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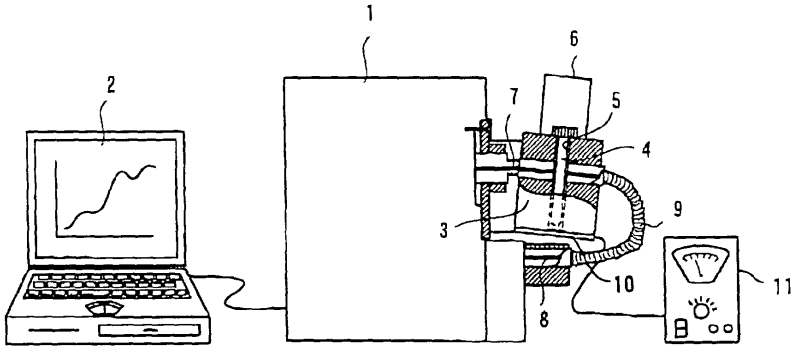
(54) Analytical method and apparatus for blood using near infrared spectroscopy

(57) First, monochromatic near infrared light in a wavelength range of 700nm-1100nm from the slit of the near infrared apparatus 1 is applied to a ceramic plate through the optical fiber 7 to measure a transmitted light intensity of the ceramic plate which is a reference material for spectrum measurement. Next, in place of the ceramic plate, a blood collection tube 4 containing a blood sample of which the temperature has been adjusted at a predetermined temperature by a water bath and the like is inserted into the housing portion 5. The transmitted light intensity of the blood sample is thus measured using the same procedure as above. A so-called near infrared absorption spectrum in which absorbance has been plotted against wavelengths is displayed on the screen of the computer 2. Information about object characteristics is extracted from the spectrum data using a calibration equation.

FIG. 1



EP 1 199 554 A2

Description

[0001] The present invention relates to an analytical method and apparatus using near infrared (NIR) spectroscopy which determine chemical components and physicochemical characteristics (hereinafter referred to as "object characteristics") of the blood such as red blood cells, hematocrit, hemoglobin, total protein, total cholesterol and blood sugar.

[0002] In the prior art, the collected blood has been separated into blood plasma and red blood cells by centrifugal separation, and the blood plasma which is the supernatant liquid has been analyzed by an automatic blood analyzer and the like to determine the object characteristics of the blood.

[0003] Simple analytical methods which are disclosed in the National Publication of the Translated Version of PCT Application Nos. Hei 5-506171 and Hei 7-503863 are also known.

[0004] According to these methods, it is possible to determine the components of the blood, e.g. glucose concentration in the blood, by applying near infrared light to a finger or an ear to measure a reflectance spectrum without collecting blood from the human body.

[0005] The method for separating the blood into blood plasma and red blood cells to automatically analyze the blood plasma that is the supernatant liquid is lacking in promptness. Such analysis not only requires the use of many reagents, but also a great deal of skill. It is therefore a problem as an analytical method of the blood carried out on site by an unskilled operator.

[0006] On the other hand, the methods that have been disclosed in the National Publication of the Translated Version of PCT Application Nos. Hei 5-506171 and Hei 7-503863 are simple, but the blood is not measured directly. Accordingly, much noise is generated and there is a problem in measurement accuracy.

[0007] It is therefore an object of the present invention to provide an analytical method and apparatus which can simply and precisely carry out an analysis of the object characteristics of the blood.

[0008] To attain the above-mentioned object, according to the present invention, an analytical method for blood is provided, which comprises the steps of applying near infrared light to the blood in a translucent blood containment means, such as a collection tube or bag, from the outside through the blood containment means, detecting diffusely reflected light, diffusely transmitted light, or diffusely transmitted and reflected light from the blood in the blood containment means using an optical sensor to measure a near infrared absorption spectrum of the blood, and applying a calibration equation, which has been made in advance from a spectrum measured using the same method as above, to the measured value so as to determine the object characteristics of the blood.

