ray and the normal) to the angle of refraction θ_2 (angle between the refracted ray and the normal):

$$n_1 \sin\theta_1 = n_2 \sin\theta_2 \tag{17.2}$$

From Snell's law it can be derived that in order to minimize refraction, the angle of incidence θ_1 should be as close as possible to 0 degree in which case $\sin 0° = 0$. This is the reason why collimated beams perpendicular to any kind of surfaces in the optical (focusing, filtering) systems, aimed to guide the light through, are utilised in the spectrophotometers helping to minimize loss of light from the source.

B. Reflection at the Surface

A portion of the photons is reflected at the surface due to the difference in the index of refraction of the two media and the angle of incidence of the photons. The measure of the fraction of light that is reflected by a material is called its reflectance. Specular reflection (mirror-like) is the reflection in which incident parallel rays remain parallel after reflection and the angle of reflection equals the angle of incidence. Specular reflection occurs when the size of the surface irregularities is less than the wavelength of the incident radiation (Sliney and Wolbarsht 1980).

In the case of unpolarised light, the specular reflectance R_s, is given by Fresnel's formula (Welch et al. 1995).

$$R_s = \frac{1}{2}\left(\frac{\tan^2(\theta_1 - \theta_2)}{\tan^2(\theta_1 + \theta_2)} + \frac{\sin^2(\theta_1 - \theta_2)}{\sin^2(\theta_1 + \theta_2)}\right) \tag{17.3}$$

Diffuse surface reflection occurs when the surface irregularities are randomly oriented and are much greater than the wavelength of the incident

radiation (Sliney and Wolbarsht 1980). Diffuse surface reflection gives the reflected rays that are scattered in a random fashion and are not parallel (Ryer 1998). The isotropic diffuse surface reflectance follows Lambert's law stated by Lambert in 1760 (Regan and Parrish 1982) which is also known as the Cosine law of Reflection (Sliney and Wolbarsht 1980; Regan and Parrish 1982; Born and Wolf 1975; Ishimaru 1978).

$$I = \frac{P\rho\cos\theta}{\pi d^2} \tag{17.4}$$

Where I is the reflected irradiance from the surface, P is collimated beam power upon the surface, θ is the angle between the incident ray and the surface's normal, ρ is the diffuse reflection coefficient for the given wavelength and d is the distance from the diffuse reflection spot on the surface to the detector (Sliney and Wolbarsht 1980).

It is important to notice that diffuse and specular reflections at the surface are highly wavelength dependent. Many reflections are a combination of both components. One manifestation of this is a spread reflection, which has a dominant directional component that is partially diffused by surface irregularities (Ryer 1998) (see Fig. 17.3).

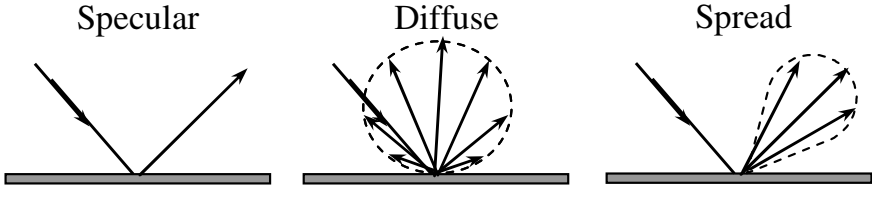


Fig. 17.3 Specular, diffuse and spread reflection from a surface

C. Transmission

The measure of the fraction of light that is penetrated (transmitted) into a medium is called transmission, T (Saleh and Teich 1991). For a unit of irradiance the light transmitted into a medium is (Welch et al. 1995).

$$T = 1 - R \tag{17.5}$$

where R is the reflectance.

17.2.2.2 Photon Propagation Inside a Medium

Photons that propagate inside a medium can be absorbed by the molecules in the sample.

A. Absorption

During absorption spectroscopy an incident photon can be absorbed by a molecule and then the photon energy is converted into an excitation of that molecule's electron cloud. This type of interaction is sensitive to the internal structure of the molecule, since the laws of quantum mechanics only allow for the existence of a limited number of excited states of the electron cloud of any given chemical species. Each of these excited states has a defined energy; the absorption of the photon has to bridge the energy gap between the ground state (lowest energy state) and an allowed excited state of the electron cloud. Molecules can therefore be identified by their absorption spectrum: Their wavelength-dependent capacity for absorbing photons depends on the energy spacing of the states of their electron cloud. If the frequency of the radiation matches the vibration frequency of the molecule then radiation will be absorbed, causing a change in the amplitude of molecular vibration. Molecules which strongly absorb Vis light, appear coloured to the human eye and are therefore called, chromophores, i.e. "carriers of colour."

B. Fluorescence

In absorption, the signal of molecules in a sample is direct but it can be done with higher sensitivity by using an indirect approach, fluorescence detection. Then the ingoing light will give the absorbing molecules excited states of their electron cloud (higher energy). From this state the molecule can shift, “relax”, to the electronic ground state by transforming the excess energy into an outgoing emitted light having longer wavelengths than the ingoing light. Different molecules have different emitted spectrum which is utilized during the fluorescence measurements (Lakowicz 2006).

17.2.2.3 Photon Propagation from a Medium

Photons can also pass through the sample and the transmitted photons can be measured.

A. Transmittance

A part of photons can pass the medium without interacting with it at all. Those photons are transmitted through the medium and are sources to

measure a quantity called transmittance (Saleh and Teich 1991). Transmittance in spectroscopy is defined mathematically later in this chapter.

B. Spectrophotometer

The basic parts of a spectrophotometer are a light/radiation source, a sample holder of transparent material e.g. cuvette of glass, a monochromator or diffraction grating ("wavelengths selector"), a photo/radiation detector and finally a signal processor. Fig. 17.4 shows a UV-Vis-NIR spectrophotometer schematically including the basic components. The radiation sources are often a tungsten filament (300-2500 nm) or a halogen lamp and a deuterium lamp, which has continuous radiation intensity over the UV region (190-400 nm). Recently, light emitting diodes (LED) and Xenon Arc Lamps are introduced (Skoog et al. 2007). The detector is typically a photodiode or a charge-coupled device (CCD). Photodiodes are used with monochromators, which filter the EM so that only EM of a single wavelength reaches the detector. Diffraction gratings ("beam splitter") are used with CCDs, which collects light of different wavelengths on different pixels.

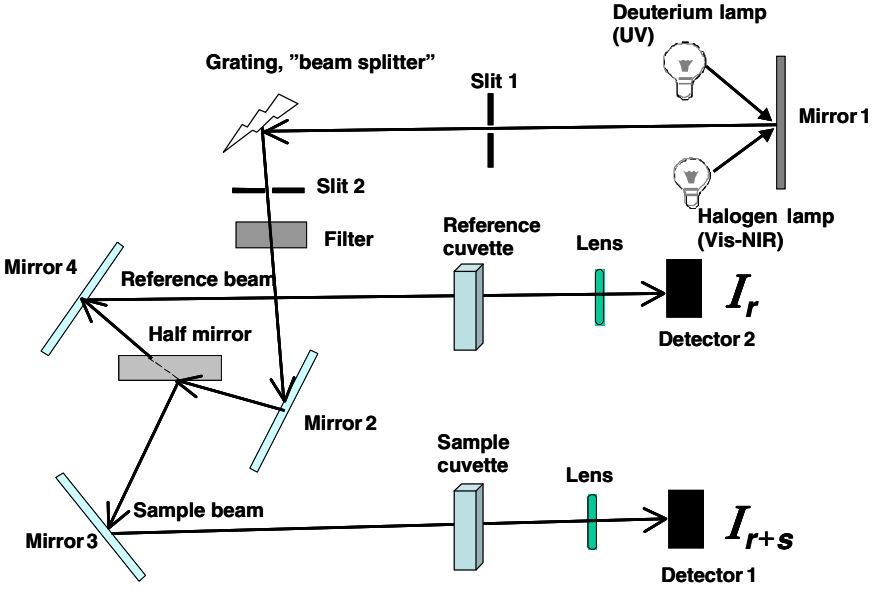


Fig. 17.4 Schematically illustration of components in a double beam UV/Vis/NIR spectrophotometer

A spectrophotometer can be either single beam or double beam. In a single beam instrument, all of the light passes through the sample cell. The ingoing light (I_o) must be measured by removing the sample. In a double-beam instrument, the light is split into two beams by a half mirror before it reaches the sample (see Fig. 17.4). One beam is used as the reference; the other beam passes through the sample. Some double-beam instruments have two detectors (photodiodes), and the sample and reference beam are measured at the same time.

Samples for UV/Vis/NIR spectrophotometry are most often liquids, although the absorbance of gases and even of solids can also be measured. Samples are typically placed in a transparent cell, a cuvette. Cuvettes are often rectangular in shape, commonly with an internal width (path length, l) of 1 cm (see Fig. 17.5). The type of sample container used must allow radiation to pass over the spectral region of interest. The most widely applicable cuvettes are made of high quality fused silica or quartz glass because these are transparent throughout the UV, Vis and NIR regions. Glass and plastic cuvettes are also common, although glass and most plastics absorb in the UV, which limits their usefulness in this region (Skoog et al. 2007). A complete spectrum of the absorption at all wavelengths of interest can often be produced directly by a more sophisticated spectrophotometer. In a spectrophotometer different wavelengths can be selected as single ones or scanned over an optical wavelength range.

Instrumentation for NIR spectroscopy is partially similar to instruments. There is a source, a detector, and a dispersive element (such as a prism, or, more common, a diffraction grating) to allow the intensity at different wavelengths to be recorded. Fourier transform (FT) NIR instruments using an interferometer are also common, especially for wavelengths above ~1000 nm. Depending on the sample, the spectrum can be measured in either reflection or transmission. Common quartz halogen light bulbs are most often used as broadband sources of NIR radiation for analytical applications. LEDs are also used; they offer greater lifetime and spectral stability and reduced power requirements.

Qualitative and quantitative spectroscopic methods typically require the application of multivariate calibration algorithms and statistical methods (i.e. chemometrics) to model spectral response to chemical or physical properties of the samples used for calibration. The method relies on the spectra-structure correlations existing between a measured spectral responses caused by harmonics of the fundamental vibrations occurring at infrared frequencies. These harmonic vibrations occur at unique

frequencies depending upon the quantity of absorber (analyte), type of absorbing molecules present within the sample, and the sample thickness. Quantitative methods are possible where changes in the response of the spectrophotometer are proportional to changes in the concentration of chemical components, or in the physical characteristics (scattering/ absorptive properties) of samples undergoing analysis.

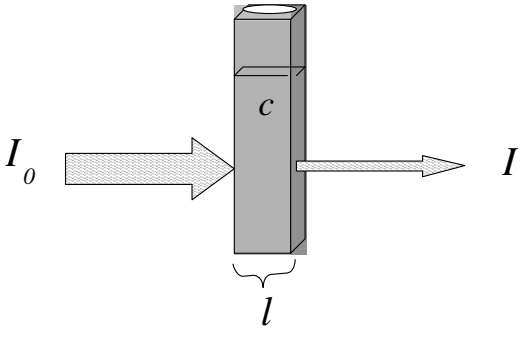


Fig. 17.5 Cuvette with a sample containing an absorber with concentration (c). Ingoing light (I_0) and outgoing light (I) after absorption in the sample when passing through the cuvette with the optical path length, l.

C. Transmittance and Absorbance

The amount of transmitted and absorbed portion of EM radiation in the medium (e.g. in a dialysate sample) can be characterised by the parameters transmittance T, and Absorbance A in the spectrophotometer. In order to utilize EM radiation for measurement of constituents in a fluid, the sample is applied in an optical cuvette (see Fig. 17.5). Through the calibration and measurement procedures one determines the amount of the ingoing light illuminating the sample symbolized by I_0 and the outgoing light I symbolizing the intensity of the light after passing the sample as the remaining ingoing light partly absorbed by the sample (see Fig. 17.5). Having knowledge about those parameters one can determine transmittance T, and Absorbance A as:

Transmittance:
$$T = \frac{I}{I_0} \quad \Rightarrow \quad \%\,T = \frac{I}{I_0} \cdot 100\% \tag{17.6}$$

Absorbance:
$$A = -\log T = -\log \frac{I}{I_0} = \log \frac{I_0}{I} \tag{17.7}$$

Example 17.1

For the following given transmittances, determine the corresponding absorbance

a) T = 1.00 (100 %T)
b) T = 0.10 (10 %T)
c) T = 0.001 (0.1 %T)

Solution

The absorbance can be calculated according to Eq. (17.7) as follows:

a) $A = -\log 1 = 0.00$
b) $A = -\log 0.1 = 1.00$
c) $A = -\log 0.001 = 3.00$

The intensity often has the units of W/m^2 (Tuchin 2000; Sliney and Wolbarsht 1980; Saleh 1991) but can also be expressed in W if measured as the radiant power (Mann et al. 1974).

