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(54) Title: OPTICAL METHOD AND DEVICE FOR MEASURING CONCENTRATIONS OF SUBSTANCES IN BIOLOGICAL FLUIDS

(57) Abstract: A method and a device for measuring concentration of substances, preferable uric acid, in biological fluids. Measurements are performed optically-utilizing spectrum of the biological fluid, the Savitzky-Golay algorithm, and a concentration calculation algorithm containing the transforming function to determine the concentration of the substances in specimens in vitro or flowing fluids on-line. The method and device determines the concentration of the substances in-vitro or on-line utilizing a measuring cuvette suitable for specified measurements.

OPTICAL METHOD AND DEVICE FOR MEASURING CONCENTRATIONS OF SUBSTANCES IN BIOLOGICAL FLUIDS

TECHNICAL FIELD

This invention relates to a novel method and a device for measuring concentration of substances, preferable uric acid, in biological fluids. More specifically, the present invention relates to an optical method utilizing spectrum of the biological fluid and the Savitzky-Golay algorithm to determine the concentration of the substances on-line.

BACKGROUND ART

Early monitoring of the important biological constituents in biological fluids from the medical point of view can prevent serious pathological conditions and decrease mortality of patients. There is a need for a simple, compact, inexpensive, mobile, reliable method for measuring concentration of substances in biological fluids. As an example, a method and apparatus for the quantitative determination of optically active substances, particularly glucose, by polarimetry, is proposed in US5357960, and specifically related to dialysis in US2007023334.

Uric acid, a final product of the metabolism of purine, is very important biological molecule present in body fluids. It is mostly excreted from human body through the kidneys in the form of urine. The concentration of uric acid in blood increases when the source of uric acid increases or the kidney malfunctions. Hyperuricemia is a symptom when the uric acid concentration is above 7 mg/dL. Uric acid is hard to dissolve in blood and will crystallize when supersaturated. The uric acid crystallites deposit on the surface of skin, in joints, and especially in toes and results in gout. The analysis of the uric acid concentration in blood helps to diagnose gout. In addition to gout, hyperuricemia is connected with lymph disturbance, chronic hemolytic anemia, an increase of nucleic acid metabolism and kidney malfunction. High caloric foods and alcohol as well as disturbances of organs and tissues are the main causes of hyperuricemia and even gout. Harm can be prevented and reduced by an early diagnosis and monitoring. A simple and inexpensive detecting system helps patients to detect the uric acid concentration on their own.

Uric acid can be determined in several different ways. Two of the most common methods are (1) reduction method by the reaction of uric acid with alkaline phosphotungstate, and (2) the enzymatic method by the enzyme urease.

In the first method, uric acid is estimated by reducing alkaline phosphotungstate to tungsten blue and measuring the colored product in a colorimeter. The demerits of this method include: 1) some compounds similar to uric acid and ascorbic acid contained in the sample of biological fluid affect the test accuracy; 2) the operation is complex, needs lots of agents which are hard to keep, and should be operated by professionals; 3) the sample must be de-protein pretreated; and 4) the necessary equipment is expensive.

The second method detects uric acid by optical colorimetry and electrochemistry and is classified into uricase-ultraviolet absorption, uricase-peroxidase, uricase-catalase and uricase-electrode methods, wherein the former three methods make use of the color of reaction products and quantitatively detect uric acid of by colorimetry. The decrease in absorbance is proportional to the amount of uric acid initially present. The automatic bio-analyzers used in central bio-laboratories of hospitals detect uric acid by optical colorimetry. An improving system for quantification of biochemical components in biological fluids during analysis where a component reacts with an analyte is described in US6121050.

The blood sample should be pretreated to be serum or plasma first. The merits of the automatic bio-analyzers reside in mass detecting, automation and quickness. However, an automatic bio-analyzer cannot be applied in household detecting because it requires professionals to operate, is expensive, and is particularly hard to store the detecting agents. The uricase-electrode method detects uric acid by electrochemistry. The electrodes can be divided as enzymatic and non-enzymatic. The former produced by a complex production process is hard to store and thus is only suitable for research.

