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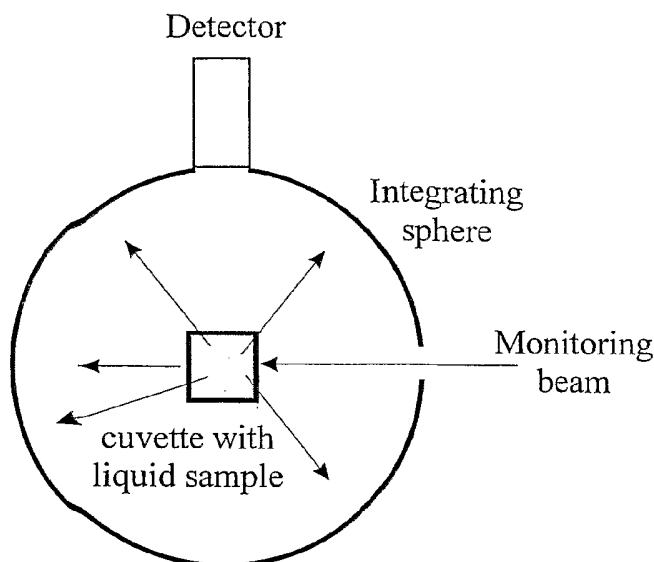
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[Continued on next page]

(54) Title: METHOD AND APPARATUS FOR DETERMINING THE ABSORPTION OF WEAKLY ABSORBING AND/OR SCATTERING LIQUID SAMPLES



(57) Abstract: The invention relates to a method and apparatus for determining the absorption of weakly absorbing and/or scattering liquid samples. The method according to the invention comprises the steps of filling the sample into a hollow measuring body having a wall with reflecting inner surface and an opening for receiving the sample and an opening or surface not coated with reflecting lining associated with a light source, illuminating the sample inside the hollow body with the light of the light source, measuring the absorbance spectrum of light received from the sample, with the further steps of transmitting the light of the light source into the sphere and from the absorbing and/or scattering sample to a detector through a light conducting means, generating a homogenous diffuse light for illuminating the sample inside the measuring body, and correcting the measured absorbance spectrum using a mathematical model. The apparatus according to the invention comprises a hollow measuring body having a wall with reflecting surface and at least one opening for receiving the sample and an opening or window not coated with reflecting lining for illuminating

the hollow body and for collecting light from the hollow body the opening or window being associated with a light source (L) and a detector (D) for receiving the collected light. The light conducting means is applied between the opening or transparent window and the light source (L) and the detector (D) respectively. A dielectric overcoating is applied to the reflective coating layer on the wall of the hollow body and a diffuser is applied to the light conductor means at the end facing to the hollow body.

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METHOD AND APPARATUS FOR DETERMINING THE ABSORPTION OF
WEAKLY ABSORBING AND/OR SCATTERING LIQUID SAMPLES

5 FIELD OF THE INVENTION

The invention relates generally to a method and apparatus for determining the absorption of weakly absorbing and/or scattering liquid samples.

- 10 More specifically the invention relates to a method for determining the absorption of weakly absorbing and/or scattering liquid samples comprising the steps of
- filling the sample into a hollow measuring body having a wall with reflecting surface and an opening for receiving the sample and an opening or surface not coated with reflecting lining associated with a light source,
 - 15 - illuminating the sample inside the hollow body with the light of the light source, and
 - measuring the absorbance spectrum of light received from the sample.

The invention also relates to an apparatus for determining the absorption of weakly absorbing and/or scattering liquid samples comprising a hollow measuring body having a wall
20 with reflecting surface and at least one opening for receiving the sample and an opening or window not coated with reflecting lining for illuminating the hollow body and for collecting light from the hollow body the opening or window being associated with a light source and with a detector for receiving the collected light.

25 BACKGROUND OF THE INVENTION

Conventionally, spectrophotometry is a convenient and reliable technique for measuring moderately absorbing optically clear samples. However, if the sample absorbs weakly and/or is not optically clear either lack of sensitivity or losses due to scattering the accuracy
30 of the measurements will decrease and the determination of the absorbance properties will not be possible. An important application is the determination of the chlorophyll absorb-

ance in freshwaters, where the chlorophyll concentration (c), carried by photosynthetic particles, is typically $c = 10^{-9} \text{ M}^{-1}$, with an absorption coefficient (ϵ) of a chlorophyll molecule of about $\epsilon = 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ and a pathlength (l) of 1 cm would imply an absorbance $A = \epsilon c l = 10^{-4}$, well under the sensitivity threshold of a conventional spectrophotometer. Other important applications include the detection of weakly allowed or forbidden transitions, flash- or chemically induced absorbance transients in dilute systems.