17.2.3 Beer- Lambert Law

The law describing the amount of incident radiation, absorbed by a homogeneous medium placed in a collimated beam of radiation, was first developed in 1729 by a priest at Le Havre, Pierre Bouguer, and later elaborated by Johann Lambert in 1760 (Regan and Parrish 1982). They demonstrated that the proportion of radiation, absorbed by a homogeneous medium, is independent of the intensity of the radiation. In 1852 August Beer found that the transmission of radiation by a homogeneous medium is dependent only upon the number of molecules through which the radiation travels. He showed that the number of exchanges of energy was directly proportional to the number of absorbing molecules (Regan and Parrish 1982). The derivation and the mathematical expression of both Beer's laws can be found in several sources (Brown 1980; Glasser 1961; Regan and Parrish 1982). The Bouguer-Lambert law may be combined with Beer's law to describe how the fraction of the incident radiation absorbed is related to the concentration and the path length through the solution (Maass 1977; Regan and Parrish 1982; Brown 1980; Björn 1965; Bashford 1987). This law has come to bear the name Beer-Lambert law, or simply Beer's law (Mann et al. 1974).

The Beer- Lambert law states that the absorbance of light intensity is proportional to the concentration of the substance. It means that the amount of e.g. UV-light absorbed when passing through a cuvette

(manufactured by UV-transparent material such as quartz) with spent dialysate is linearly dependent on the concentration c [mol/L] of the absorbing solute, the optical pathlength in (l) [m] (depth of the cuvette) and the extinction coefficient ε [m^{-1} $(mol/L)^{-1}$], even called the molar absorptivity at a certain wavelength (Glasser 1961). If I_0 is the intensity of the incident light and I is the intensity transmitted light through the medium, the absorbance (A), dimensionless, is:

$$A = \log_{10}\left[\frac{I_0}{I}\right] = \varepsilon \cdot c \cdot l \tag{17.8}$$

If ε is known for a substance the absorbance (A) can be calculated by multiplying the path length and the concentration of the substance. If ε is known for a substance and A is obtained from a measurement, it is possible to derive the concentration as:

$$c = \frac{A}{\varepsilon \cdot l} \tag{17.9}$$

In our case, when the spent dialysate contains several different absorbing compounds, the overall extinction coefficient is the linear sum of the contributions of each compound. However all the components are not identified and probably there is interference between different substances which make it difficult to separate and determine the concentrations of each solute. Absorbance of a solution, obtained by a double beam spectrophotometer, is given by the Lambert -Beer law as (Hahn et al. 1979; Zwart et al. 1984):

$$A = \log\frac{I_0}{I_{r+s}} - \log\frac{I_0}{I_r} = \log\frac{I_r}{I_{r+s}} \tag{17.10}$$

Where I_0 is the intensity of incident light from the light source I_r is the intensity of transmitted light through the reference solution (e.g. pure dialysate) and I_{r+s} is the summated intensity of transmitted light through the reference solution mixed with the solution (e.g. pure dialysate + waste products from the blood).

The common assumptions to utilise absorbance calculated according to the Beer-Lambert law to determine concentration are:

1) The radiation is monochromatic (Regan and Parrish 1982; Mann et al. 1974; Glasser 1961; Björn 1965).
2) The irradiating beam is parallel (collimated) across the sample (Regan and Parrish 1982; Glasser 1961).
3) The absorption of radiation for a given species is independent of that of other species (Regan and Parrish 1982; Glasser 1961).

4) Only the non-scattered and not-absorbed photons are detected from the medium (Welch and Gemert 1995)
5) The incident radiation and the concentration of the chromophores are not extremely high (Mann et al. 1974; Togawa et al. 1997).

The Beer-Lambert law for monochromatic light can be derived solving a differential equation for a solution with a finite depth containing chromophores and is given in detail in many sources (Mann et al. 1974; Regan and Parrish 1982; Sliney and Wolbarsht 1980). Analysis of a mixture is based fundamentally on the fact that the absorptions, at each wavelength, of separate components in the mixture are additive, provided that chemical or interfering physical reactions between the components do not occur (Glasser 1961), and the solute concentrations are not very high (not usually found in biological media). In this case, in a medium containing n different absorbing compounds with the concentrations of $c_1 ... c_n$ [mol/L] and the extinction coefficients of $\varepsilon_1 ... \varepsilon_n$ [m^{-1} $(mol/L)^{-1}$], the overall extinction coefficient is simply the linear sum of the contributions of each compound:

$$A = \log_{10}\left[\frac{I_0}{I}\right] = \left(\varepsilon_1 c_1 + \varepsilon_2 c_2 + ... + \varepsilon_n c_n\right) l \qquad (17.11)$$

17.3 DIALYSIS DOSE MONITORING UTILIZING OPTICAL TECHNIQUES

Several solutes in spent dialysate identified as uremic toxins by Prof Vanholder and colleagues in the European Uraemic Toxin (EUTox) Work group have been measured utilizing UV absorbance in HPLC technique (Vanholder et al. 2008, 2004, 2003a, b). Their research has been followed with great interest for fronting the question: Could some of these identified molecules be measured with optical techniques on-line? At the present two optical techniques for dialysis dose monitoring have been investigated in several studies, UV absorbance and NIRS. Other approaches have also been investigated but more limited so far, e.g. utilizing the Vis region for measurement. In this chapter, the UV absorbance and the NIRS technique will be discussed that up to day have been developed further and have presented several promising results as potential monitoring tools for hemodialysis.

17.3.1 UV – Spectroscopy

The first available publication about how UV-transmittance of the spent dialysate has been measured at 254 nm, believed to be the best wavelength to monitor the efficacy of hemodialysis, was written by an Hungarian group (Gal and Grof 1980). Also, there was concluded that the hardly diffusible constituents had a higher elimination rate compared to the removal rate of small, more readily diffusible components. Many of those things have shown to be different according by the knowledge we have today. Almost two decades later, a work aiming to monitor the dialysis liquid during hemodialysis by UV absorbance, was presented by Vasilevski et al from St:Petersburg (Vasilevski and Kornilov 1999). This group discussed also UV extinction as an indicator of nucleic acid metabolism (Vasilevsky and Konoplyov 2005).

In a very short time, in 2001, independent studies from abovementioned about on-line monitoring of solutes in dialysate using UV absorbance were published (Fridolin et al. 2001; Fridolin et al. 2002), investigating possibility to monitor removal of different uremic solutes in the spent dialysate, and firstly describing how to estimate urea Kt/V by UV absorbance, as an illustrative example. The first clinical study, connecting the UV-technology with a real clinical application - with dialysis dose calculated as Kt/V using UV absorbance - was reported by Uhlin et al (2003). Apparently, since this, there was no clinical study published, where dialysis dose calculated as Kt/V or URR using UV absorbance had been presented. A fruitful and exiting collaboration between Uhlin and Fridolin et al. within the field of optical UV absorbance dialysis dose monitoring has been followed. In connection with commercialisation of the UV-technology for dialysis dose monitoring during last couple of years, new interest has arisen towards the field. Recently, some works related to spectroscopical analysis of uremic substances in dialysate are presented by the groups from Japan (Umimoto et al. 2007a, b; Umimoto et al. 2009), and from St:Petersburg (Vasilevski and Konoplev 2009). The clinical validation of a clinical prototype device (Luman et al. 2009a), and the commercially available UV absorbance dialysis dose monitor have been also presented recently (Castellarnau et al. 2010).

17.3.1.1 Clinical Setting during UV Absorbance Monitoring

During the on-line clinical experiments using UV absorbance a commercial available double beam spectrophotometer was connected to the fluid outlet of the dialysis machine and ended in a tank equipped with a

scale were all spent dialysate was collected (see Fig. 17.6). Total Dialysate Collection (TDC), in the tank (see Fig. 17.6), started when the blood filled the dialyser and ended when the blood was returned to the patient (after 3-5 hours) at the end of the dialysis. After its weight was recorded and the collected spent dialysate was carefully stirred, a TDC sample (D_{total}) sample location 2 (see Fig. 17.6), was sent to the laboratory, and urea concentration (mmol/L) was measured. Urea concentrations in the dialysate (D_{urea}) later used for correlation analyses and mathematically transformations, were also determined at scheduled times during dialysis: at 5, 15, 30, 60, 90, 120, 180 and 240 min (also at 270 and 300 min, if the treatment was longer than 240 min) from samples taken from the dialysate drain tube, sample location 1 (see Fig. 17.6).

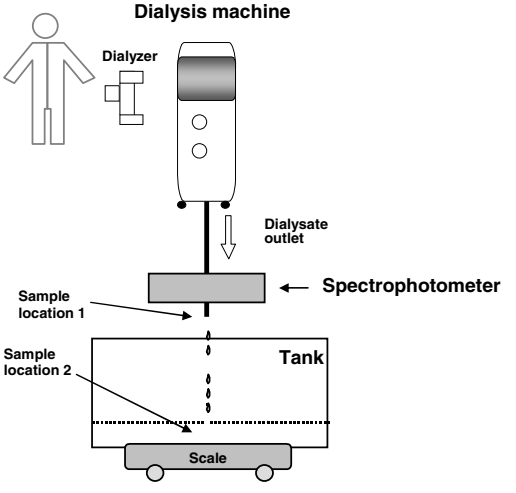


Fig. 17.6 Schematical set up during the clinical UV absorbance experiments. Sample location 1 = Drain sample (flowing dialysate) and sample location 2 = Tank sample (accumulated dialysate)

An optical flow cuvette with a path length of 10 mm, Fig. 17.7a, was connected to the drain tube and placed at the sample holder in the spectrophotometer (see Fig. 17.7b); the reference holder was left empty during on-line measurements.

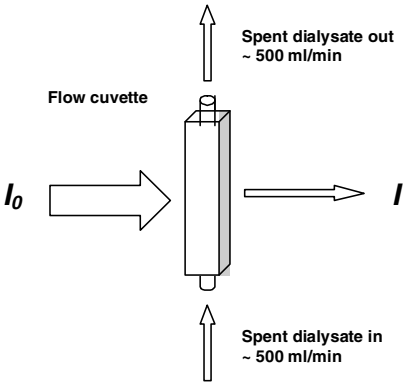


Fig. 17.7a The optical flow cuvette schematically.

Fig. 17.7b Photo of the optical flow cuvette inserted in an UV/Vis/NIR spectrophotometer where drain tube is connected. A window for the light beam is also seen on the black wall behind the cuvette.

Before start of study treatment, UV absorbance was measured on pure dialysate from the dialysis machine and applied as a zero baseline for the reference.

17.3.1.2 Visualization of the Clearance Process with UV Absorbance

During the experiments, one single wavelength was selected during the entire dialysis treatment. The obtained UV absorbance curve from on-line measurements of spent dialysate, Fig. 17.8, was used to estimate urea

based clinical parameters such as the dialysis dose (Kt/V) and total removal of urea (TRU), which will be described later in this chapter. UV absorbance drops and peaks, which normally occurred at predestined intervals during the dialysis, correspond to self-test of the dialysis machine. The drops and peaks corresponds to periodical self-tests of the dialysis machine when dialysate is set in by pass mode and no fresh dialysis fluid is pumped through the dialyzer.

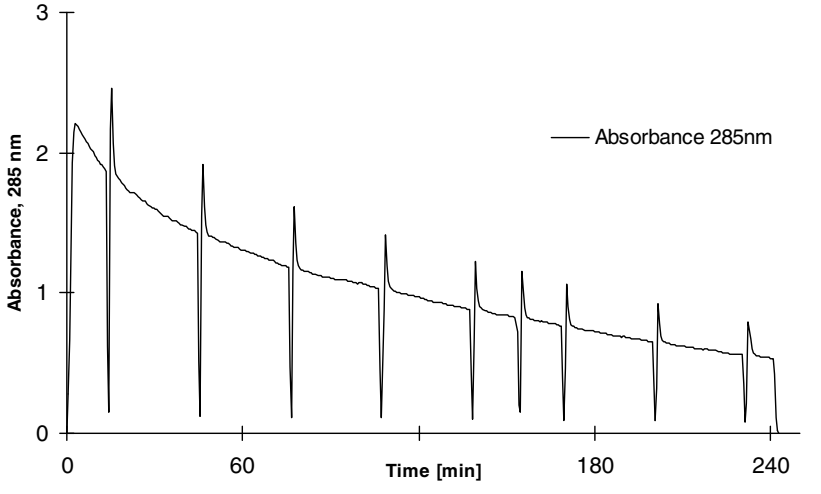
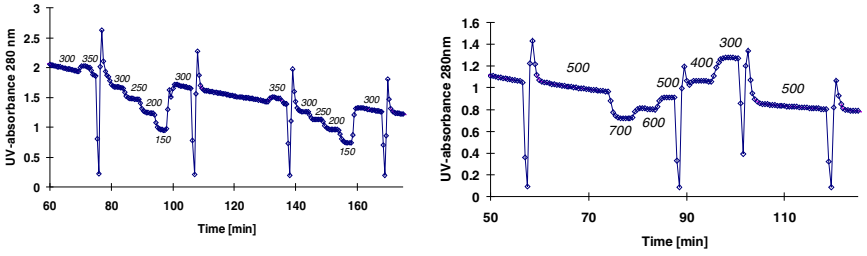


Fig. 17.8 A typical on-line UV absorbance curve during a single hemodialysis treatment where UV absorbance, at the wavelength 285 nm, is plotted against time

When utilizing the UV absorbance technique, unique information about the solute clearance process could be visualized in real time (Uhlin et al. 2006a, 2006b). Variations in clearance due to e.g. alarm situations and problems with blood access can be noticed and effects after interventions in order to increase clearance can be evaluated. Figures 17.9a and 17.9b show the result of changes in UV absorbance when manipulating the blood- and dialysate flow respectively.