[0009] According to conventional near infrared spectroscopy, near infrared light in a wavelength range of 1100nm - 2500nm has been used. It is therefore necessary to prepare a special crystal sample cell with an optical path length of 0.1 - 2mm. Operations such as cleaning, drying and filling of the sample are therefore troublesome and require time. Further, because of the narrow optical path length, nonuniformity of the sample and existence of impurities have a great influence on measured results. However, even in the case of near infrared light, if near infrared light in a short wavelength range of 700nm - 1100nm is used, its penetration force is 10 to 100 times as large as that in a long wavelength range (1100nm - 2500nm). Accordingly, when the near infrared light in the short wavelength range is used, the optical path length can be maintained at a level of 1 - 2cm and the blood analysis can be carried out with the blood contained in the blood collection tube or bag.

[0010] When the near infrared light is applied to an object (the blood), only a specified wavelength light is absorbed in proportion to the number of molecules out of various molecules contained in the object. The wavelength of the light absorbed varies with the structure of the molecule (kind of molecule). The blood contains various kinds of components and generates a complicated absorption phenomenon in which absorptions overlap. The near infrared absorption spectrum is obtained by plotting the absorbance (i.e. the degree to which the light is absorbed) against wavelengths.

[0011] To conduct quantitative analysis by using this near infrared absorption spectrum, a regression equation (a calibration equation) that relates a value of the object characteristics (the concentration or the characteristic value) to spectrum data is required. Usually, the spectrum of a sample of which the value of the object characteristics is known is measured. Based on the spectrum data and the object characteristics value, the calibration equation can be made by a chemometrics technique such as multiple linear regression (MLR), principal component regression (PCR) and PLS regression (PLS).

[0012] Further, to attain the above-mentioned object, according to the present invention, the apparatus for blood analysis is provided, which comprises a block provided with a housing portion for a translucent blood collection tube or bag, a near infrared apparatus provided with a spectroscope for dispersing near infrared light and an optical sensor for detecting the near infrared light from a source of light or a light from a sample, light conduction means for conducting the near infrared light emitted from the light source or the spectroscope to the blood collection tube or bag within the housing portion and for conducting, directly or through the spectroscope, diffusely reflected light, diffusely transmitted light, or diffusely transmitted and reflected light from the blood within the blood collection tube or bag to the optical sensor, and control means for outputting a measurement command of a spectrum to the near infrared apparatus and for applying a calibration equation, which has been made in advance, to the measured value thereby computing object

characteristics (chemical components or physiochemical characteristics) of the blood to be measured.

[0013] As the light source, it is preferable to use a metal halide lamp (a white light source) such as a tungsten halogen lamp because of its high intensity. A diode array is considered preferable as the optical sensor because it is easy for the diode array to be compacted and there is also some possibility that the diode array will be widely used from now on.

[0014] Further, when the monochromatic near infrared light is used as the light source, it is preferable to use a silicon detector or a lead sulfide detector that is commonly used as the optical sensor.

[0015] As the light conducting means, it is preferable to use an optical fiber (a single fiber) or an optical fiber bundle (a bundle of optical fibers).

[0016] It is also possible to realize a high precision measurement if the block is provided with a temperature control means for stabilizing the blood within the blood collection tube or bag at a predetermined temperature.

[0017] The above and other objects, features and advantages of the present invention will become more apparent from the following description when taken in conjunction with the accompanying drawings.

Fig. 1 is a general view of one example of an apparatus for carrying out an analytical method for blood according to the present invention;

Fig. 2 is a partially enlarged cross-sectional view showing a condition in which the apparatus analyzes a reference material;

Fig. 3 is a partially enlarged cross-sectional view showing a condition in which the apparatus analyzes the blood;

Fig. 4 is a view showing a near infrared absorption spectrum of arterial blood and venous blood measured by a diffuse transmittance method;

Fig. 5 is a view showing the near infrared absorption spectrum of venous blood measured by a diffuse reflectance method;

Fig. 6 is a view showing an example of measurement of hemoglobin (Hb) of the blood by a near infrared spectroscopy; and

Fig. 7 is a view showing an example of measurement of hematocrit of the blood by a near infrared spectroscopy.

[0018] A preferred embodiment of the present invention will now be described with reference to the accompanying drawings. Fig. 1 is a general view showing one example of an apparatus for carrying out an analytical method for blood according to the present invention. Fig. 2 is a partially enlarged cross-sectional view showing a condition in which the apparatus analyzes a reference material and Fig. 3 is a partially enlarged cross-sectional view showing a condition in which the apparatus analyzes the blood.