Another method for determination of the amount of waste products in the dialysis liquid during dialysis treatment to control the dialysis machine in order to adapt the dialysis treatment to the patient is described in US6666840, and in the reference (Fridolin, Magnusson et al. 2002). The measurements of a concentration of a certain substance or a combination of substances in the dialysis liquid are obtained continuously or regularly on a sample from outgoing dialysis liquid from a dialyzer during dialysis treatment. The measurements are performed spectrophotometrically by means of UV-radiation (wavelength in the range 180-380 nm). At least one parameter for the dialysis treatment is adjusted depending on the measurement of the concentration of the substance or combination thereof. The merits of the described method are that it does not need blood samples, no

disposables or chemicals, and is fast. However, the described method is general and does not specify methodology to measure exclusively a single compound and is meant to apply only for dialysis monitoring. Moreover, no results about the concentration measurements are presented. More exact description about the uric acid and urea measurements using the abovementioned method is given in a scientific papers (Uhlin, Lindberg et al. 2005), (Uhlin, Fridolin et al. 2005).

Another method relates to a method for dialysis monitoring method and apparatus using near infrared radiation, described in WO9819592. The merits of the described method are similar to that of the UV-radiation. However, the described method does not measure uric acid and utilizes near infrared radiation spectrometry with different technical and optical considerations. For near infrared radiation spectrometry the principial component analysis using calibration and prediction stage is described in US5886347.

OBJECTS AND SUMMARY OF THE INVENTION

The objective of the invention is, therefore, a new method and a device for measuring concentration of substances, such as uric acid, in biological fluids. More specifically, the present invention relates to an optical method utilizing optical spectrum of the biological fluid and the Savitzky-Golay (or Savitsky-Golay) algorithm, and concentration calculation algorithm containing the transforming function to determine on-line the concentration of the substances, which can be effected directly at the bed-side and which avoids the disadvantages caused by the analysis in a laboratory.

Another object of the present invention is to provide a novel and practical optical uric acid detecting method and device which detects concentration of uric acid in the biological fluids and can be represented directly and easily on the monitor or screen printed. The novel method and device does not require any chemical disposables, neither expensive uricase nor both L-ascorbic acid oxidase, and can be easily made and mass-produced providing an environment-friendly optical method.

A still further object of the present invention is to provide a method for assessing routine clinical monitoring in order to face risks of higher mortality in patients (e.g. in dialysis).

A still further object of the present invention is to provide a novel, rapid, convenient and safe method for detecting concentration of substances in a liquid sample. The liquid sample can be

directly dropped on the detecting cuvette for in-vitro measurements or sent a flowing stream of fluid through a flow-cuvette for on-line monitoring. The method is suitable for household use when being applied to detect the concentration of substances in the biological fluids.

The features and advantages described herein are not all-inclusive and, in particular, many additional features and advantages will be apparent to one of ordinary skill in the art in view of the drawings, specification, and claims. Moreover, it should be noted that the language used in the specification has been principally selected for readability and instructional purposed, and not to limit the scope of the inventive subject matter.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts a block diagram of one embodiment of the invention.

Fig. 2 depicts a block diagram of another embodiment of the invention applied for uric acid concentration measurements during dialysis.

Fig. 3 shows the linear relationship between uric acid (UA) concentration measured by a known method and at the laboratory.

Fig. 4 shows the linear relationship between uric acid (UA) concentration measured by the invented (new) method and at the laboratory.

DETAILED DESCRIPTION OF THE INVENTIONS

The device according to one embodiment of the invention is shown in Fig. 1. The device for measuring concentration of certain substances (e.g., uric acid) 5 in a biological fluid 1 comprises:

an optical module 2, comprising a spectrophotometrical system, comprising a light source and a light detector;

a measuring cuvette for holding a sample of the biological fluid so that the light can be led through the sample;

a signal processing module 3 comprising a data acquisition module and a spectra processing module, and

a data representing module 4.

The light source can be either a broadband light source or a narrowband light source. If broadband light source is used, either a broadband detector and a filter can be used, or narrowband detectors. According to one embodiment, the optical module is operating in the ultra violet region (wavelength range 190-330 nm).