(a) (b)

Fig. 17.9 Response to manipulations during two dialysis treatments; (a) changes in blood flow, and (b) changes in dialysate flow. The changes lasting for 5 min each. The blood- and dialysate flow rate in mL/min is presented in the figure. [Reprinted with permission from Uhlin et al. 2006a]

Figure 17.10 shows clinical situations and the effect visualized in changes in UV absorbance, the effect of interventions (adjustment of needle position) to increase the blood flow in the artery needle (Uhlin et al. 2006a).

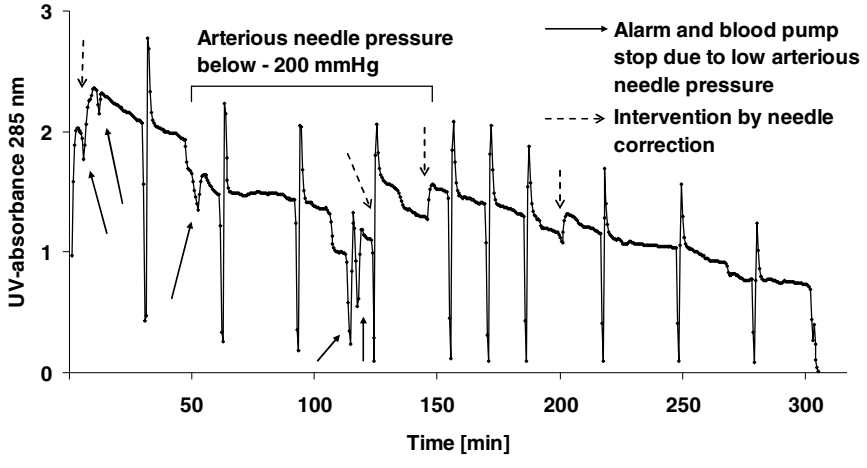


Fig. 17.10 A problematic treatment, where the main problem was bad flow in the artery needle. The blood pump flow was set to 300 ml/min throughout the treatment

This restricted flow resulted in several interruptions and alarms and an abnormal curve shape can be visualized (compare to Fig. 17.8). This treatment had a low reduction ratio of waste solutes when blood samples pre-and post-dialyses were analyzed, compared to a non-problematic treatment of the patient.

17.3.1.3 UV-Spectra of the Spent Dialysate and UV Chromophores in the Uremic Fluids

To detect the UV absorbance spectra of spent dialysate, samples were taken from the drain tube of the dialysis machine at different times during treatment (sample location 1, see Fig. 17.6). It is known that during the first 60 minutes the removal rate of water soluble solutes is highest compared to later period in the dialysis treatment resulting from so called double-pool behaviour of the solute removal during dialysis. Therefore the samples were taken more frequent in the beginning of treatments. Figure 17.11 shows an UV absorbance spectrum where samples taken at different times during a treatment are scanned over the entire UV range.

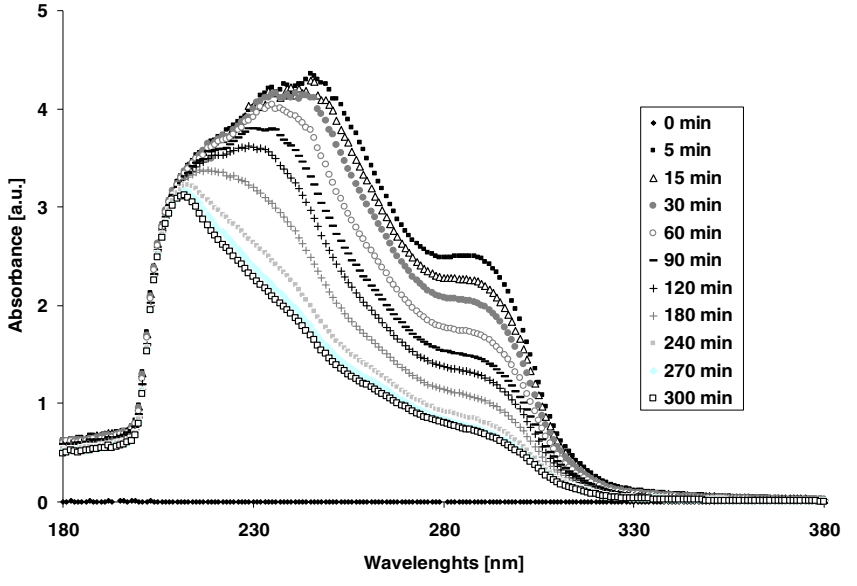


Fig. 17.11 Scanning of collected spent dialysate taken at different times during one dialysis session over the UV range when using pure dialysate as a reference fluid

As shown the UV absorbance is highest for the samples taken early in treatment. For reference solution, pure dialysate was used taken before the start of the dialysis treatment, marked as 0 min in Fig. 17.11 (absorbance ≈ 0). In spent dialysate the absorbance in the upper half of the UV region (260-330 nm) is easily to measure due to a suitable dynamic range of the absorbance signal. But in the lower half of the UV-range

(approx. 200- 250 nm) the absorbance value could be often too high with the optical path length of 10 mm.

A number of the higher prevalent peaks on the HPLC profiles of the uremic serum from the earlier HPLC studies indicate that there exists a group of compounds – UV chromophores – which are the main cause of the cumulative and integrated UV absorbance (Schoots et al. 1989; Vanholder et al. 1992). Recent HPLC studies investigated the uremia related UV absorbance profiles of the serum and the spent dialysate and removal of uremic retention solutes in connection with optical dialysis adequacy monitoring (Lauri et al. 2006; Lauri et al. 2010a, b). The chromatographic peaks were detected by a UV detector at wavelengths of 254 and 280 nm. Those studies indicated that the main solute responsible for the UV absorbance in the spent dialysate is a low-molecular-weight water-soluble non-protein bound compound uric acid (UA) (see Fig. 17.12). Spiking experiments and UV spectra between 200-400 nm allowed recognize predominant uremic toxins in 5 chromatographic peaks identified as creatinine (CR), uric acid (UA), hypoxanthine (HX), indoxyl sulphate (IS), and hippuric acid (HA) (Lauri et al. 2010a, b). Moreover, 2 persistent, but non-identified peaks - peak 1 (P1) and peak 2 (P2) - were detected (Lauri et al. 2010b). The relative contribution from the area of 10 main peaks to the total area of all detected peaks in percentage was about 90 % at 280 nm (Lauri et al. 2006).

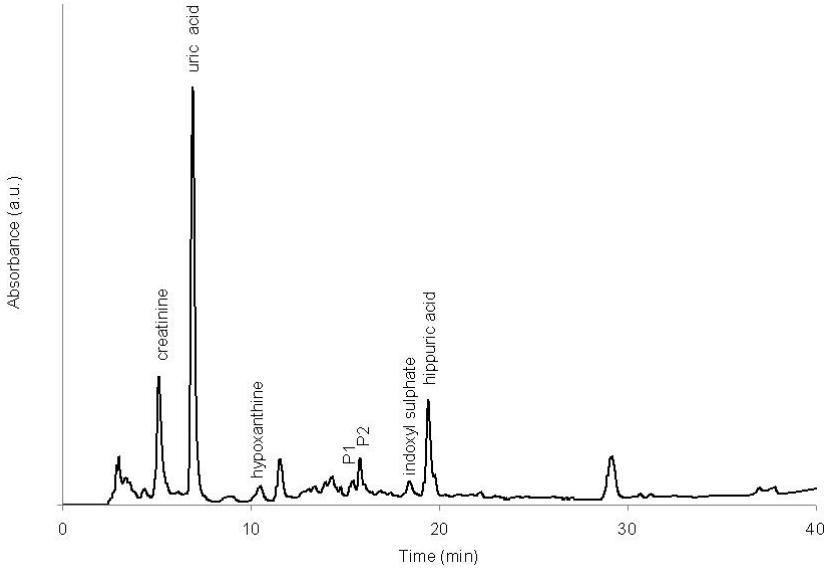


Fig. 17.12 The representative HPLC chromatogram of the serum monitored at the wavelength of 254 nm. Identified peaks are presented.

The number of detected HPLC peaks was not significantly different for a serum filtered through the 3 kDa or 70 kDa cut off filters, and was lower for the spent dialysate, indicating that the MW of the main UV chromophores in the uremic fluids do not exceed 3 kDa (see Fig.17.13).

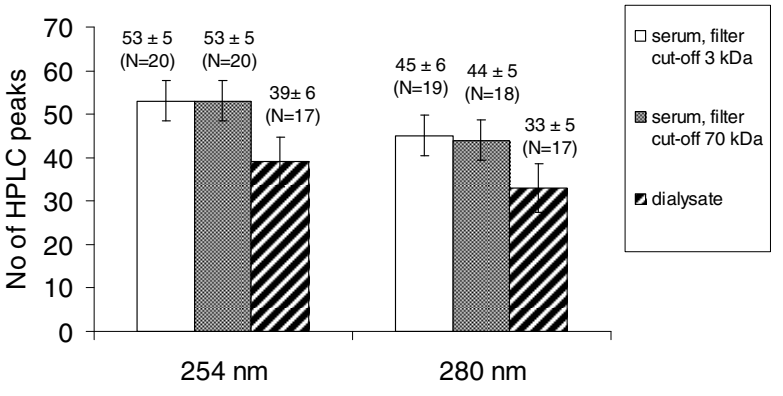


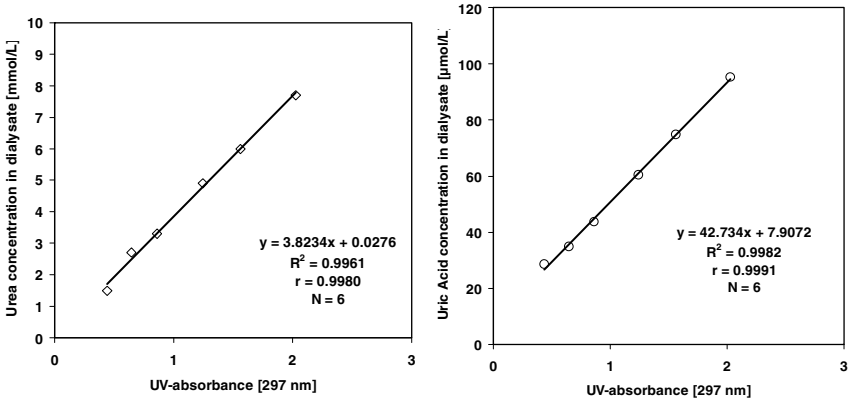
Fig. 17.13 Number of detected HPLC peaks at wavelengths 254 nm and 280 nm in the serum (filter cut-off 3 kDa and 70 kDa, respectively) and in the spent dialysate.

The reduction ratio (RR) estimated by the total area of HPLC peaks at 254 nm and 280 nm in the serum and by the on-line UV absorbance at 280 nm was best related to the removal of small water-soluble non-protein bound solutes like urea, creatinine and UA (Lauri et al. 2010a). This indicates that the UV absorbance is following the behaviour of the UV absorbing compounds – the low-molecular-weight water-soluble non-protein bound uremic toxins, which contribute most to the total UV absorbance in the serum and in the spent dialysate (see Fig. 17.13).

17.3.1.4 UV Absorbance and Uremic Solutes

Known from the HPLC studies, UV absorbance do not measure a single solute. The absorbance signal reflects contribution of several UV absorbing compounds in the spent dialysate, and the strongest influence comes from the low-molecular-weight water-soluble non-protein bound compounds (Lauri et al. 2010a). If a solute concentration is correlated with the total UV absorbance of the solutes then functional calibration

(transformation) models can be constructed for the solute. High correlation between UV absorbance at the wavelengths of 280-320 nm and concentration of some solutes in spent dialysate has been demonstrated such as urea, creatinine and uric acid (Fridolin et al. 2002). The correlations are close to 1.0 in every single treatment and even in several treatments of the same patient. The slope values are different for different solutes; single solutes have different relationships to the UV absorbing solutes that the UV-signal represents. Figures 17.14a and 17.14b show the correlation between UV absorbance and concentrations of urea and uric acid respectively in the same dialysis session.