[0019] An analytical apparatus for carrying out an analytical method for blood of the present invention is, as shown in Fig. 1, provided with a dispersive type of near infrared apparatus 1 and a computer 2 to control it. The near infrared apparatus 1 is provided therein with a spectroscope for dispersing the near infrared light and an optical sensor for detecting the near infrared light from the white light from a source of light. An aluminum-made block 3 is attached to the near infrared apparatus 1.

[0020] This block 3 is formed with a housing portion 5 which can contain a blood collection tube 4 therein. The upper surface of the housing portion 5 is open and a cap 6 is arranged to prevent light from entering this open portion.

[0021] One end of an optical fiber 7 is connected to the spectroscope which is provided within the near infrared apparatus 1 and the other end thereof faces the inside of the housing portion 5. One end of an optical fiber 8 is connected to the optical sensor provided within the near infrared apparatus 1 and the other end thereof faces a position opposite to the other end of the optical fiber 7 on the inside of the housing portion 5. The optical fibers 7and 8 are protected by a bellows tube 9.

[0022] Provided under the block 3 is a heating apparatus 10 such as a panel heater for stabilizing the blood within the blood collection tube 4 at a predetermined temperature. A controller 11 is arranged to control this heating apparatus 10.

[0023] A spectrum measurement procedure for a blood sample using the analytical apparatus stated above will now be explained.

[0024] First, a ceramic plate 12 which is a reference material for spectrum measurement is set within the housing portion 5 of the aluminum block 3 and a light shielding cap is set to cover the housing portion 5. The computer 2 is then operated to measure the transmitted light intensity of the ceramic plate 12. Namely, the monochromatic near infrared light in a range of 700nm - 1100nm from slits of the near infrared apparatus 1 is applied to the ceramic plate 12 through the optical fiber 7. The light diffusely transmitted through the ceramic plate 12 is detected through the optical fiber 8 by the optical sensor provided within the near infrared apparatus 1.

[0025] The near infrared apparatus 1 can scan a predetermined wavelength range in about 0.5 seconds. The near infrared apparatus usually repeats the scan about 50 times and the measurements are averaged to obtain the transmitted light intensity of the ceramic plate 12 at each wavelength.

[0026] Next, in place of the ceramic plate 12, the blood collection tube 4 containing a blood sample adjusted at a

predetermined temperature by a water bath and the like is inserted into the housing portion 5. The transmitted light intensity of the blood samples is then measured using the same procedure as above.

[0027] The absorbance as shown by a formula (1) is computed by the computer 2 and a so-called near infrared absorption spectrum in which the absorbance has been plotted against wavelengths is displayed on the screen of the computer 2.

$$A(\lambda)=\log \{Er(\lambda)/Es(\lambda)\} \quad (1)$$

wherein,

A (λ) : absorbance at the wavelength ofλnm
Er (λ) : intensity of light transmitted through the ceramic plate at the wavelength of λnm
Es (λ) : intensity of light transmitted through the blood sample at the wavelength of λnm

[0028] Fig. 4 shows the near infrared absorption spectra of arterial blood and venous blood of a goat measured in a diffuse transmittance method and Fig. 5 shows the near infrared absorption spectrum of the venous blood of the goat measured in a diffuse reflectance method. An absorption band of water of 970nm is observed in each spectrum. In the venous blood, an absorption band of 760nm due to reduced hemoglobin can be seen. It is not possible to clearly observe the absorption band due to the object characteristics, but the information for the object characteristics is also included in the same spectra. Thus, to extract the information for each object characteristic from the spectrum data, a calibration equation that relates each object characteristic to the spectrum data is necessary.

[0029] The calibration equation for measuring the object characteristics of the blood sample, e.g. the hemoglobin concentration, will be described below.