For weakly absorbing samples, for a given concentration the only remedy is to increase the optical pathlength of the measuring beam traversing the sample. Leaving out of account the possibility of excessively long sample holders, which are incompatible with most modern spectrophotometers, the pathlength can be made longer in multi-path cuvettes. Indeed, such a solution has been presented in an article by Haggquist GW and Naqvi KR (A simple method for prolonging the effective pathlength in laser kinetic spectroscopy, Review of Scientific Instruments, 65 (7): 2188-2189 JUL 1994).

They suggested a 1 cm rectangular cuvette that was coated from the outside with specularly reflecting layer in a geometry shown in Figure 3. With this arrangement the optical pathlength could be multiplied by a factor of about 10. Certain technical limitations arise from the facts that the beam is reflected both from the unmirrored and the mirrored surface, and from an attenuation by the absorbance of the cell walls.

Conventionally, the absorbance spectra of turbid samples are determined by placing a cuvette containing the sample inside an Ulbricht's integrating sphere, the internal surface of which is coated with a diffusely reflecting material and multiple reflections direct a fraction of the transmitted and scattered light to a detector (Figure 1). The absorption spectrum of the sample can be determined by measuring the intensity of the detected light as a function of the wavelength of the measuring beam. A photometer sphere using this principle is disclosed in US 4,310,246. This procedure thus eliminates scattering artifacts in the measured absorbance spectra. However, this method is applicable only for moderately absorbing samples.

An important development, allowing a sensitivity enhancement of more than 100x and the elimination of the scattering artifacts, was the introduction of a spherical cuvette the outer surface of which, apart from two windows serving as entrance and exit of the monitoring beam and the opening for filling/emptying, was coated by reflective or diffusely reflective material (Ketskemény et al. 1969, 1970). However, as pointed out by Ketskemény and Kozma (1969) "repeated total reflections on the wall" will lead to leakage through the entrance and exit windows. Furthermore, investigations have shown, these improvements came at the cost of distortion of the shape of the measured absorbance spectra.

10 It can be concluded from the foregoing comments that the best candidates for the ultrasensitive spectrometry are the multipath rectangular cuvettes and the Ketskemény type cavity.

Our invention aims at the elimination of principal drawbacks of these systems and elaboration of a correction procedure for retrieving the true absorption spectra, and adapting these techniques to the modern versions of spectrophotometers, laser technology, and extending their use in flash photometry and other forms of transient spectroscopy.

SUMMARY OF THE INVENTION

20

According to the invention an improved method is provided the improvement of which comprises:

- transmitting the light of the light source into the sphere and from the absorbing and/or scattering sample to a detector through a light conducting means,
- 25 - generating a diffuse light for illumination inside the measuring body, and
- correcting the measured absorbance spectrum using a mathematical model.

According to an aspect of the invention the mathematical model takes into account the geometry and material constant of the cell, and absorbance of the sample.

30

In accordance with the invention also an apparatus for determining the absorption of weakly absorbing and/or scattering samples is suggested the improvement of which comprises:

- 5 - a light conducting means applied between the opening or transparent window and the light source and the detector respectively,
- a dielectric overcoating applied to the reflective coating layer on the wall of the hollow body and
- a diffuser applied to the light conductor means at the end facing to the hollow body.

10 According to an aspect of the invention the apparatus further comprises a hollow body which is made of two parts each of them having a spherical wall with a reflecting surface and at least one opening for receiving the sample and a light conducting means for illuminating the hollow body and for collecting light from the hollow body wherein the two parts are fitted together to build a uniform spherical hollow body.

15

According to another aspect of the invention the light conducting means is applied to the hollow measuring body at the opening for filling and/or emptying the body with the sample.

20 In the apparatus according to the invention the light conducting means applied to the hollow measuring body at the opening for filling and/or emptying the body with the sample may be a bifurcated light guide.