(a) (b)

Figs. 17.14 The correlation between concentrations of urea (17.14a), uric acid (17.14b) and UV absorbance respectively in a single treatment (the same treatment), r is high, but slope and intercept are different

The correlation is lower when several patients are included in the plot (see Fig. 17.15) due to different concentration of chromophores in the uremic fluids from different patients.

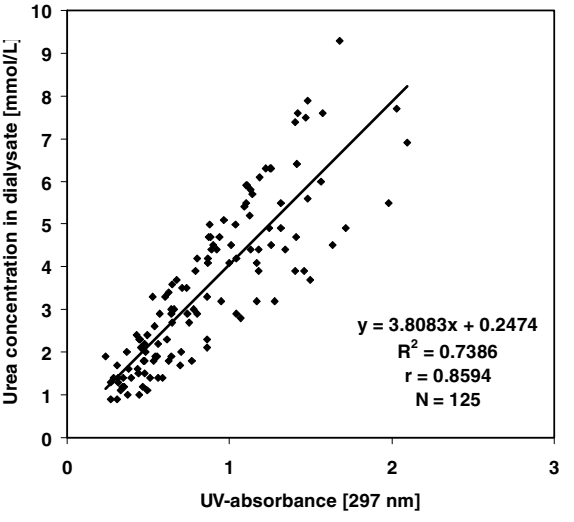


Fig. 17.15 The correlation between urea concentration and UV absorbance in spent dialysate, in a group of 7 patients and 3 treatments each. The same type of dialyzer was used in all sessions

17.3.1.5 Choice of Wavelength for Dialysis Monitoring

Studies of relation between UV and urea indicated that the wavelength range 280 to 320 should be preferred for on-line measurement when small water soluble molecules such as urea should be estimated (Fridolin et al. 2002). At this wavelength range a relatively strong linear relationship exists between the UV absorbance and concentrations of urea, creatinine and uric acid even for the larger patient groups. The wavelengths in this range, which have been mostly used in several studies, are 280 nm, 285 nm and 297 nm (Fridolin et al. 2002; Uhlin et al. 2003; Uhlin et al. 2006a; Jerotskaja et al. 2010a). The reason for this good relationship can be explained by the prevalent contribution of the low-molecular-weight water-soluble non-protein bound compounds to the total UV absorbance (Lauri et al. 2010a, b). Fig. 17.16 shows the sum of individual contribution to the absorbance signal of six waste solutes in the spent dialysate (Fridolin and Lindberg 2003).

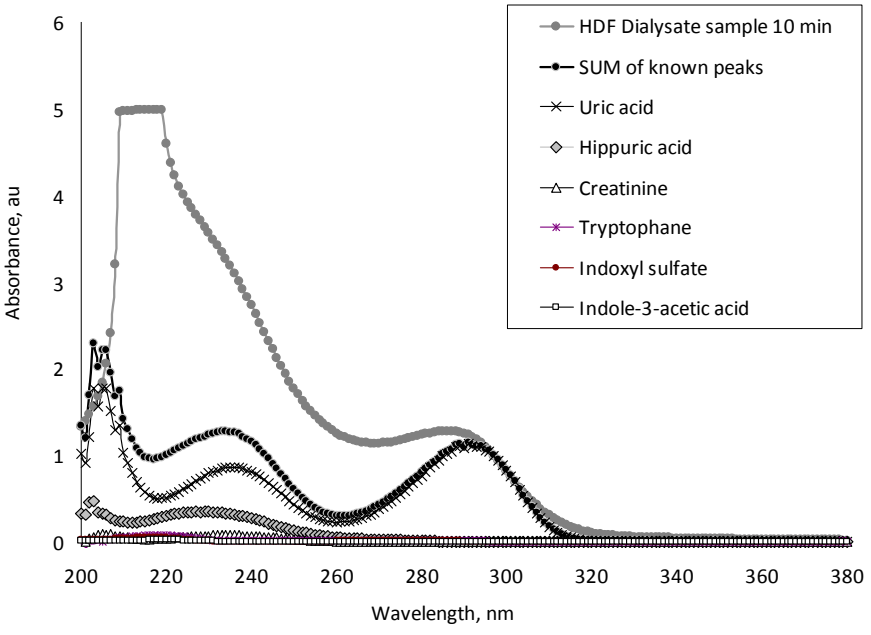


Fig. 17.16 Solutes' individual contribution to the UV-absorbance signal

The remaining part of the total absorbance signal is the sum of other; partly unknown, contributing solutes (see Fig. 17.16). As mentioned earlier, uric acid has the highest contribution to the absorbance signal (see Fig. 17.12) and that urea does not absorb UV light in the chosen wavelength range. Other wavelengths can be also considered depending on particular uremic solutes (Jerotskaja et al. 2007). More advanced approaches, utilising multiple wavelengths to monitor uremic solutes, are shortly described later in this chapter.

17.3.2 Near Infrared Spectroscopy (NIRS)

Another method to measure urea in the spent dialysate using NIR radiation was firstly described in a PhD-thesis by Kupcinskas (2000), followed by the publications by Eddy and Arnold (2001), by Jensen and Bak (2002), and developed further by both last mentioned groups (Eddy et al. 2003; Jensen et al. 2004a; Olesberg et al. 2004; Cho et al. 2008). The method demonstrated to be able to perform online urea concentration measurements in the spent dialysate (Olesberg et al. 2004). No clinical study with dialysis dose calculated as Kt/V or URR using NIR radiation has been published so far.

Infrared energy is the electromagnetic energy of molecular vibration. Absorption of NIR light is based on molecular overtone and combination vibrations when NIR is passing through a sample. As a result, the molar absorptivity in the NIR region is typically quite small. Optical measurements of physiological solutes, often solved in water, utilizing NIR has usually water several orders of magnitude larger absorbance signal than the solute itself. The signal from the solute concentration could be far below the noise level of the measurement system. Despite of this promising attempts have been made to monitor an uremic solute like urea by NIR in the wavelength region of 12 500 – 8600 1/cm (0.80 – 1.16 microm) (Eddy and Arnold 2001), 6730 1/cm (1.486 microm) (Kupcinskas et al. 1998), 5000-4000 1/cm (2.0 – 2.5 microm) (Eddy and Arnold 2001; Olesberg et al. 2004; Cho et al. 2008) and mid IR band - region 1550-1000 1/cm (6.45 – 10.0 microm) (Jensen et al. 2004b). Since mostly the NIR wavelength range has been used, the common name "Near Infrared spectroscopy (NIRS)" was chosen for this approach. To eliminate the interfering factors during NIRS measurements somewhat more advanced technology has to be implemented than the standard double-beam spectrometer utilized during UV absorbance dialysis dose monitoring.

Two kinds of spectrometers have been utilized for the uremic solute determination by NIRS in the spent dialysate: 1) dual-beam interferometer-based spectrometer or so called Fourier transform near-infrared (FT-NIR) spectrometer (Eddy and Arnold 2001; Eddy et al. 2003; Olesberg et al. 2004; Jensen et al. 2004a); and 2) acousto optical tunable filter (AOFT) based spectrometer (Cho et al. 2008).

A FT-NIR spectrometer contents a Michelson interferometer, has high wavelength accuracy and instrumental signal-to-noise ratios. However, the interferometer-based spectrometers are sensitive to environmental parameters, such as temperature and vibrations, they are expensive to manufacture, and difficult to miniaturize (Cho et al. 2008).

An AOFT based spectrometer contents an acousto optical tunable filter, which uses pressure waves to create a periodic modulation of the refractive index in a tellurium dioxide crystal. The periodic index modulation creates a diffraction grating. The period of the grating, which determines the wavelength of light diffracted by the crystal, can be tuned by varying the frequency of the pressure wave (Tran 1992). Because AOTF is less expensive, more compact, and a solid state device that can be scanned fast (in milliseconds) over a wide wavelength range, cheaper and more robust NIR spectroscopic sensors could be constructed. At the same time the root mean square (RMS) noise level is higher for spectra collected with the AOFT spectrometers (Cho et al. 2008).

NIR-spectra consist of generally overlapping vibrational bands that may appear non-specific and poorly resolved. The use of chemometric mathematical data processing and multiple harmonics can be used to calibrate for qualitative or quantitative analysis despite these apparent spectroscopic limitations. In order to convert the optical signal into a solute concentration principal component regression and partial least squares regression analysis is usually carried out to establish a relationship between the measured optical signal and the solute concentration through multivariate calibration models (Eddy and Arnold 2001; Jensen et al. 2004b). For some solutes (phosphate, glucose) the second derivative spectra was utilized (Jensen et al. 2004b). To increase model robustness by excluding unimportant broad variations, that reduces the number of factors required for a multivariate calibration model, Savitzky-Golay smoothing filter was applied to the measured absorbance spectra (Olesberg et al. 2004).

17.3.2.1 Clinical Setting during Monitoring by NIRS

The NIR method includes transmitting a selected band of NIR radiation through the spent dialysate. The instrumentation of the NIR method has varied in different publications and has been designed for a clinical use. The earlier instrumentation based on interferometers was not ideal for non-laboratory measurements due to the sensitiveness of environmental parameters e.g. temperature and vibrations. We will here present the set up from the latest publication of this method (Cho et al. 2008).

During the experiments, spent dialysate was drawn continuously from the outlet dialysate tube after passing the dialyzer. The dialysate was drawn though an optical cell with an optical path length of 1 mm at 30 mL/min by a peristaltic pump. Due to temperature variations are difficult to eliminate entirely in a flow-through system no active temperature stabilization was performed. The temperature was measured just downstream from the optical cell and varied between 28-32 ˚C. Spent dialysate sample were collected at predestinated times at 5, 15, 30, 60, 120, 180 and 240 min after start of dialysis treatment and later sent to laboratory for measurement of concentrations of urea creatinine, glucose and lactate. Spectra were collected using a FT-NIR spectrometer. The best analytic performance correspond top measurements within the combination of the spectral range, which extended from 2.0 to 2.5 μm (5000-4000 cm^{-1}). A tungsten halogen lamp, a beam splitter dividing source into a reference and sample channel, and a two-stage thermoelectrically cooled extended-wavelength photodiode detector. The dialysate sample was contained within a tube of thin-wall Teflon with an

inner diameter of 1.1 mm. NIR spectra was collected by focusing the incident light through the Teflon tubing flow-through cell (Cho et al. 2008).

17.3.2.2 NIR Spectra of the Spent Dialysate and NIR Chromophores in Uremic Fluids

NIR is sensitive for variations of different origin that occur in spent dialysate during hemodialysis. Savitzky–Golay filtering is usually used for data smoothing e.g. to remove baseline variations and also high-pass filtering is used to remove the broad spectral features attributable to temperature variations while preserving the higher-frequency analyte signal. This smoothed baseline is then subtracted from the original spectrum; leaving only the high-frequency components (see Fig. 17.17)

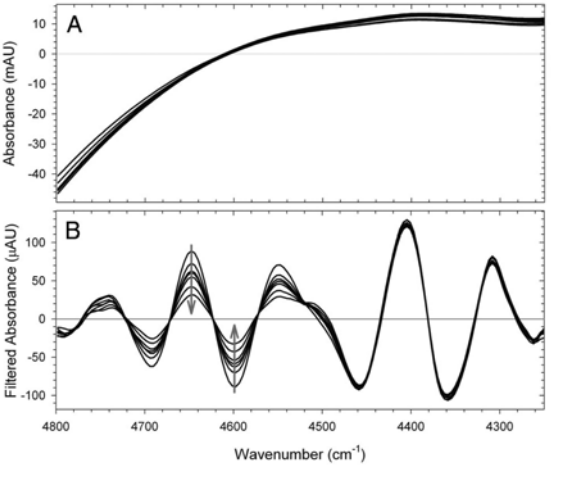


Fig. 17.17 Online absorbance spectra of spent dialysate recorded during a dialysis treatment. (A), unfiltered spectra rationed to water; (B), spectra after the application of a second-order Savitzky–Golay filter of width 200 cm^{-1}. The features between 4700 and 4500 cm^{-1} are primarily attributable to urea, whereas those between 4500 and 4300 cm^{-1} are primarily attributable to glucose. The arrows in B indicate the direction of change with time. [Reprinted with permission from Clin Chem, Olesberg et al. 2004]

Subtraction of the smoothed baseline, local absorbance minima give negative absorbance features in the final absorbance spectra (see Fig. 17.17b) which then excludes unimportant broad variations and reducing the number of factors required for a multivariate calibration model.

The benefit of filtering is to increase model robustness rather than accuracy. The magnitudes of the spectral features in the filtered spectra (see Fig. 17.17b) are more than two orders of magnitude smaller than the broad variations attributable to sample temperature (see Fig. 17.17a). Filtered spectra of the NIR active dialysate components considered in Olesberg et al (2004) are shown in Fig. 17.18. Urea is absorbing in the NIR region. The urea absorption spectrum is distinct because it is dominated by features in the 4700–4500 cm^{-1} range.