(A) Prepare at least 100 specimens of the blood sample having a wide range of hemoglobin concentrations.
(B) Collect each blood sample in the blood collection tube 4 and adjust the temperature of the blood collection tube at a predetermined temperature by a water bath. Measure the near infrared absorption spectrum of the blood sample according to the method stated above. Repeat this operation for the number of specimens.
(C) Analyze the hemoglobin concentration of each blood sample by the conventional chemical method.
(D) Input the analyzed hemoglobin concentration in the corresponding spectrum data file.
(E) Divide the spectrum data with the hemoglobin concentration into two data sets for calibration and validation.
(F) Carry out MSC treatment, derivative treatment and the like on the spectrum data of calibration set as a pre-treatment for the near infrared absorption spectrum as occasion demands.
(G) Using the pretreated spectrum data of calibration set, prepare a plurality of relational equations (regression equations) which can be candidates for the calibration equation by a chemometrics technique such as MLR, PCR and PLS.
(H) Using the spectrum data of validation set which has not been used for calibration, evaluate the performance of the relational equations made in the preceding paragraph by standard error of prediction (SEP). Adopt the equation with the smallest SEP as the calibration equation at the time of the routine analysis.

[0030] Table 1 shows a result of the PLS using the second derivative spectra of the blood measured by the diffuse reflectance method.

[Table 1]

Results of PLS regression using the second derivative spectra of the blood					
Object Characteristics	F	R	SEC	SEP	Bias
Hemoglobin (%)	3	0.99	0.26	0.28	0.00
Hematocrit (%)	3	0.99	0.81	0.86	0.04
Oxygen (%)	4	0.90	1.95	2.34	-0.03

F: Number of factors used in the calibration equation
R: Multiple correlation coefficient
SEC: Standard error of calibration
SEP: Standard error of prediction
Bias: Difference between a mean value of the values according to a conventional method and a mean value of

NIR values

[0031] The correlation coefficient between an actual value of hemoglobin (Hb) analyzed by a known chemical method and NIR measured value is 0.99 and the SEC is 0.26%. The SEP is 0.28%. In the case of the routine analysis, measurement is made with the error of this SEP value.

[0032] In the case of the routine analysis, the following calibration equation is used to measure, for example, the hemoglobin (Hb).

$$Hb\ (\%) = F1 \bullet q1 + F2 \bullet q2 + F3 \bullet q3 + F4 \bullet q4 \quad (2)$$

where,

$$Fi = \Sigma A(\lambda) \bullet Wi(\lambda)$$

Fi : i^{th} factor (where, i = 1 - 4)
A (λ) : original spectrum of the blood (absorbance at λnm)
Wi (λ) : i^{th} loading weight (where, i = 1 - 4)
qi : i^{th} regression coefficient (where, i = 1 - 4)

[0033] The concentration of hemoglobin (Hb) can be computed using the formula (2) from absorbance in each wavelength because the regression coefficient qi and the loading weight Wi (λ) are the constants to be determined according to the object characteristics. Quantitative analysis can also be carried out for hematocrit, oxygen, and other object characteristics using the same method as above.

[0034] In the routine on-site analysis and the like, an analytical procedure for the hemoglobin concentration of the blood is as follows. However, the above-mentioned calibration equation shall be stored in the near infrared apparatus 1 or the computer 2.

(A) The electrical supply of the near infrared apparatus 1 is switched on. After the near infrared apparatus 1 is stabilized, a reference spectrum is measured using the reference ceramic plate 12.
(B) The blood sample contained in the blood collection tube 4 is adjusted to a predetermined temperature by the water bath.
(C) The blood collection tube 4 containing the blood sample of which the temperature has been adjusted is loaded in the housing portion 5 of the aluminum block 3. The computer 2 is operated to allow the near infrared apparatus 1 to measure the spectrum.
(D) After the spectrum measurement is completed, the computer 2 computes the hemoglobin concentration based on the calibration equation stored therein and the spectrum obtained, and displays the hemoglobin concentration on the screen.
(E) Repeat the operations (B) - (D) for the number of samples. Time required for the operations of the steps (C) and (D) is about 30 seconds.

[0035] The relationship between an actual value analyzed by the known chemical method and the NIR value in the event that the contents of hemoglobin (Hb) and hematocrit of the blood are analyzed by a routine analysis is shown in Figs. 6 and 7, respectively.

[0036] In the above-mentioned embodiment, the object characteristics of the blood are measured using the diffuse transmittance method, but it is also possible to use the diffuse reflectance method or the transflectance (transmittance + reflectance) method. In such a case, the arrangement of the optical fiber of course differs from the above.