Another embodiment of the invention is shown in Fig. 2. The device is applied for determining the concentration of substances such as uric acid during dialysis in spent dialysate 6. The device comprises spectrophotometer 7, a signal processing unit 8 for smoothing and derivate calculation, a unit 9 for calculating concentration 10 of the substance in the spent dialysate.

The measuring cuvette can be, e.g., adapted for in-vitro measurements, or designed for the on-line measurements.

According to one embodiment, the spectra processing module is adapted to execute the Savitzky-Golay algorithm for smoothing and calculating the derivate of the measured spectra. The spectra processing module may be further adapted to execute a concentration calculation algorithm comprising a transforming function calculating the concentration of certain substance in the biological fluid.

The transforming function is based on the regression analysis in order to transform UV-absorbance (dimensionless) into uric acid concentration [mmol/L]. In the presence of a linear relationship the transforming function has the form "uric acid concentration [mmol/L] = UV-absorbance*Slope + Intercept".

The data representing module is adapted to execute a program for data representation and comprises or is connected to a data visualization module, e.g., a monitor, a display, or a printing device.

EXAMPLE

Concentration measurements of a certain substance, uric acid, in the spent dialysate is given as an example of the present invention.

Subjects: Ten uremic patients, three females and seven males, mean age 62.6 ± 18.6 years, on chronic thrice-weekly hemodialysis were included in the study at the Department of Dialysis

and Nephrology, North-Estonian Regional Hospital. The dialysate flow was 500 mL/min and the blood flow varied between 245 to 350 mL/min. The type of dialysis machine used was Fresenius 4008H (Fresenius Medical Care, Germany).

Methods: The optical module consisted of a double-beam spectrophotometer (SHIMATSU UV-2401 PC, Japan) with an accuracy of ±1% on the dialysate samples taken at pre-determined times during dialysis. Spectrophotometric analysis over a wavelength range of 190-380 nm was performed by a cuvette with an optical path length of 1 cm. The data acquisition module consisted of a PC incorporated in the spectrophotometer using UV-PC software (UV-PC personal spectrophotometer software, version 3.9 for Windows). The obtained UV-absorbance values were processed and presented by a signal processing module using Savitzky-Golay smoothing algorithm, and the derivative calculating algorithm and EXCEL (Microsoft Office Excel 2003) software (concentration calculation algorithm containing
the transforming function calculating the concentration of certain substance in the biological fluid). The data representing module was either the computer screen or a printer.

Seven dialysate samples were taken during the dialysis: in the beginning, 10, 60, 120 and 180 minutes after the start of the dialysis session, and immediately at the end of the treatment (210 or 240 minutes). Also sample from the total dialysate collection, marked as "Mixture" was included into analysis. Pure dialysate was collected before the start of a dialysis session, used as the reference solution, when the dialysis machine was prepared for starting and the conductivity was stable.

The concentrations of a substance such as uric acid (UA), were determined at the Clinical Chemistry Laboratory at North-Estonian Regional Hospital using standardized methods. On the basis of the results linear correlation coefficient was determined.

The results obtained by the closest existing method for determination of the amount of waste products in the dialysis liquid during dialysis treatment described in WO9962574, US6666840 is referred here as the „known method". Those results are compared to the results by the method subject to this invention noted as the „new method". The systematic error and random error were calculated for the known and new method using concentrations from the laboratory as the reference.

Results: The determined values of UA concentration (μmol/l) by the known method (Fig. 3) and by the new method (Fig. 4) compared to the values measured at the chemical laboratory by biochemical methods in the spent dialysate are presented. It was discovered that the linear correlation coefficient (R) and the R-squared value (R^2) between the UA concentration from the optical method and concentration of UA increases when the new method was applied.

As seen from the Table 1 determination of uric acid concentration can be done much more precisely applying the "new method". The systematic error and random error are decreased about twice compared to the known method.

Table 1: Summary results for the different methods to measure concentration of the uric acid.

	Known method	New method
N	158	158
Syst. Error [%]	-13.4	-5.8
Random Error [%]	78.3	39.9

This means that utilizing the new method the concentration of the uric acid can be predicted more accurately in terms of systematic and random error.