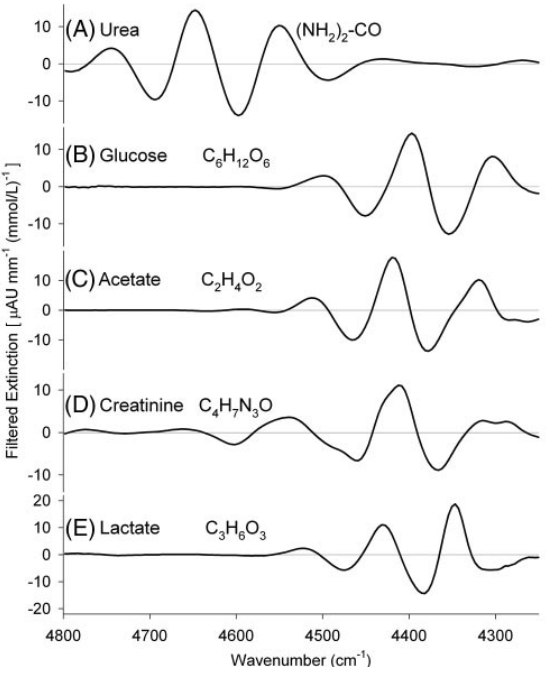


Fig. 17.18 Filtered pure-component extinction spectra of the five principal solutes found in spent dialysate. [Reprinted with permission from Clin Chem, Olesberg et al. 2004]

These characteristics are attributable to nonlinear combinations of vibration modes of the N–H bond in the urea molecule, whereas the characteristics between 4500 and 4300 cm^{-1} are attributable to combinations of C–H bond modes in Fig. 17.18. Only urea and creatinine contain N–H bonds, although the creatinine absorption spectrum is dominated by C–H characteristics (Olesberg et al., 2004). The shapes of the filtered online spectra, shown in Fig. 17.17b, are dominated by absorption of urea and glucose. The glucose concentration remains relatively constant, while the urea concentration

decreases by a factor of 3 during the dialysis treatment. According to existing infrared characteristics, the potential interfering substances with urea in the spent dialysate are glucose, creatinine, phosphate, lactate, bicarbonate and acetic acid (Eddy and Arnold 2001; Jensen and Bak 2002; Jensen et al. 2004b; Olesberg et al. 2004). Also, strong water absorption bands effect the dynamic measurement range and wavelength selection.

17.3.2.3 NIRS and Uremic Solutes

Urea is the first and so far the only uremic solute that has been investigated and monitored on-line by NIRS in the spent dialysate. First a calibration model had to be built and verified by using a subset of spectra for the analytes whose concentrations are known. Then the model was tested for one dialysis treatment, not used when building the model, to calculate urea concentration over time (see Fig. 17.19) (Olesberg et al. 2004). Predicted urea concentrations in mmol/L from the NIR measurement, measured at once a minute, are shown as a solid line. The circles indicate the urea concentrations measured by the standard chemical assays, and show an excellent agreement (Olesberg et al. 2004).

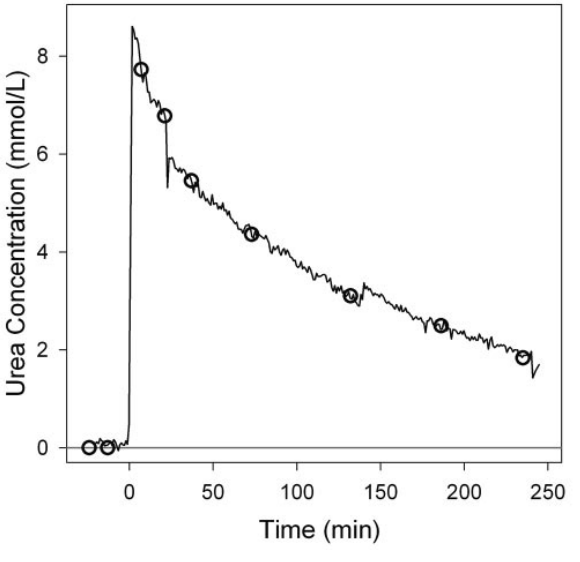


Fig. 17.19 Concentration of urea in the spent dialysate stream at 1-min intervals during dialysis treatment and urea concentration measured using standard methods (circles). [Reprinted with permission from Clin Chem, Olesberg et al. 2004]

There are visible changes in the urea concentration at 20 and 140 min after the start of the treatment. This was due to changes of the blood flow rate. The blood flow rate was reduced from 330 to 260 mL/min at 20 min and later increased to 300 mL/min at 140 min because patient had problems with cramps. Predicted values from the NIR-method follow the expected trend of approximately exponential decrease in urea concentration over time. A direct calibration where the net analyte signal was computed by standard methods was utilized and no adjustment of the slope of the actual vs. predicted concentration line to match the online spectra (see Fig. 17.20) (Olesberg et al. 2004).

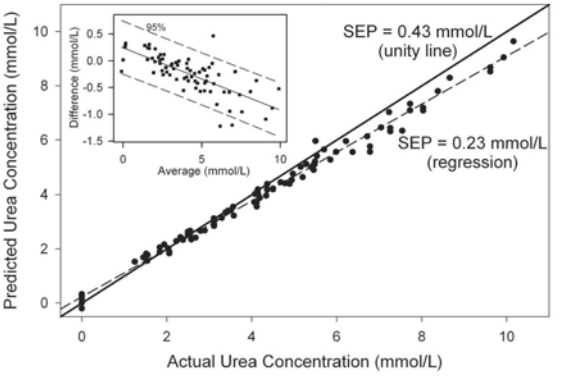


Fig. 17.20 Predicted vs. actual urea concentration using the net-analyte signal calibration. The dashed line is a linear regression of the calibration and prediction points. (Inset), Bland–Altman plot comparing the optical and chemical assays, indicating a clear proportional error. The solid line in the inset is a regression line; the dashed lines represent the 95% confidence limits. [Reprinted with permission from Clin Chem, Olesberg et al. 2004]

The standard error of prediction (SEP) was 0.43 mmol/L (1.2 mg/dL urea nitrogen), which was larger (60%) than the standard error (SE) of the partial least squares regression (PLS) calibration model. In Figure 17.20 a Bland–Altman plot is also shown which presents that the error is attributable to a proportionality error. The proportionality error is probably a result of a combination of errors in the concentration of the reference urea solution, variations in the optical path length of the sample cell between collection of the pure component spectra and collection of the dialysate sample spectra, and unaccounted for solutes in the sample. A linear regression is used to correct for the proportionality error.

17.3.3 Pros and Cons of UV and NIR Spectroscopy

In this section three areas that distinguish the two methods are discussed.

17.3.3.1 Signal Strength and Interfering Factors

Technically, the UV absorbance technique is practically easy to handle and the signal level is high for the uremic solutes in spent dialysate which makes influence of the interfering factors (e.g. temperature, pH, movement of water molecules etc.) minimal. The small absorptivities of biomolecules in the NIR spectral range generate relatively small absorbance values at clinically relevant concentrations (Cho et al. 2008), e.g. in mAU unprocessed intensity and microAU processed (e.g. Savitzky-Golay) intensity (Olesberg et al. 2004; Jensen and Bak 2002). This in turn results that the measurements regarding signal-to-noise ratio, determining accuracy of solute concentrations, are highly temperature sensitive. Thus, even the latest AOTF based technology seems to be overcome the need for thermostated samples utilised in earlier FT-NIR measurements, still the AOTF sensor and the detectors must be thermo-controlled (Cho et al. 2008) making the technology more complex and demanding.

17.3.3.2 Selectivity to Solutes

The UV absorbance technology, as applied today, does not measure a single solute. Instead, the signal reflects the sum of all UV absorbing compounds in the spent dialysate. This makes difficult to select a specific uremic solute separately for monitoring in an easy way. On the other hand, the correlation is high to several water soluble solutes in spent dialysate, which gives the possibility to estimate the removal rate and amount of the particularly solute by satisfactory accuracy. By utilizing this good relationship between UV absorbance and a known solute, estimation of the removal is possible even when the solute is not UV-active at all e.g. in case of urea. Urea is absorbing in the IR region, which makes possible estimate urea removal by NIRS. According to existing IR characteristics, potentially interfering substances with urea in the spent dialysate are glucose, creatinine, phosphate, lactate, bicarbonate and acetic acid (Eddy and Arnold 2001; Jensen and Bak 2002; Jensen et al. 2004; Olesberg et al. 2004; Cho et al. 2008), which can be seen as a possibility to monitor several solutes by NIRS. Also, strong water absorption bands effect the dynamic measurement range and wavelength selection (Eddy and Arnold 2001). The spectral bands seen in the UV and NIR region are typically very broad, leading to complex spectra; it can be difficult to assign specific features to specific chemical components. Multivariate (multiple

wavelength) calibration techniques (e.g., principal components analysis, partial least squares, or artificial neural networks) are needed to extract the desired chemical information. Careful development of a set of calibration samples and application of multivariate calibration techniques is essential.

17.3.3.3 Simplicity and Robustness

To implement NIR technology based on FT-NIR or AOTF sensor as a simple and robust is far more complicated because the underlying optical principle where interference or diffraction is utilized (Jensen and Bak 2002; Cho et al. 2008). UV- method is more straightforward and is not so demanding considering source and detector characteristics, and other technological modules. A simplified comparison of the advantages and disadvantages of the optical dialysis dose monitoring systems for UV and NIR spectroscopy is given in Table 17.1.

Table 17.1 Comparison of the advantages and disadvantages of the optical dialysis dose monitoring systems for UV and NIR spectroscopy

Method/Parameter	Signal strength, interfering factors	Selectivity to solutes	Simplicity, robustness
UV	+	-	+
NIR	-	+	-

Finally, despite being not absolutely perfect, manifold additional values are created based on the advantages inherent with the optical dialysis dose monitoring technologies. In general, the optical systems allow the end user (the doctor) to evaluate the dialysis quality more precisely and diversely. This, in turn, will help to keep the dialysis patients healthier, prolong lifespan, and maintain ability to work. Since the treatment lasts for up to decades, the quality of the treatment in crucial to provide the patient with as long and complication-free life as possible. Also, decrease of complications offers economic frugality instead of hospital costs and treatment of after-effects.

17.4 CLINICAL PARAMETERS FROM OPTICAL DIALYSIS DOSE MONITORING

The two described optical techniques, UV absorbance and NIRS, have both investigated the possibility to estimate urea, the most commonly used representative marker for accumulated waste solutes, in dialysis patients.

In case of the NIR methodology studies have so far focused on validation of the method to measure urea concentration in the spent dialysate. No efforts have so far been made to calculate clinical parameters from NIR. In case of the UV methodology, some clinical parameters have been estimated and compared with existing standard methods. In this section we will present how clinical parameters based on urea such as Kt/V, URR, TRU and PCR/PNA can be estimated optically utilising UV absorbance.

17.4.1 Urea Kinetic Modelling Based on Optical Methods

Urea kinetic modelling (UKM), i.e. where urea is used in different equations, with the attempt to provide quantitative assessments of dialysis and nutrition adequacy in dialysis patients. The high correlation between urea concentration and measures of UV absorbance gives consequently the possibility to utilize the UKM equations for UV absorbance likewise. During the UV absorbance on-line measurements, the pure dialysate was used as the reference (see Beer-Lambert law I_r = pure dialysate) and the wavelength was fixed for the entire treatment. The absorbance baseline level was, after the pure flowing dialysate had been stabilized in temperature and conductivity, set to zero when the pure dialysate was flowing through the cuvette prior treatment (baseline correction).

17.4.1.1 How to Estimate Kt/V from UV Absorbance

From the differential equation, describing urea mass balance during a dialysis session, it can be determined that the average value of the urea clearance (K) in mL/min divided with urea distribution volume in the body (V) in mL (K/V) during a session may be approximated as the slope from the natural logarithm (ln) plot of the urea blood concentration in the blood versus time, S_B. In the same way, but instead of blood urea concentrations, the concentrations of urea in dialysate (S_D) can be used. Hence:

$$Kt / V \approx -S_B T \approx -S_D T \tag{17.12}$$

Where T is the dialysis session length in minutes. This equation would hold strictly if urea obeys fixed volume and single pool kinetics and no urea is generated during the session (Gotch and Keen 1991). In order to calculate Kt/V from the on-line UV absorbance, the slope of blood and dialysate urea concentration was replaced by the ln slope of the UV absorbance (Sa), Fig. 17.21, versus time (Kt/V ≈ - SaT), (Uhlin et al. 2003).