25 According to a further aspect of the invention the hollow body may also be a cuvette having substantially planar side walls and a bottom wall with reflecting surface at least on two opposite side walls; one opening for receiving the sample and at least one window not coated with reflecting lining for illuminating the hollow body and for collecting light from the hollow body the window being associated with a light conducting means and a detector for receiving the collected light.

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Other object feature and advantages of the present invention will become apparent from a consideration of the following detailed description, and from the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

5

FIG. 1 is a schematic view of a conventional integrating sphere;

FIG. 2 is a schematic view of an integrating sphere according to the invention;

FIG. 3 is a schematic view of a multipath cuvette according to the invention;

FIG. 4 is a schematic view of assembling the parts of the integrating sphere for performing
10 a measurement according to the invention;

FIG. 5 is a schematic view of three single-beam photometer arrangements;

FIG. 6 is a schematic view of a double-beam photometer arrangement;

FIG. 7 is a schematic view of a dual-wavelength photometer arrangement; and

FIG. 8 is a schematic view of a double-beam photometer arrangement with a chopper and
15 one monochromator.

DESCRIPTION OF THE EMBODIMENTS OF THE INVENTION

The conventional arrangement of an integrating sphere (such as used by Ketskemény et al.
20 described above in more detail) as shown in Fig. 1 has been modified according to the invention as shown in Fig. 2. One improvement of the conventional arrangement used by Ketskemény et al. involves the use of a proper inner or outer coating as a diffusely or specularly reflecting surface. According to the invention a protective dielectric overcoating is applied to the reflective coating layer on the inner or outer wall of the hollow body. The
25 reflecting surface is accomplished by a reflective layer of any reflective metal such as silver or aluminium. The dielectric layer may be a composite layer of two dielectric layers with a different index of refraction. The two layers are applied as a pair of alternating layers in a number of up to 10 or up to one hundred pairs. The use of inner layers results in a higher sensitivity. Because of the direct and permanent contact with the liquid sample the protective
30 layer should provide a suitable protection against mechanical and chemical erosion as well. When using outer layers the sensitivity will be lower because of the losses caused by

the wall of the hollow measuring body but only mechanical protection is required as no chemical agents contact the layers permanently.

By these means unwanted optical effects (absorption, reflection, refraction, polarization distortion, etc.) at the walls can be eliminated.

According to another improvement a light conducting means (a light guide) is applied between the opening for filling the cavity with the liquid sample (or emptying) and the light source or illuminating the liquid sample inside the cavity. Instead of using the opening for filling/emptying a transparent surface (window) can also be used for illuminating the liquid sample inside the cavity. In this case the illuminating light guide need not to be removed while filling or emptying the integrating sphere. Another light guide is used between a second opening or transparent surface (window) and the detector. These light guides make it possible to arrange the light source and the detector at a more distant location. The flexibility of the light guide enables also the relative movement of the integrating sphere with respect to the light source and the detector. By using a bifurcated light guide (see Fig. 4 to Fig. 8), fitted into the opening for filling/emptying the cavity, the need for the windows (transparent surfaces) is eliminated. This simplifies design and further enhances the performance of the apparatus according to the invention.

It is essential for the invention that the light illuminating the liquid sample generates a homogenous photon gas distribution inside the cavity. This can be achieved by using a diffuser at the light entrance of the cavity. The diffuser may be a multifaceted prism which is attached to the end of the light guide facing the hollow body.

The sensitivity of absorption measurements can be increased by a factor of up to three orders of magnitude using the integrating sphere according to the invention due to the multiple reflections of monitoring beam inside the sphere. The measuring light can be continuous or pulsed (e.g. train of flashes).

30

The multipath cuvette of Hegguist and Naqvi can be improved by depositing mirrors, with dielectric overcoat, on the internal or external surface of the cuvette (Fig. 3). By this means, unwanted optical effects (absorption, reflection, refraction, polarization distortion, etc.) at the walls can be eliminated. For non-scattering sample much longer pathways can be obtained by using a well-collimated beam, such as emerging from a laser, and optimizing the entrance and exit windows and the angle of incidence. Proper design of the exit beam would permit the use of polychromators and multichannel detectors, which makes the instrument easy to use and/or to adopt it to commercially available diode array spectrophotometers or flash photolysis instruments.