[0037] As described above, according to the present invention, it is possible to measure the object characteristic value of the blood with the blood contained in the translucent container such as the blood collection tube and bag. Accordingly, it is also possible to get the information about the main components of the blood on-site for blood collection. With this construction, nutritional diagnosis, medical examination or the like is realized on the spot.

Claims

1. An analytical method for blood using near infrared spectroscopy comprising the steps of:

applying light to the blood in a translucent blood containment means from the outside through the blood containment means;
detecting diffusely reflected light, diffusely transmitted light, or diffusely transmitted and reflected light from the blood in the blood containment means by an optical sensor to measure a near infrared absorption spectrum of the blood; and
applying a calibration equation, which has been made in advance from a spectrum measured using the same method as above, to the measured value so as to determine object characteristics of the blood.

2. The analytical method for blood according to claim 1, wherein the wavelength of near infrared light applied to the blood in the blood containment means is 700nm- 1100nm.

3. The analytical method for blood according to claim 1, wherein the calibration equation is made by a chemometrics technique such as multiple linear regression (MLR), principal component regression (PCR) and PLS regression.

4. The analytical method for blood according to any one of the preceding claims, wherein the translucent blood containment means is a blood collection tube or bag.

5. An analytical apparatus for blood comprising:

a block provided with a housing portion for a translucent blood containment means;
a near infrared apparatus provided with a spectroscope for dispersing near infrared light from and an optical sensor for detecting the near infrared light from a source of light or a light from a sample;
light conduction means for conducting the near infrared light emitted from the light source or the spectroscope to the blood containment means within the housing portion and for conducting, directly or through the spectroscope, diffusely reflected light, diffusely transmitted light, or diffusely transmitted and reflected light from the blood within the blood containment means to the optical sensor; and
control means for outputting a measurement command of a spectrum to the near infrared apparatus and for applying a calibration equation, which has been made in advance, to the measured spectrum thereby computing object characteristics of the blood to be measured.

6. The analytical apparatus for blood according to claim 5, wherein a white light source such as a tungsten halogen lamp is used as the light source, and a diode array is used as the optical sensor.

7. The analytical apparatus for blood according to claim 5, wherein the monochromatic near infrared light is used as the light source, and a silicon detector or a lead sulfide detector is used as the optical sensor.

8. The analytical apparatus for blood according to claim 5, wherein the light conduction means is an optical fiber or an optical fiber bundle.

9. The analytical apparatus for blood according to claim 5, wherein the block is provided with a temperature control means for stabilizing the blood within the blood containment means at a predetermined temperature.

10. The analytical apparatus for blood according to any one of claims 5 to 9 wherein the blood containment means is a blood collection tube or bag.