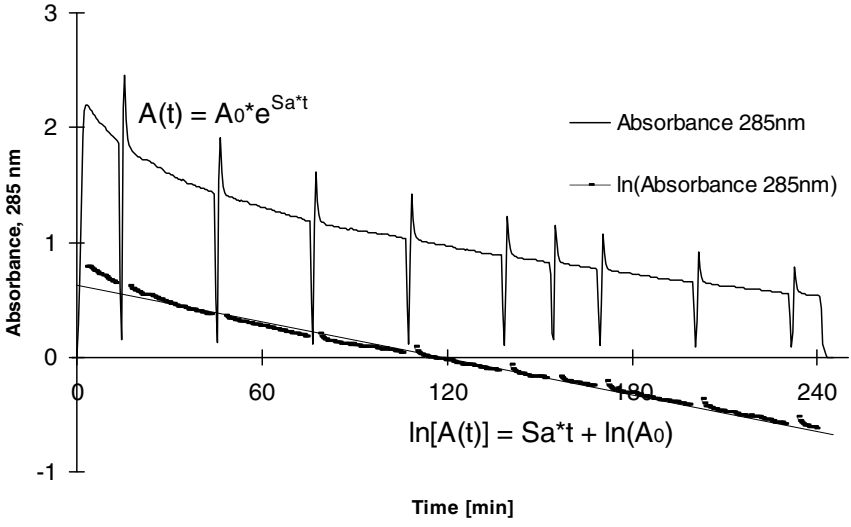


Fig. 17.21 On-line absorbance curve during a single hemodialysis treatment where UV absorbance at the wavelength 285 nm is plotted against time. The corresponding natural logarithmic (ln) fitting line is also shown, used for Kt/V calculation

Assuming that urea is distributed in a single pool volume in the body, that urea generation rate and ultrafiltration are negligible during the session and that the ratio K/V remains constant over the dialysis the following equation holds (Barth 1988; Gotch and Sagent 1985):

$$\mathrm{Kt/V} = -\ln\frac{C_t}{C_0} \tag{17.13}$$

Where C_t is the post and C_0 is the pre dialysis urea blood concentration respectively.

According to Eq. (17.12) we obtain from Eq. (17.13):

$$\frac{C_t}{C_0} \approx \exp\left(-\mathrm{Kt/V}\right) \approx \exp\left(S_B T\right) \approx \exp\left(S_D T\right) \approx \exp\left(S_a T\right) \tag{17.14}$$

If the slopes are used instead of the blood urea concentrations. This approximation is equivalent to the equation when two measuring points are used, and the previously mentioned assumptions are fulfilled. Using the

absorbance slope values (S_a), according to Eq. (17.14), the Daugirdas based monocompartmental (single pool, sp) Equation (Daugirdas 1995):

$$\mathrm{sp(Kt/V)} = -\ln\left(\frac{C_t}{C_0} - 0.008\frac{T}{60}\right) + \left(4 - 3.5\frac{C_t}{C_0}\right)\frac{UF}{BW} \qquad (17.15)$$

Equation 17.15 can be written as (Uhlin et al. 2003):

$$\mathrm{sp(Kt/Va)} = -\ln\left(\exp(S_a T) - 0.008\frac{T}{60}\right) + \left(4 - 3.5\exp(S_a T)\right)\frac{UF}{BW} \qquad (17.16)$$

Where UF and BW is the ultrafiltration volume in liters (L) and the patient's dry body weight in kg.

The equilibrated Kt/V from UV absorbance, eKt/Va, according to the rate adjustment method (Daugirdas 1995), is predicted from the rate of dialysis (K/V) and the sp(Kt/Va) as:

$$\mathrm{eKt/Va} = \mathrm{sp(Kt/Va)} - \frac{0.6}{(T/60)}\mathrm{sp(Kt/Va)} + 0.03 \qquad (17.17)$$

The rate adjustment method predicts that the urea rebound is related to the rate of dialysis or dialysis efficiency (Daugirdas et al. 1997).

Example 17.2

During a dialysis session lasting 4.5 hours, the urea concentration in blood was 25.6 mmol/L at the start of dialysis and 9.1 mmol/L after dialysis. Weight of the patient was 89.0 kg before dialysis and the prescribed post dialysis weight was 87.0 kg, the estimated amount of fluid intake (drinking) during dialysis was 0.3 liters. Calculate sp(Kt/V) according to Daugirdas second generation formula (Daugirdas 1995) and according to Uhlin formula (Uhlin et al. 2003).

Solution

The sp(Kt/V) according to Daugirdas second generation formula (Daugirdas 1995) is calculated according to Eq. (17.15) as follows:

$$\mathrm{sp(Kt/V)} = -\ln\left(\frac{9.1}{25.6} - 0.008\frac{270}{60}\right) + \left(4 - 3.5\frac{9.1}{25.6}\right)\frac{2.3}{87} = 1.22$$

In similar way sp(Kt/V) for UV absorbance, sp(Kt/Va) can be calculated using the ln slope of the absorbance values during dialysis.

$$\mathrm{sp(Kt/V)} = -\ln\left(\exp(-0.003835\cdot 270) - 0.008\frac{270}{60}\right) + \left(4 - 3.5\cdot\exp(-0.003835\cdot 270)\right)\frac{2.3}{87} = 1.22$$

K/V was determined as a linear fitting curve of the ln slope of the UV absorbance values = Sa, Fig. 17.21. In this example ln [A(t)] = - 0.003835 x + 0.7681, where Sa corresponds to 0.003835. Sa was then inserted into Eq. (17.16) to obtain sp(Kt/Va).

17.4.1.2 Simplified eKt/V Calculation from UV Absorbance

For determination of the dialysis dose from UV absorbance can be simplified when instead of the slope of UV absorbance only two values are utilized, similar to calculations from blood values. For example, when measured by the DIAMON prototype (Luman et al. 2009a), eKt/V_{DIAMON}, instead of the pre-and post-dialysis blood urea concentrations, the maximum UV absorbance value in the beginning and the minimum UV absorbance value at the end of dialysis (A_0 and A_t) were utilized. The sp(Kt/Va) was calculated as:

$$\mathrm{spKt/Va} = -\ln\left(\frac{A_t}{A_0} - 0.008\frac{T}{60}\right) + \left(4 - 3.5\frac{A_t}{A_0}\right)\frac{UF}{BW} \qquad (17.18)$$

sp(Kt/Va) was used to obtain the eKt/V_{DIAMON} according to Eq. (17.17).

17.4.1.3 Estimation of Urea Removal Using UV Absorbance

One way to estimate Total Removed Urea (TRU), assuming that the dialysate flow, $Q_d(t)$, is constant and the total UF is known, is to use the following equation:

$$\mathrm{TRU(mmol)} = \overline{\mathrm{Urea}}\cdot\left(\mathrm{Qd}\cdot\mathrm{T} + \mathrm{UF}\right) \qquad (17.19)$$

where $\overline{\mathrm{Urea}}$ in mmol/L is the mean urea concentration in spent dialysate of a particular hemodialysis session (Raj et al. 1997). For the TRU calculations $\overline{\mathrm{Urea}} = D_{total}$ can be utilized as reference, where D_{total} is the urea concentration in the collection tank, (Tank sample, Fig. 17.6), after end of dialysis. Q_d is the rate of the dialysate flow in L/min, T is the

dialysis session length in minutes and UF is the total ultra-filtrated volume in L during the session.

Under the condition that a good correlation exist between UV absorbance and concentration of a urea (Fig. 17.14a and 17.15) it is possible to utilize this relationship. Therefore in a similar way TRU may be calculated from the on-line UV absorbance (TRUa) curve as (Uhlin et al. 2005):

$$\mathrm{TRUa(mmol)} = \left(\alpha \cdot \bar{A} + \beta\right) \cdot \left(\mathrm{Qd} \cdot \mathrm{T} + \mathrm{UF}\right) \tag{17.20}$$

Where the $\bar{A}$ is the mean of all UV absorbance (A) values from the start to the end of the dialysis, The regression line between the UV absorbance and concentration of in spent dialysate (Drain sample, Fig. 17.6) from on-line measurement gives the Slope (α) and the Intercept (β) inserted in Eq. (17.20) when determining TRUa from either an individual model (one for each patient, Fig 17.14a) or a general model (preferable) based on the entire group of patients (e.g. see Fig. 17.15). TRU from the Total Dialysate Collection (Fig 17.6), TDC (reference) was calculated as D_{total} (mmol/L) multiplied with collected weight (kg), assuming that 1kg =1L of the dialysate. TRU from these methods was compared regarding mean values and showed no statistically significant difference (Uhlin et al. 2005).

Example 17.3

According to example 17.2, we can get additional information from the UV absorbance curve namely; $\bar{A}$ = 1.279. A transformation/calibration model based on a group of seven dialysis patients distributed on 21 dialysis sessions gave the relationship (correlation) between urea concentration and UV-absobance in spent dialysate α = 3.8083 and β = 0.2474 (Fig. 17.15). Calculate TRUa for this particularly session.

Solution

Total Removed Urea (TRU) is calculated according to Eq. (17.20) as follows:

$$\mathrm{TRUa(mmol)} = \left(3.8083 \times 1.279 + 0.2474\right) \times \left(0.5 \times 270 + 2.4\right) = 703.2$$

If we compare this result to the reference value; calculated as urea concentration in the collection tank after end of dialysis, $\overline{\mathrm{Urea}}$ = 5.2 mmol/L multiplied with the volume in the tank of 137.4 L (i.e. the last part of the formula; $Q_d \cdot T + UF$) we get a TRU value of 714.5, which is similar to the result estimated using UV absorbance.

17.4.1.4 Estimation of Nutrition Parameters from UV Absorbance

High blood concentration of urea is not necessarily related with a poor dialysis outcome if urea removal is sufficient (Blumenkrantz et al. 1982), but also protein-energy malnutrition is frequently present in patients undergoing hemodialysis therapy. Several studies have suggested that malnutrition is an important risk factor for morbidity and mortality in HD patients (Lindsay et al. 1994). In order to optimize the diet of patients with renal diseases, dietary protein intake has to be controlled. Protein nitrogen appearance (PNA), formerly protein catabolic rate (PCR) (European Best Practice Guidelines on Hemodialysis 2007), is easily obtainable from UKM and in patients who are not markedly catabolic or anabolic, the normalized PNA (nPNA) correlates closely with dietary protein intake (Flanigan et al. 1995, Keane and Collins 1994). These parameters can be calculated from TRU.

The PCR calculation, from TDC and UV absorbance, was based on a theory by Garred et al (1995), where a calculation of urea removal is expressed as a fraction of the week's urea generation. The fraction varies with the day of the week and was found to be essentially constant among patients on a given day (Garred et al. 1995). The amount of urea could therefore be approximated from measuring urea concentration from only one of the three treatments and PCR could be calculated as (Uhlin et al. 2005):

$$\mathrm{nPCRw} = \mathrm{Factor}_{1,\,2\text{ or }3}\left(\frac{\mathrm{TRU}_{1,\,2\text{ or }3}}{\mathrm{BW}}\right) + 0.17 \qquad (17.21)$$

Where TRU 1, 2 or 3 (expressed in grams of urea nitrogen)[1] is the TRU from the first (1), midweek (2) or last dialysis in week (3) and Factor 1, 2 or 3 is the fractional factor for the first (1), midweek (2) and last treatment (3) of the week respectively; factor 1 = 2.45; 2 = 2.89; 3 = 3.10 (Garred et al. 1995). Obligatory loss of dietary protein in stools and via skin shedding represents the constant term 0.17 (g protein/kg body weight/day). The dry body weight (BW) was used for normalization of PCR (nPCRw). Observe that these fractional factors relays to a treatment schedule of three times a

[1] First we have to convert urea in mmol into gram by multiplying with the molecule weight of urea, 60.06, and then divided with 1000. BUN (g) = TRU (g) 6.23/13.4.

week. More frequent dialysis treatments are more common today whereas the factors are not appropriate.

Example 17.4

Using the same patient data as in Example 17.2; the dialysis treatment of this patient was performed on a mid-week. Calculate nPCRw according to Garred´s formula.

Solution

Since the fractional factor for mid-week session is 2.89, then nPCRw can be calculated according to Eq. (17.21) as follows:

$$\text{nPCRw} = 2.89\left(\frac{19.64}{87}\right) + 0.17 = 0.82 \text{ g/kg/day}$$

17.4.1.5 Dialysis Dose and Nutrition Mapping of the HD Patients

Simultaneous analysis of Kt/V, URR and nPNA permits to get a picture of: 1) the adequacy of dialysis treatment; and 2) dietary protein intake related to nutritional status of the patients. This enables the dialysis team to make a choice between increasing dialysis effectiveness, dietary counselling, or both. In order to take account those important parameters, UKM is suggested e.g. presenting the patients' status on a diagram incorporating Time-average concentration of urea (TAC), nPNA, and spKt/V (Lindsay et al. 1994). However, UKM is performed by tedious and time consuming calculations using special and complicated software. Recently, a new, simplified mapping of dialysis dose and nutrition was tested by using only two parameters: Kt/V and nPNA, Fig. 17.22, suitable for automatic and time-efficient way to review the performed HD strategy based on the data from on-line dialysate side dialysis monitors (Luman et al. 2009b).