10

Fig. 4 shows schematically the steps of assembling the parts of the apparatus according to the invention. In the first step (on the left) two hemispheres are provided with equal dimensions. One of the hemispheres (upper part) is also provided with a filling/emptying opening attached to a short filling/emptying pipe. Both the upper and the lower part is provided with a diffusely or specularly reflecting layer and a protective dielectric overcoat on the inside or outside surface. In some embodiments it might be advantageous to leave one or more windows (transparent areas) on the surface for transmitting light.

15

20

In the second step (in the middle) the two parts are fitted together facing each other with the hollow side and attached to each other permanently by gluing, sealing or the like. The integrating sphere is then filled up with the sample through the filling aperture.

25

In the third step (on the right) a bifurcated light guide is being mounted onto the sphere. A diffuser prism is attached to the penetrating (common) end of the light guide in order to foster a homogenous distribution of the photon gas in the cavity. On the other side one end of the bifurcated light guide is connected to a light source for illuminating the liquid sample inside the cavity and the other end of the light guide is connected to a detector for receiving the reflected and/or scattered light. The illumination can be continuous or pulsed (e.g. trains of flashes).

30

Fig. 5A-5C shows schematic diagrams of three different single-beam photometer arrangements: a conventional photometer arrangement with one monochromator (Fig. 5A); with a polychromator and diode-array (Fig. 5B); and with double-monochromator (Fig. 5C).

5 Fig 5A: Single-beam spectrophotometer has a light source L with the necessary collimation optics C, a dispersing element (a prism or a diffraction grating) and aperture or slit to select a narrow bandwidth quasi-monochromatic light. The light is directed into the integrating sphere IS with the aid of a light guide. A photo detector D (photomultiplier or photodiode) measures the absolute intensity of outgoing light. The photo detector D is connected to a
10 data acquisition and analysis unit. The data acquisition and analysis unit also serves as a controller for the light source L and the monochromator M. Light-induced absorption changes could also be measured using e.g. a flash lamp or other actinic illumination, which is directed into the cavity by an additional light guide (grey line). For chemically induced absorbance transients, the same opening can serve for injecting different reagents.

15

Fig. 5B: Similar arrangement as described above. Here a broadband illumination is used and the spectral resolution is provided by the combination of a polychromator and a diode array on the detection side. The illumination can be a continuous light source or a flash lamp. In this latter case the detector performs intensity measurements synchronously with
20 light emission from the flash lamp. The necessary synchronicity is controlled by the data acquisition and analysis unit, which is connected to the diode array and the light source. Light-induced absorption changes can also be measured using e.g. an additional flash between two monitoring flashes (dashed vertical line). Again, the light is directed to and from the sphere with a light guide.

25

Fig. 5C: With this arrangement one can perform three different types of measurements. (i) The conventional absorption spectrum can be measured on non-fluorescing samples by the simultaneous scanning of the two monochromators M1 and M2 on the illumination and the detection side, respectively. In this case the monochromators are set to the same wave-
30 lengths).

(ii) The emission spectrum of a fluorescent sample can be measured if the wavelength of the illuminating light is fixed (by setting M1) within a specific range where the sample can absorb it and the monochromator on the detection side (M2) scans through the wavelength range where the fluorescent sample emits light. (iii) The excitation spectrum of a fluorescent sample can be measured if the detection wavelength is fixed (by setting M2) to a specific value where the sample emits fluorescent light and the wavelength of the illuminating light is scanned through the range where the sample can absorb it. The data acquisition and analysis unit (signal processing and control unit) is connected to the monochromators M1, M2 and the detector D.

10

Fig. 6 shows a schematic drawing of a double-beam photometer arrangement. The light of light source L passes through collimator lenses C1, C2 and a monochromator M before reaching at a beam splitter B. The beam-splitter B divides the measuring beam, which is then directed into the sample and the reference path, respectively. Since the two beams are not combined after passing through the sample and the reference, two detectors D1, D2 are needed to measure the intensities. Using an attenuator (which can be either another integrating sphere or a neutral density filter) in the reference path the intensity of the sample and reference beam could be brought into similar ranges. This makes the intensity measurements more reliable because the same sensitivity range of the detectors could be applied for the sample and the reference beams. Light-induced absorption changes can also be measured if one is using another light guide to direct the actinic light (e.g. train of flashes) into the sphere (grey line). The data acquisition and analysis unit is connected to the detectors D1, D2 and the light source L.