Although this invention is described with respect to a set of aspects and embodiments, modifications thereto will be apparent to those skilled in the art. The foregoing description of the embodiments of the invention has been presented for the purposes of illustration and description. It is not intended to be exhaustive or to limit the invention to the precise form disclosed. Many modifications and variations are possible in light of this disclosure. It is intended that the scope of the invention be limited not by this detailed description, but rather by the claims appended hereto.

Patent references:

US5357960 Method and apparatus for the quantitative determination of optically active substances, SCHMIDTKE G et al;

US2007023334, An apparatus, a system and a method relating to hemodialysis, hemodiafiltration, hemofiltration or peritoneal dialysis. HALLSTADIUS H,

BERTINSSIN G I;

US6121050, Analyte detection systems, HAN CHI-NENG A;

US6666840 Method for determining waste products in the dialysis liquid in dialysis treatment, FALKVALL T et al;

WO9819592, Dialysis monitoring method and apparatus, KEMENY G J, MAYNARD J D;

US5886347 Analytical method for multi-component aqueous solutions and apparatus for the same, INOUE M.

Non-patent references:

Fridolin, I., Magnusson, M., et al. (2002). "On-line monitoring of solutes in dialysate using absorption of ultraviolet radiation: technique description." The International Journal of Artificial Organs 25(8): 748-761.

Uhlin, F., Fridolin, I., et al. (2005). "Estimating total urea removal and protein catabolic rate by monitoring UV absorbance in spent dialysate." Nephrol Dial Transplant. 20((11)): 2458-2464.

Uhlin, F., Lindberg, L. G., et al. (2005). Total Removed Uric Acid During Dialysis Estimated by On-line Ultra Violet Absorbance in the Spent Dialysate. 3rd European Medical & Biological Engineering Conference, EMBEC'05, Prague, Czech Republic, IFMBE Proceedings 11, CD-ISSN: 1727-1983, 6 pages, IFMBE Proceedings

CLAIMS

1. A device for measuring concentration of substances in a biological fluid, the device comprising:
a measuring cuvette for holding a sample of the biological fluid;
an optical module comprising a spectrophotometrical system, comprising a light source for introducing light into the sample, and a light detector for receiving light from the sample;
a signal processing module comprising a data acquisition module, a spectra processing module and a data representing module, the data representing module adapted to execute a program for data representation and comprising a data visualization module.

2. A device as in claim 1, the light source and the light detector are operating in wavelength range 190-330 nm.

3. A device as in claim 1, the light source and the light detector are in the wavelength range 270-330 nm, suitable for uric acid measurements.

4. A device as in claim 1, wherein the spectra processing module is adapted to execute Savitzky-Golay algorithm for smoothing the measured spectrum and for calculating a derivate of the measured spectra.

5. A device as in claim 1, wherein the spectra processing module is adapted to execute a concentration calculation algorithm containing the transforming function calculating the concentration of certain substance in the biological fluid.

6. A device as in claim 1, wherein the optical module comprises a broadband light source.

7. A device as in claim 6, wherein the optical module comprises a filter and a broadband detector.

8. A device as in claim 6, wherein the optical module comprises narrowband detectors.

9. A device as in claim 1, wherein the optical module comprises a set of narrowband light sources, and a broadband detector.

10. A device as in claim 1, wherein the cuvette is a flow-cuvette for receiving a flowing stream of the biological fluid.

11. A device as in claim 1, wherein the cuvette is adapted for in-vitro measurements.

12. A device as in claim 1, wherein the cuvette is disposable.

13. A method for measuring concentration of a substance in a biological fluid, the method comprising:

introducing the sample of the biological fluid into a cuvette;

applying light with predetermined wavelengths to the sample and recording an optical spectrum of the sample;

using Savitsky-Colay algorithm for smoothing the optical spectrum and for calculating a derivate of the measured spectra; and

calculating the concentration of the substance in the sample from the derivate.

14. A method as in claim 13, comprising introducing flowing stream of the biological fluid through flow-cuvette.

15. A method as in claim 13, comprising outputting the concentration of the substance to a display device or to a printer.