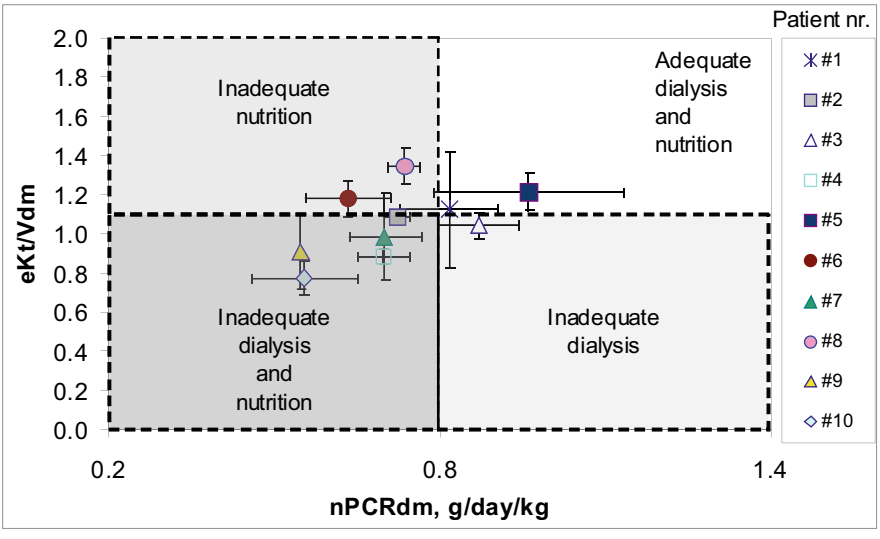


Fig. 17.22 Simplified mapping of dialysis dose and nutrition applied by using only two parameters - Kt/V and nPNA, suitable for automatic and time-efficient way to review the performed HD strategy based on the data from on-line dialysate side dialysis monitor (dm)

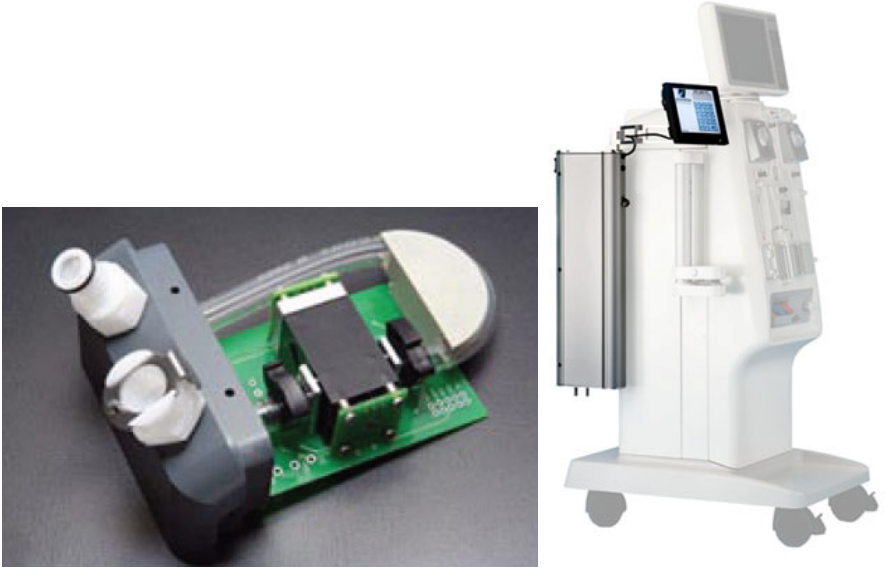
The eKt/V-nPNA plots yielded the results in concordance with the variable volume single-pool (VVSP) UKM presented as TAC = f (nPNA) for all patients and outlined similarly the critical patients regarding the delivered dialysis dose and nutrition.

17.4.1.6 Current Commercial Systems for Optical Dialysis Dose Monitoring

The first commercial systems for optical dialysis dose monitoring are available today. Two kinds of the systems can be distinguished: 1) external, and 2) internal (integrated) modules; depending on if the systems have been designed either according to "stand-alone" or "built-in" architecture. The modules can currently estimate only urea based dialysis quality parameters, Kt/V and URR.

The first sensor for optical dialysis adequacy monitoring in the world is DiaSens, Fig. 17.23a (Ldiamon AS, Estonia). This is the main functional

element of DiaHub - an external "stand-alone" module which can be connected to dialysis machine and perform continuous on-line measurement of Kt/V and URR during the treatment, Fig. 17.23b (Ldiamon AS, http://www.ldiamon.eu/index.php?act=page&id=2).



(a) (b)

Fig. 17.23 a) An optical sensor DiaSens. b) The external module DiaHub connected to a dialysis machine. [Reprinted with permission from Ldiamon AS]

The first integrated or "in-build" dialysis dose monitor in the world, utilising the UV-technology, is the Adimea system (Option Adimea, B.Braun Avitum AG) (B. Braun Avitum AG 2010). The heart of the Adimea system is the optical sensor DiaSens, integrated into the HD machine, delivering values of real-time Kt/V or URR for the dialysis team during a treatment (see Fig. 17.24).

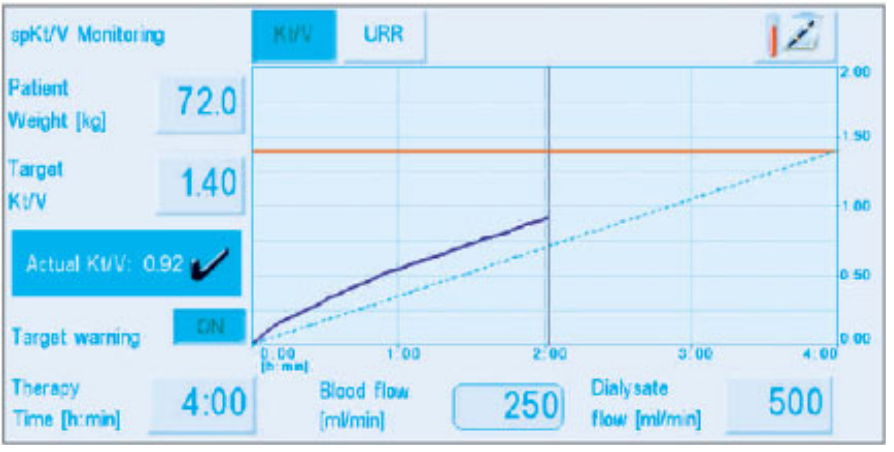


Fig. 17.24 Graphic display to present dialysis dose monitoring progress by the Adimea system for clinicians [Reprinted with permission from BBraun Avitum AG]

The obtained data about the dialysis efficiency are available to be saved on the patient therapy card and the data management system. Validation studies of real-time Kt/V by the Adimea system have shown that the results by the UV-technology are indistinguishable from blood based Kt/V (Castellarnau et al. 2010).

17.5 MONITORING UREMIC TOXINS BEYOND UREA

The facts that: (i) urea is a nontoxic substance and only a marker for uremic retention solutes, and (ii) the European uremic toxins group (EUTox) has identified several more relevant uremic toxins utilizing optical analysis methods, arise a question connected to further development of optical techniques – "Could some of these identified uremic toxins be measured optical and on-line?". Of the 90 compounds that have been identified as uremic toxins by the EUTox group (Vanholder et al. 2003a, 2003b) we have, from spectroscopic databases, identified 36 to be UV absorbing and among them approximately 25 to be absorbing near 297 nm. Besides these 90 compounds mentioned as uremic toxins, there are even more solutes in the dialysate that are optically active at 297 nm and that add to the measured UV absorbance signal. Spent dialysate contains several different absorbing compounds and concentrations of solutes declines differently during a session for individuals. The UV absorbance curve may therefore be an individual “clinical print” of the patient’s sum of several UV

absorbing solutes and therefore a possible parameter for monitoring total solute removal during dialysis. Investigations still remain to find the single solute's individual contribution to the absorbance signal, which is also dependent on which wavelengths are used (Fridolin and Lindberg 2003). Earlier knowledge from the correlation analysis between UV absorbance and a few solutes-uremic toxins has shown that it is possible to estimate removal of such solutes which have a high correlation to UV absorbance. This removal beyond urea may have stronger impact on dialysis outcome compared to urea or urea alone.

17.5.1 Uric Acid

Uric Acid (UA) is a water–soluble compound (molecular weight of 168.1) that is the final metabolite of purine in humans. Elevated serum UA contributes to endothelial dysfunction and increased oxidative stress within the glomerulus and the tubulo-interstitium, with associated increased remodeling fibrosis of the kidney (Hayden and Tyagi 2004). A high level of serum UA, hyperuricemia, has been suggested to be an independent risk factor for cardiovascular and renal disease especially in patients with heart failure, hypertension and/or diabetes (Feig et al. 2008; Viazzi et al. 2006; Høieggen et al. 2004) and has been shown to cause renal disease in a rat model (Nakagawa et al. 2006). UA is mostly associated with gout but studies have implicated that UA affects biological systems (De Smet et al. 1997) and also could influence risks of higher mortality in dialysis patients (Perlstein et al. 2004) but the pathogenic role of hyperuricemia in dialysis patients is not completely established (Navaneethan and Beddhu 2009).

In previous studies a good correlation between UV absorbance in the spent dialysate and the concentration of several solutes both in the spent dialysate and in the blood of dialysis patients has been presented, indicating that the technique can be used to estimate the removal of retained substances (Fridolin et al. 2002). A study was performed in two dialysis centers in two different countries The purpose was to find out if it would be possible to create a specific model for UA while still using the same wavelength (285 nm) that earlier was used when urea removal was estimated (Uhlin et al. 2005). The fact that UA is a UV absorbing solute (Jerotskaja et al. 2007) made that study even more interesting. The results show the possibility to estimate Total Removed Uric Acid (TR_{UA}) by using UV absorbance technique generated transformation models in two different dialysis centers in two countries, Estonia and Sweden. The mean values of TR_{UA} obtained using the UV-model were not statistically different to TR_{UA} calculated from TDC (reference method) at the two centers (N=56) (see Fig. 17.25).

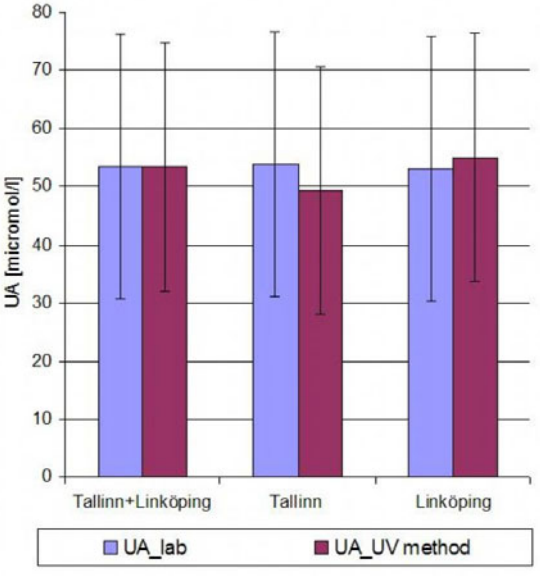


Fig. 17.25 Mean values of UA for the total material and for different centers

Important issues to be considered, which have emerged from this study, are that the standardized optical parameters (wavelength, optical flow cuvette, optical path length, etc.) should be defined to build more accurate general models. Other areas that influence the measurements are type of dialyzer (ultrafiltration coefficient, surface area), and probably even some patient dependent parameters (Jerotskaja et al. 2010a). The high correlation between UV absorbance and UA in every single patient (Fig. 17.14B) could be explained by a dominant absorbance for UA, compared to other compounds in spent dialysate at the wavelength 280 nm (Jerotskaja et al. 2007). This is due to relatively high millimolar extinction coefficients of UA 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 (Fridolin and Lindberg, 2003). The absorbance around 292 nm is characteristic for UA and is utilized for UA concentration determination by the enzymatic degradation method (Praetorius and Poulson 1953).

17.5.2 Creatinine

Beside the marker molecule urea-based dialysis patient nutrition parameter nPNA two promising alternative parameters (i) Creatinine index (CI); and (ii) Lean Body Mass (LBM), calculated from the small molecular

water soluble uremic retention solute – uremic toxin creatinine (Cr) have been proposed. CI and LBM are shown to be predictors of long-term survival in HDF patients (Desmeules et al. 2004). CI and LBM have several pros compared to the urea based dialysis quality and nutrition parameters:

- CI and LBM are related to the preservation of muscle mass and protein nutritional status and could be seen as markers of protein-energy malnutrition.
- CI and LBM predict survival better than Kt/V and nPNA.
- CI and LBM are more stable than nPNA which is highly dependent on protein intake and dialysis dose.

Recently, a new method to estimate LBM in hemodialysis patients by UV absorbance measurements in the spent dialysate was presented (Fridolin et al. 2010). A good linear relationship between UV absorbance and dialysate Cr concentration at the wavelength range of 230 - 300 nm was used for generating a model to estimate Cr concentration in the spent dialysate. The LBM using the blood samples (LBM_b), and LBM utilizing the Cr concentration values estimated by the UV absorbance (LBM_uv), were obtained according to the method described by Desmeules et al (2004). Comparison between the blood based lean body mass (LBM_b) and the UV absorbance method (LBM_uv) exhibited a good agreement (see Fig. 17.26).