FIG. 1

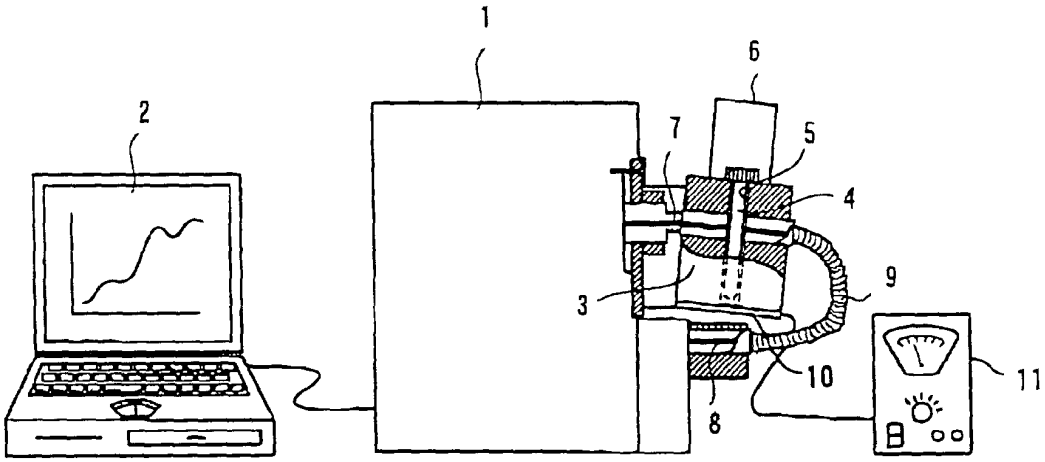


FIG. 2

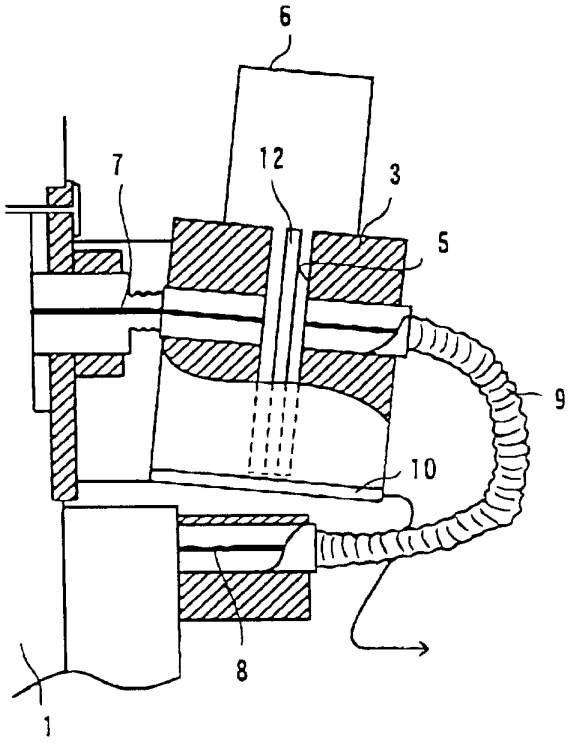


FIG. 3

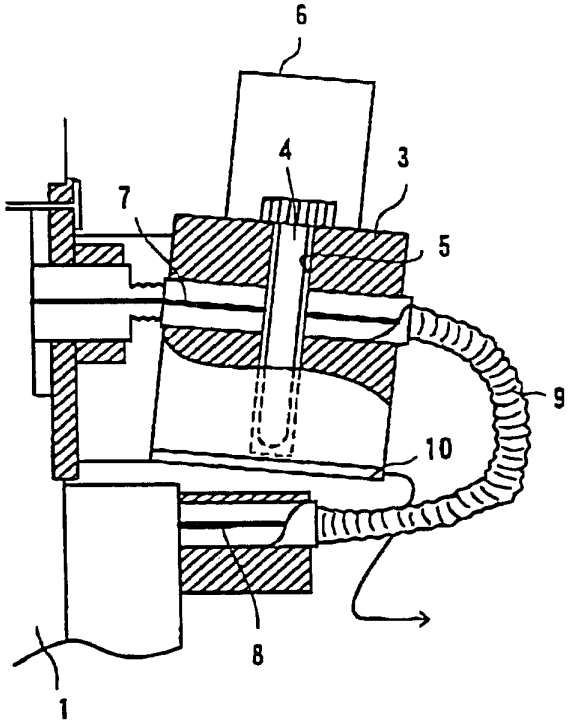


FIG. 4

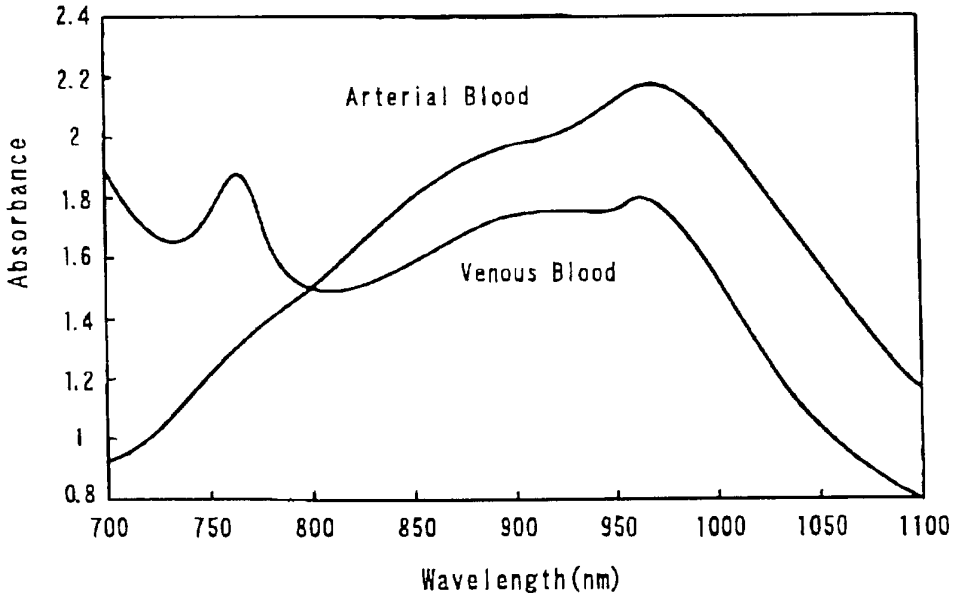

2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