25

Fig. 7 shows a schematic diagram of a dual-wavelength photometer. In this mode the light of the light source L passes through a collimator lens C and is projected onto a rotating mirror or chopper CH, which alternately directs the beam through the two monochromators M1, M2 several times per second. The difference in the absorbance of a sample is measured at two selected wavelengths provided by the two monochromators M1 and M2. Dual-wavelength spectroscopy is automatically performed by alternating two incident light beams of different wavelengths. One beam is fixed at a specific wavelength while the other

30

- 10 -

is scanned over a given wavelength range. Light-induced absorption changes can also be measured if a further light guide is used to direct the actinic light (e.g. a train of flashes) into the sphere (grey line). As in other arrangements, the absorption changes upon the addition of chemical reagents can also be measured using an additional injector opening on the sphere. The data acquisition and analysis unit is connected to the detector D, the monochromators M1, M2, the rotating mirror CH and the light source L in order to control them according to a measuring program.

Fig. 8 shows a schematic diagram of a double-beam photometer arrangement with a chopper CH and one monochromator M. In a double-beam spectrophotometer the light passes through collimator lenses C1, C2, a monochromator M and a beam chopper CH, which alternately directs the beam through the sample or an attenuator several times per second. Using an attenuator in the reference pathway the intensity of the sample and the reference beam can be set in similar range and this makes easier the intensity measurement because the same sensitivity range can be applied in the detector D for measuring light intensity of the sample and reference beam. Light- or chemically-induced absorption changes can also be measured as in other arrangements, e.g. using a flash, which is guided into the sphere by an additional light guide (grey line).

As the arrangement of the different embodiments provide an intensity signal received from the sample and converted by the detector which is combined with distortions in the measured spectrum, the measuring procedure requires a correction of this distortion in order to provide correct absorption spectra.

Therefore the method according to the invention also involves a mathematical correction procedure, the empirically determined parameters of which depend on the geometry and material constant of the cell, and on the absorbance of the sample. We have found at least two simple relations which fit the data extremely well, and permit us to convert the measured absorbance to the true value.

30

Let $A^*(\lambda; C)$ denote the measured absorbance at wavelength λ and a solute concentration C . We found that the concentration dependence of the measured absorbance can be fitted to the following expression

$$A^*(\lambda; C) = \frac{aC}{b+C} \quad (1)$$

5 Note the following limits:

$$A^* \xrightarrow{C \rightarrow 0} \frac{aC}{b} = A \quad (2)$$

$$A^* \xrightarrow{C \rightarrow \infty} \frac{aC}{C} = a \quad (3)$$

Rewrite Eq. (1) as

$$10 \quad A^*(\lambda; C) = \frac{aC}{b+C} = \frac{aC/b}{1+C/b} = \frac{A}{1+A/a} \quad (4)$$

From Eq. (4), it follows that

$$A = \frac{aA^*}{a - A^*} \quad (5)$$

For known values of A^* and a , we can determine the true absorbance A . The true absorbances so calculated should all be superimposable (when multiplied by a normalizing factor
15 which accounts for the concentration dependence); this seems to be the case.

CLAIMS:

1. Method for determining the absorption of weakly absorbing and/or scattering liquid samples comprising the steps of
 - 5 - filling the sample into a hollow measuring body having a wall with reflecting inner surface and an opening for receiving the sample and an opening or surface not coated with reflecting lining associated with a light source,
 - illuminating the sample inside the hollow body with the light source,
 - measuring the absorbance spectrum of light received from the sample,
 - 10 characterized in comprising the further steps of
 - transmitting the light of the light source into the sphere and from the absorbing and/or scattering sample to a detector through a light conducting means,
 - generating a homogenous diffuse light for illuminating the sample inside the measuring body, and
 - 15 - correcting the measured absorbance spectrum using a mathematical model.
2. Method as claimed in claim 1, characterized in that the sample is illuminated with a flash light.
- 20 3. Method as claimed in claim 1, characterized in that a chemical agent is added to the sample.
4. Method as claimed in claims 1 to 3, characterized in that the mathematical model is used taking into account the geometry and material constant of the cell, absorbance of the
 - 25 sample.
5. Apparatus for determining the absorption of weakly absorbing and/or scattering liquid samples comprising a hollow measuring body having a wall with reflecting surface and at least one opening for receiving the sample and an opening or window not coated
 - 30 with reflecting lining for illuminating the hollow body and for collecting light from the hollow body the opening or window being associated with a light source (L) and a detector