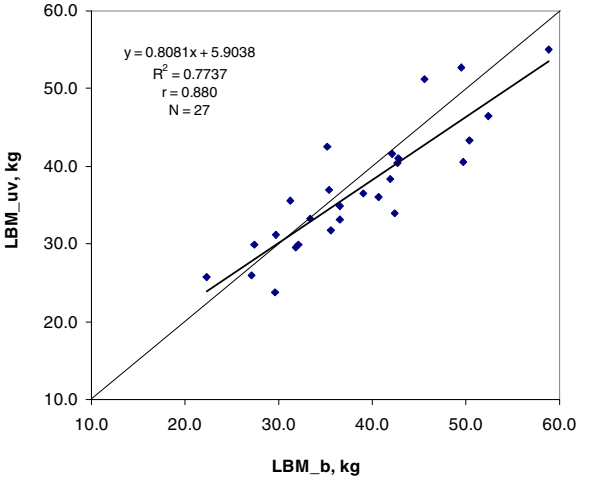


Fig. 17.26 LBM_uv estimated by UV absorbance in the spent dialysate plotted against LBM_b estimated using the blood samples (number of HD sessions N = 27). The line of identity as a dashed line is also shown. Adapted from Fridolin et al (2010).

The mean ± SD of LBM_b estimated using the blood samples was 38.6 ± 8.77 kg (N = 27), and LBM_uv 37.9 ± 8.06 kg (N = 27). The mean value of LBM from blood samples were not statistically different from the corresponding value estimated by UV absorbance in the spent dialysate (P = 0.516).Those results encourage continuing in order to develop an optical technology enabling quickly and automatically estimate the muscle mass and protein nutritional status of the hemodialysis patients.

17.5.3 Middle Molecules

Urea shows a kinetic behavior that is not representative for all uremic retained solutes, yet including other water-soluble solutes (Vanholder et al. 2004). The retained organic compounds can be be divided into three groups, small, water soluble solutes with a molecule weight (MW) < 500 D (e.g. urea, uric acid and creatinine), protein-bound solutes (e.g. hippuric acid, indoxyl sulphate and P-cresol), and middle molecules MW > 500 D (e.g. β-2 microglobulin, Cystatin C, IL 6 and Leptin) (Vanholder et al., 2003a). Many of the solutes affecting metabolism have retention and elimination characteristics that are different from the traditional markers for uremia, urea and creatinine (Vanholder et al. 2004). β2-microglobulin has been suggested to be an excellent marker for the middle molecule range which is a "real" uremic toxin with an independent impact of patient outcome (Canaud et al. 2006). In a pilot study where correlation between concentration of β2-microglobuline in mg/L and UV absorbance at 297 nm was investigated in a number of 21 HemoDiaFiltration treatments, a high correlation was found (Uhlin et al. 2009a) similar to that of urea, uric acid and creatinine (see Fig. 17.27).

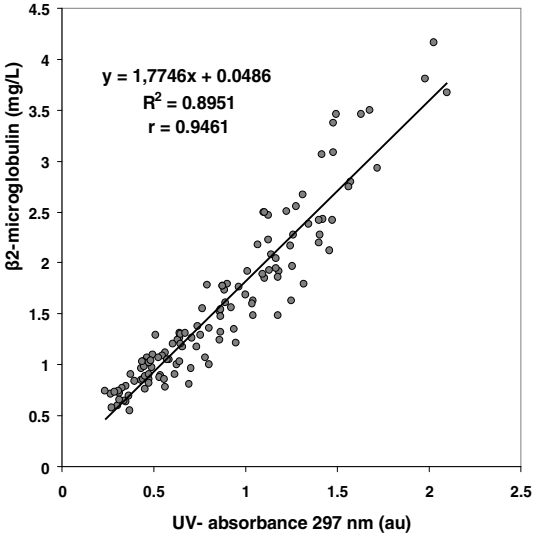


Fig. 17.27 Correlation between concentration of β2-microglobulin and UV absorbance at 297 nm in spent dialysate, (Uhlin et al. 2009a)

This relationship can be utilized to estimate total removal of during dialysis β2-microglobulin in similar way as earlier have been described in case of urea.

17.6 FUTURE DIRECTIONS IN OPTICAL MONITORING

In this last section of the chapter we will present other approaches that could be used in order to obtain new parameters from UV absorbance or increase the accuracy of earlier presented parameters.

17.6.1 Other Approaches of UV Absorbance

There are two main areas which so far have been investigated by the UV method, area under curve and multiple wavelengths.

17.6.1.1 Area under the UV Absorbance Curve (AUCa) was recently introduced as a possible complementary parameter prospected to evaluate total solute removal during dialysis (Uhlin et al. 2009b). The results indicate a strong relationship between a patient's measured AUCa and the total removal of a few well-known low molecular weight solutes (urea, creatinine, urate and phosphate) during a given session, r = 0.967-1.000.

The relationship is less strong for the entire group of 15 patients (r = 0.919-0.957). AUCa is a less sensitive parameter for deviations during treatment compared to parameters based on the slope (e.g. Kt/V). Perhaps AUCa could add valuable information in combination with other parameters when evaluating dialysis treatments.

17.6.1.2 Multiple Wavelengths are an approach which has been tested for UA estimation to increase the accuracy (Jerotskaja et al. 2010c). The first results demonstrate that using two or three wavelengths of UV absorbance instead of one improves estimation of the UA noticeably (Table 17.2). Even more reliable results are achieved when derivative spectra is used instead of the original absorbance spectra (Jerotskaja et al. 2009; Jerotskaja et al. 2010b, c). The systematic and random errors were significantly different ($p \leq 0.05$) between the methods indicating that the 1st derivate and multi wavelength algorithm enables more accurate UA estimation.

Table 17.2 Summary of results for the different methods to measure concentration of the uric acid (N=301), utilizing UV absorbance (UV_A) and derivate spectra (UV_D)

Method	Concentration of UA ± SD [micromol/l]	Syst. Error [%]	Rand. Error [%]	R^2
Lab	52.91 ± 21.5	-	-	-
UV_A_298 nm	57.07 ± 23.6	-8.89	16.5	0.87
UV_A_298+312 nm	54.15 ± 22.1	-3.31	13.4	0.91
UV_A_298+312+285 nm	51.95 ± 21.1	1.16	10.0	0.94
UV_D_307 nm	54.96 ± 22.5	-4.74	13.0	0.91
UV_D_307+284 nm	52.94 ± 21.7	-0.61	10.4	0.94
UV_D_307+284+214 nm	52.78 ± 21.8	-0.19	10.3	0.94

Multi wavelength systems, where several compounds with clinical impact could be measured simultaneously, are a vision for a time-to-come optical monitoring system, presenting a future for dialysis dose.

17.7 CONCLUSION

Optical technologies applied for dialysis dose monitoring have a potential to be a helpful tool for the dialysis team in evaluation of the dialysis process. Moreover, the optical methods offer new perspectives to ensure

dialysis adequacy and quality, aiming to be a practical toolkit for the clinicians and the dialysis team, which helps to obtain adequate dialysis targets and to meet the individual needs of each patient. This can lead to "personalized healthcare" within hemodialysis resulting decreased morbidity, longer life span and enhanced quality of life.

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QUESTIONS

1. Which optical technology is found to be of clinical acceptance for monitoring dialysis dose?
2. What is the biological fluid used to measure optically elimination of uremic solutes? Why?
3. How can biological tissues and fluids be classified according to their optical properties?
4. How electromagnetic (EM) radiation is classified by wavelength?
5. Which are the UV-regions according to ISO standard on determining solar irradiances?
6. How the infrared (IR) part of the electromagnetic spectrum is divided?
7. How the "wave number" in the context of near infrared (NIR) spectroscopy is calculated?
8. How the term “Light” is defined?
9. Describe the main events occurring when photons propagate into a medium (e.g. into a biological fluid).
10. Describe the main events occurring when photons propagate inside a medium (e.g. inside a biological fluid).
11. Describe the main events occurring when photons propagate from a medium (e.g. from a biological fluid).
12. Describe the basic parts of a spectrophotometer.
13. How the Transmittance and Absorbance are calculated?
14. What does the Beer- Lambert law state? Give the mathematical equation.
15. Give the mathematical equation for the Beer- Lambert law for a medium containing several different absorbing compounds.
16. Which are the common assumptions to utilize absorbance calculated according to the Beer-Lambert law to determine concentration?
17. Describe the technical principle for on-line monitoring of dialysis dose by the UV absorbance.
18. Which information contains UV absorbance curve from on-line measurements of spent dialysate?

19. Which are the known predominant uremic toxins contributing to UV spectra between 200-400 nm.
20. Which uremic toxins on-line UV absorbance at 280 nm is mostly following?
21. How can concentration of a particular uremic solute be estimated by the total UV absorbance?
22. Which is the criterion according to a wavelength should be chosen to estimate optically concentration of a particular uremic solute?
23. Describe the technical principle for monitoring dialysis dose by the near infrared (NIR) spectroscopy.
24. Which are the known predominant uremic toxins contributing to filtered NIR spectra in the 4700–4500 cm^{-1} range?
25. Describe the advantages and disadvantages of the optical dialysis dose monitoring systems for UV and NIR spectroscopy.
26. What marker is mostly used to evaluate dialysis dose today?
27. Which patient' status, this marker (in question 26) can also be used for, and why is it so?
28. Why is this marker (in question 26) not good enough and why efforts are being made to find additional markers?
29. Which is the fundamental difference between a single pool model and a double pool model when calculating dialysis dose?
30. Which parameters do you need when calculating Kt/V according to Daugirdas single pool model, give the mathematical equation?
31. When you are going to use UV absorbance to calculate Kt/V according to Daugirdas single pool model you have two options, which ones?
32. Calculate the sp(Kt/V) using the simplified calculation utilizing on-line UV absorbance measurements where the length of session was 4 hours, dialysate flow was 500mL/min, UF 2500mL and the patients post-dialysis weight was 95 kg . Readings from the UV absorbance curve you get the max and min absorbance value of 2, 17 and 0, 62 respectively.
33. What conditions must exist if solute removal should be estimated during dialysis utilizing an optical technique, and which constants do you need?

34. If total removed urea (TRU) is known for one single dialysis session of a patient, it's possible to estimate PCR both in case of blood and UV absorbance measurements. Which presumption concerning TRU is made if the PCR value is to be calculated?
35. Which method is used as reference during clinical studies when estimating TRU from UV absorbance? Can you think about potential risks of error with this reference method?
36. The correlation between urea concentration and UV absorbance in spent dialysate give the constants $\alpha = 3.8083$ and $\beta = 0.2474$. Calculate total removed urea (TRU) in mmol for a session where dialysate flow was 500mL/min, UF 1000mL during a dialysis lasting 3 hours. The mean absorbance value during this particularly treatment was $\overline{A} = 0.983$
37. The wavelength of 297 nm has been used in several studies using the UV absorbance technique in measurements of spent dialysate. The UV absorbance signal is not the signal of one single solute instead it's the sum of several UV absorbing solutes. How many UV-active solutes have so far been identified around this wavelength, approximately?
38. Recently, a new, simplified mapping of dialysis dose and nutrition status has been tested. What two parameters are used then?
39. What's the reason why the optical on-line curves from UV absorbance and NIR (and even concentrations of solutes) getting an exponential shape at the elimination during dialysis treatment?
40. Explain what happens to the UV absorbance signal if a decrease in blood flow occurs during dialysis, and why?
41. Explain what happens to the UV absorbance signal if decreases in dialysate flow occur during dialysis, and why?
42. If you are interested using a wavelength for on-line measurement of spent dialysate in the UV- range of approximately 240-260 nm, the UV-signal will probably be too high and reach a maximum level. How can this be solved?
43. Into which three groups are usually uremic waste solutes divided? Give one example from each group.

44. Uric acid is an absorbing solute in the UV-range; high serum level of uric acid is associated with some clinical manifestations, give examples?
45. Standardized optical parameters are important when using different set-up during studies, give expels of issues to consider in this field.
46. There has been demonstrated that the solute creatinine can be estimated using on-line UV absorbance measurements during hemodialysis. Which advantages may creatinine based parameters, such as creatinine index (CI) and lean body mass (LBM), have as a nutrition parameter compared to urea based parameters?.
47. What are the benefits by utilizing optical on-line monitoring of dialysis dose compared to monthly pre-and post-dialysis blood control of urea concentration that is the common used dialysis dose parameter today at dialysis units?
48. Give some examples about other approaches that could be used in order to obtain new parameters from UV absorbance or increase the accuracy of earlier presented parameters.
49. Calculate the absorbance A if the transmittance T is 1.0; 0.5; 0.1 and 0.01.
50. Calculate the transmittance T if the absorbance A is 4.0; 1.0; 0.5; and 0.1.
51. Which is the general benefit for the dialysis patients using the optical dialysis dose monitoring?