Absorbance
Arterial Blood
Venous Blood
700
750
800
850
900
950
1000
1050
1100
Wavelength(nm)

FIG. 5

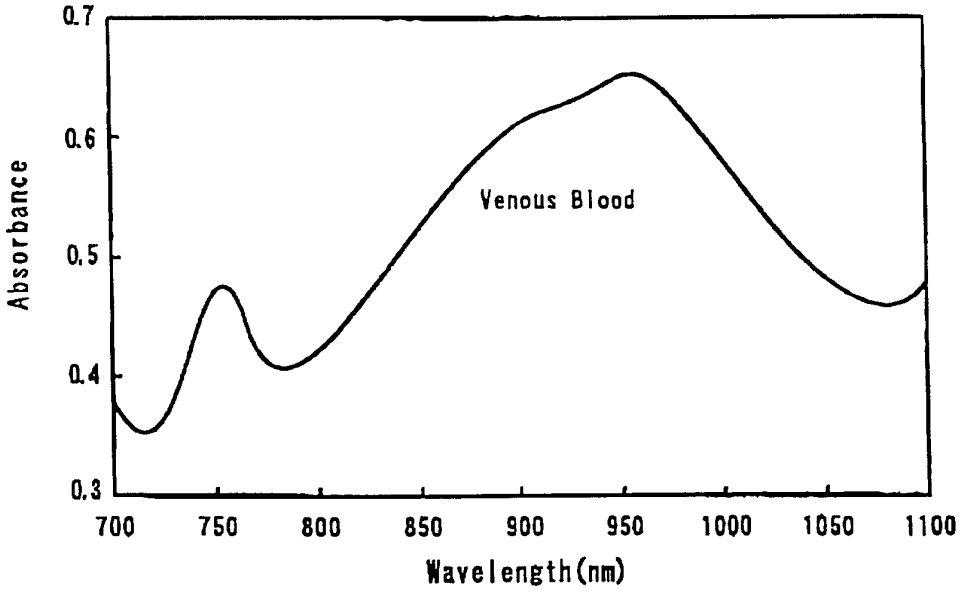

0.7
0.6
0.5
0.4
0.3
Absorbance
Venous Blood
700
750
800
850
900
950
1000
1050
1100
Wavelength(nm)