16. A method as in claim 13, wherein the substance is uric acid.

17. A method as in claim 16, wherein the wavelength is in ultra violet region.

18. A method as in claim 17, wherein the wavelength is from 270 to 330nm.

19. A method as in claim 13, wherein the calculating the concentration comprises executing a transforming function.

20. A method as in claim 13, comprising dropping the sample of the biological fluid onto in-vitro cuvette.

21. A method as in claim 13, comprising obtaining the sample and introducing the sample into cuvette in home conditions.

22. A method of clinical monitoring of a patient by monitoring a concentration of a substance in patient's biological fluid, comprising:

introducing a flow of patients biological fluid into flow-cuvette;

applying light with predetermined wavelengths to the sample and recording an optical spectrum of the sample;

using Savitzky-Golay algorithm for smoothing the optical spectrum and for calculating a derivate of the measured spectra; and

calculating the concentration of the substance in the sample from the derivate;

23. A method as in claim 22, comprising recording the concentration of the substance in a memory device.

24. A method as in claim 23, comprising generating an alarm signal, if the concentration does not fall between predetermined limits.

FIG 1

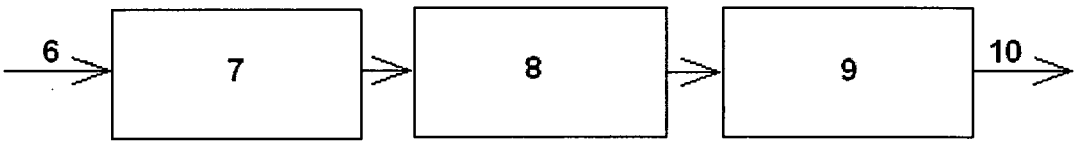

FIG 2

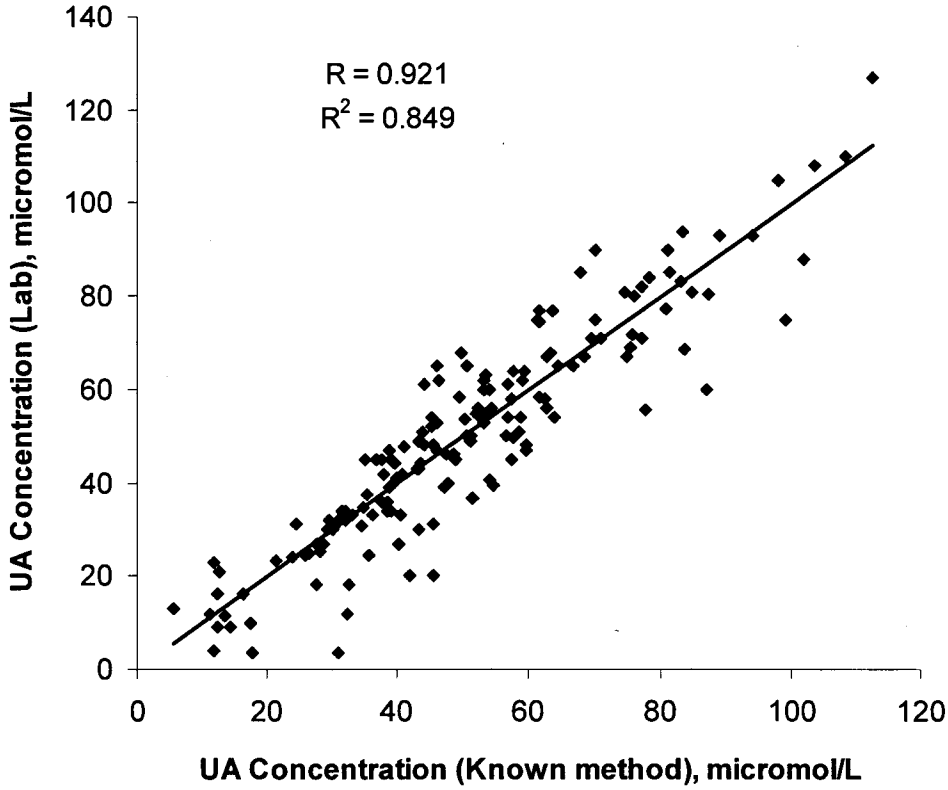
R = 0.921
R^2 = 0.849
UA Concentration (Lab), micromol/L
UA Concentration (Known method), micromol/L
0
20
40
60
80
100
120
140