FIG. 6

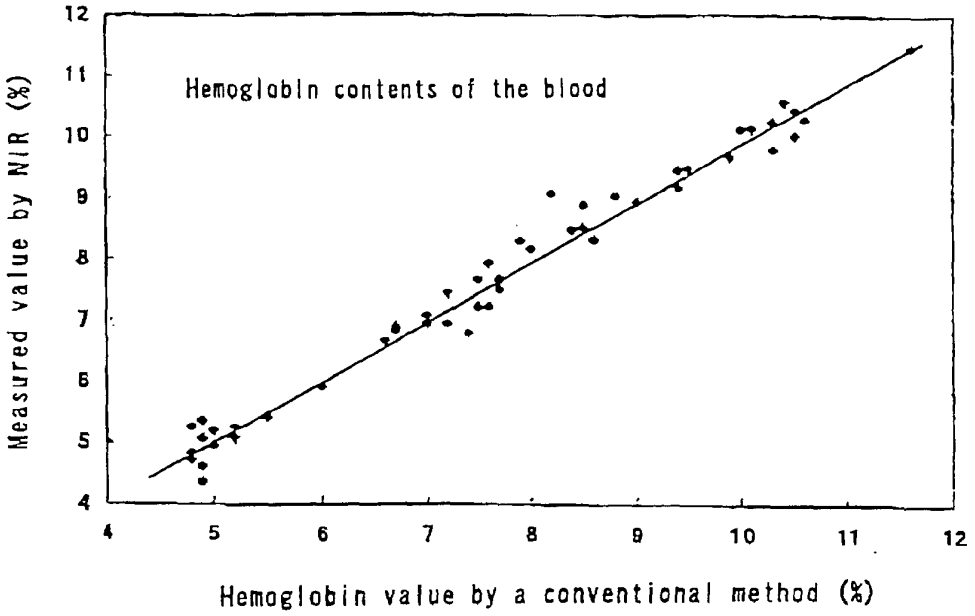
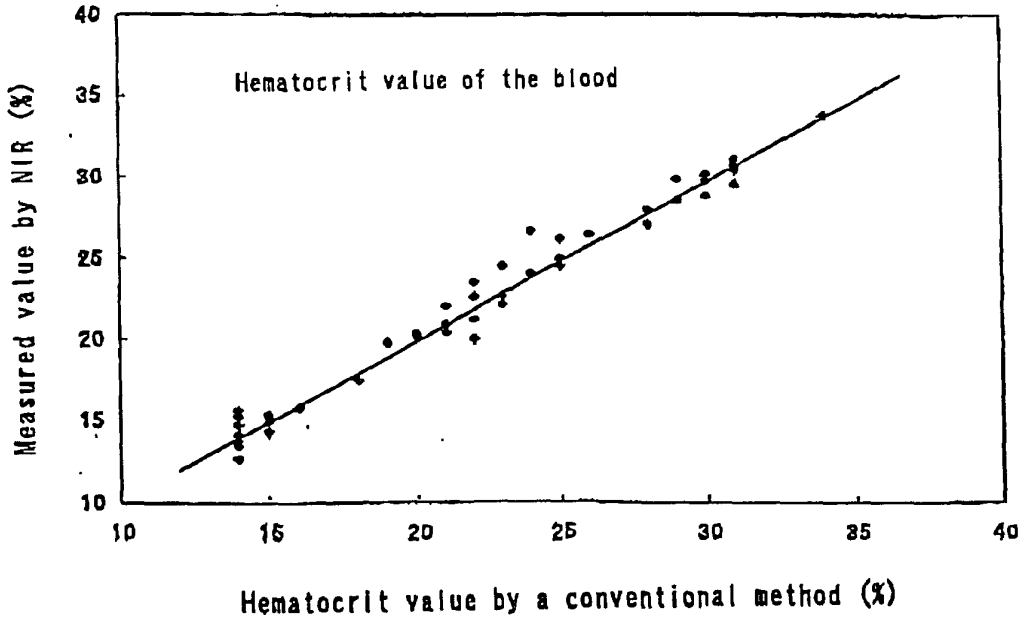

Hemoglobin contents of the blood
Measured value by NIR (%)
Hemoglobin value by a conventional method (%)
12
11
10
9
8
7
6
5
4
4
5
6
7
8
9
10
11
12

FIG. 7


Hematocrit value of the blood
Measured value by NIR (%)
Hematocrit value by a conventional method (%)
40
35
30
25
20
15
10
10
15
20
25
30
35
40

专利名称(译)	使用近红外光谱的血液分析方法和装置		
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其他公开文献	EP1199554A3		
外部链接	Espacenet		

摘要(译)

首先，通过光纤7将距近红外设备1的狭缝在700nm-1100nm波长范围内的单色近红外光施加到陶瓷板上，以测量作为参考材料的陶瓷板的透射光强度。频谱测量。接下来，代替陶瓷板，将包含血液样本的血液采集管4插入到容纳部分5中，所述血液样本的温度已经通过水浴等在预定温度下调节。因此，使用与上述相同的程序测量血样。在计算机2的屏幕上显示所谓的近红外吸收光谱，其中对波长绘制了吸光度。使用校准方程从光谱数据中提取关于物体特性的信息。

FIG. 1