FIG 3

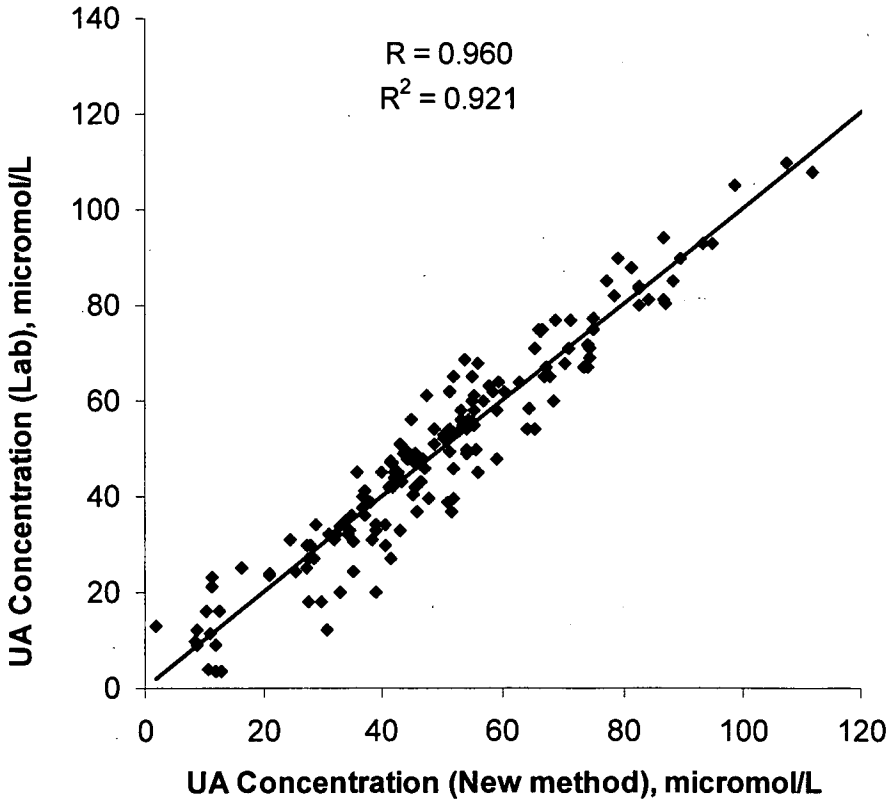
R = 0.960
R^2 = 0.921
UA Concentration (Lab), micromol/L
UA Concentration (New method), micromol/L
0
20
40
60
80
100
120
140

FIG 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EE2008/000026

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N21/33 A61M1/16 G01N33/487

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N G01J A61M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, INSPEC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FRIDOLIN I ET AL: "ON-LINE MONITORING OF SOLUTES IN DIALYSATE USING ABSORPTION OF ULTRAVIOLET RADIATION: TECHNIQUE DESCRIPTION" INTERNATIONAL JOURNAL OF ARTIFICIAL ORGANS, MILAN, IT, vol. 25, no. 8, 1 August 2002 (2002-08-01), pages 748-761, XP008101248 ISSN: 0391-3988 cited in the application abstract; figures 1-4	1-3,5-12
Y		4

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 March 2009	02/04/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Fax: (+31–70) 340–3016	Brison, Olivier

INTERNATIONAL SEARCH REPORT

International application No
PCT/EE2008/000026

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	UHLIN F ET AL: "ESTIMATING TOTAL UREA REMOVAL AND PROTEIN CATABOLIC RATE BY MONITORING UV ABSORBANCE IN SPENT DIALYSATE" NEPHROLOGY DIALYSIS TRANSPLANTATION, OXFORD UNIVERSITY PRESS, GB, vol. 20, 1 January 2005 (2005-01-01), pages 2458-2464, XP001167226 ISSN: 0931-0509 [retrieved on 2005-08-02] cited in the application figures 1,2	1-3,5, 10-12
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EE2008/000026

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