(D) for receiving the collected light, characterized in that a light conducting means is applied between the opening or transparent window and the light source (L) and the detector (D) respectively,

5 a dielectric overcoating is applied to the reflective coating layer on the wall of the hollow body and a diffuser is applied to the light conductor means at the end facing to the hollow body.

6. Apparatus according to claim 5, characterized in that the hollow body is made of two parts each of them having a spherical wall with reflecting surface and at least one
10 opening for receiving the sample and a light conducting means for illuminating the hollow body and for collecting light from the hollow body with the two parts being fitted together to build a uniform spherical hollow body.

7. Apparatus according to claim 6, characterized in that the light conducting means is
15 applied to the hollow measuring body at the opening for filling and/or emptying the body with the sample.

8. Apparatus according to claim 7, characterized in that the light conducting means
20 applied to the hollow measuring body at the opening for filling and/or emptying the body with the sample is a bifurcated light guide.

9. Apparatus according to claim 5, characterized in that the hollow body is a cuvette
25 having substantially planar side walls and a bottom wall with reflecting surface at least on two opposite side walls; one opening for receiving the sample and at least one window not coated with reflecting lining for illuminating the hollow body and for collecting light from the hollow body the window being associated with a light conducting means and a detector for receiving the collected light.

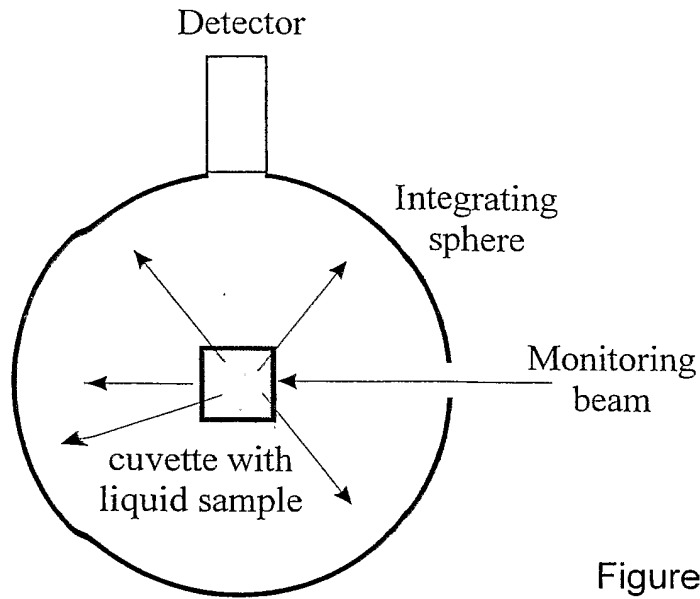


Figure 1

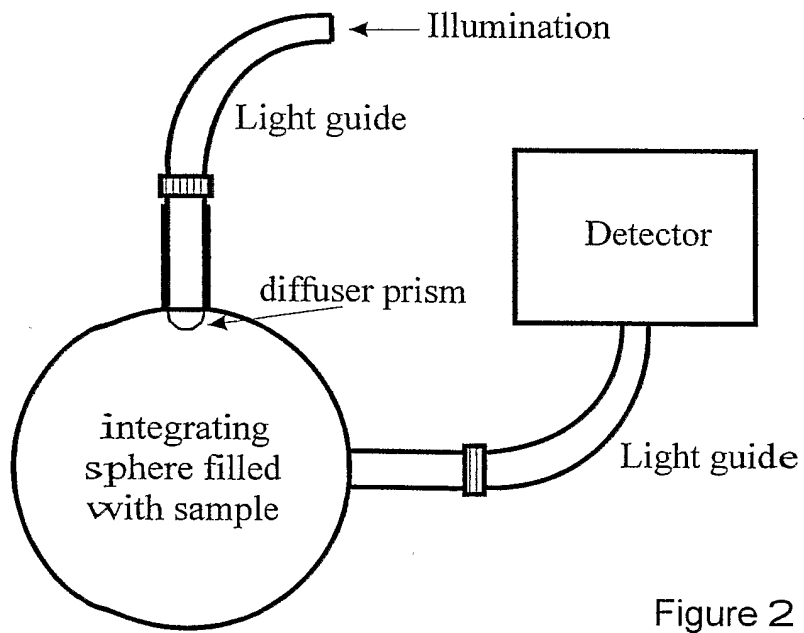


Figure 2

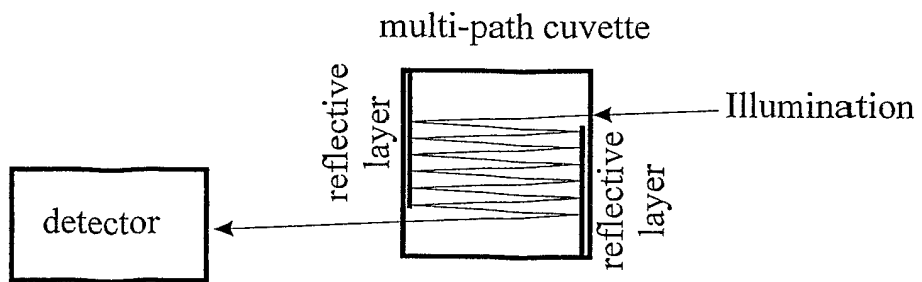


Figure 3

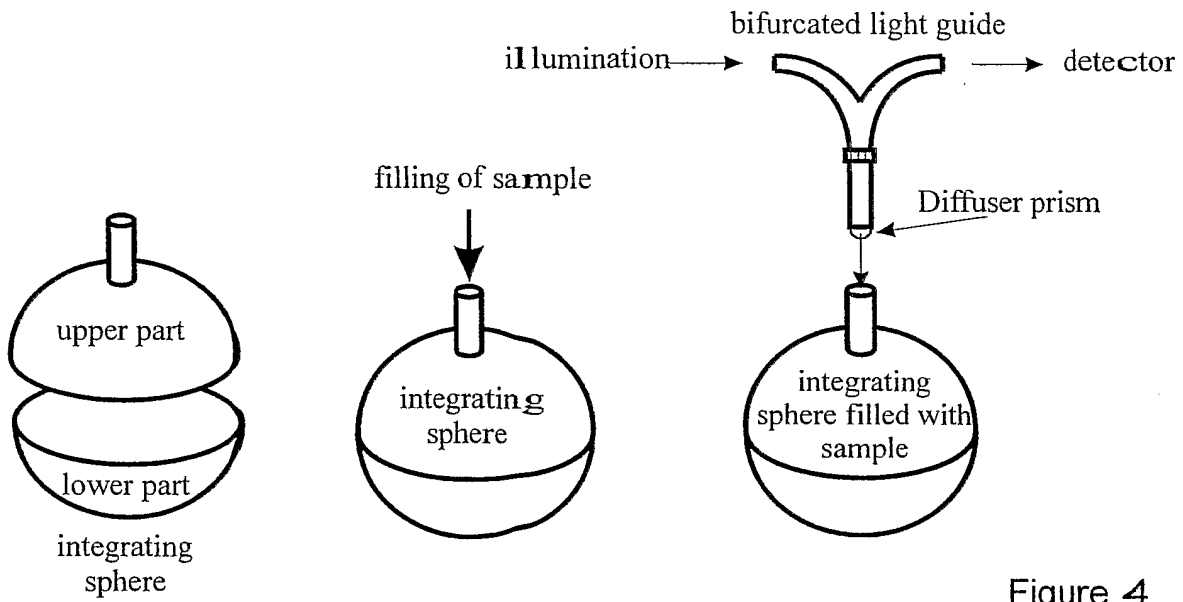


Figure 4

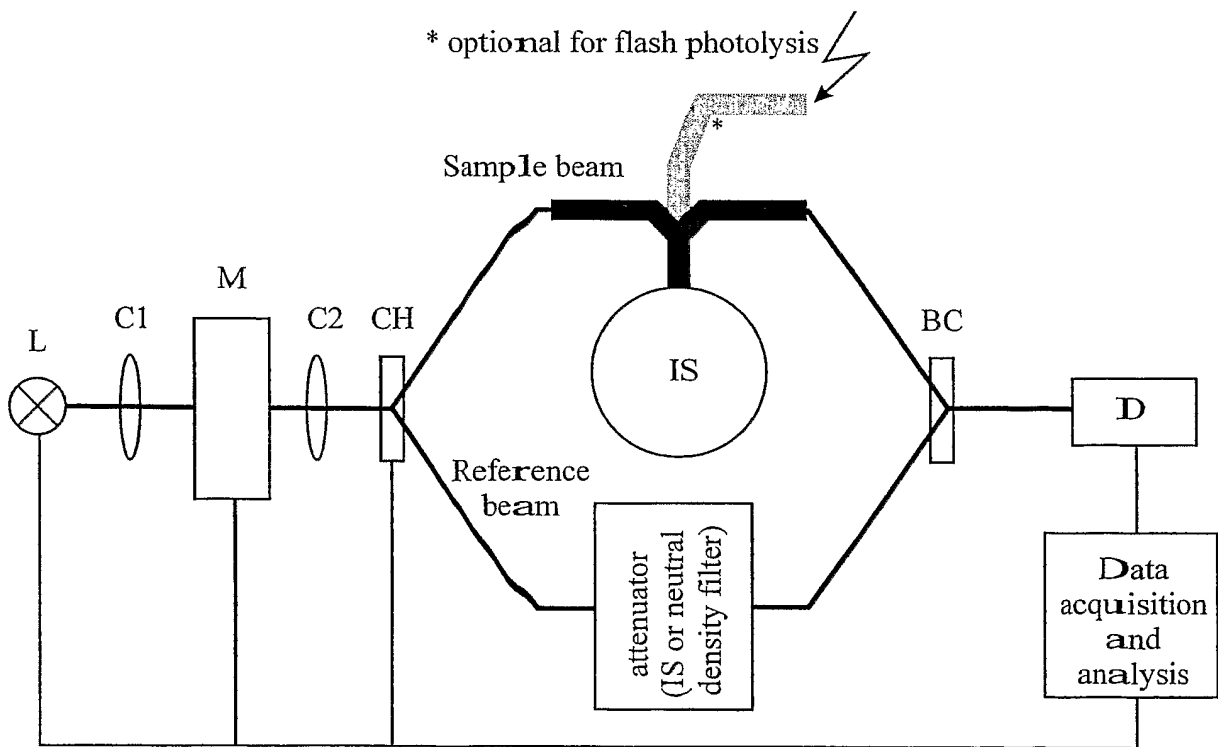


Figure 8

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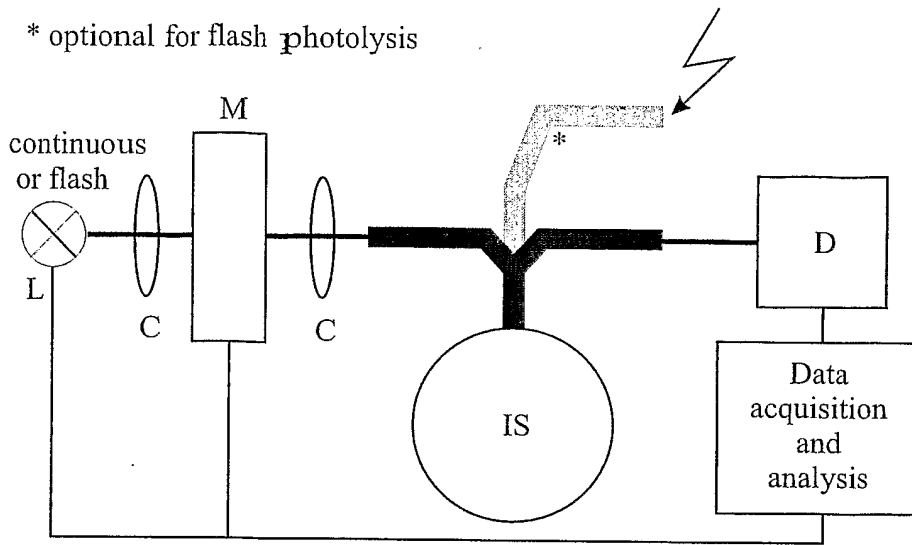


Figure 5A

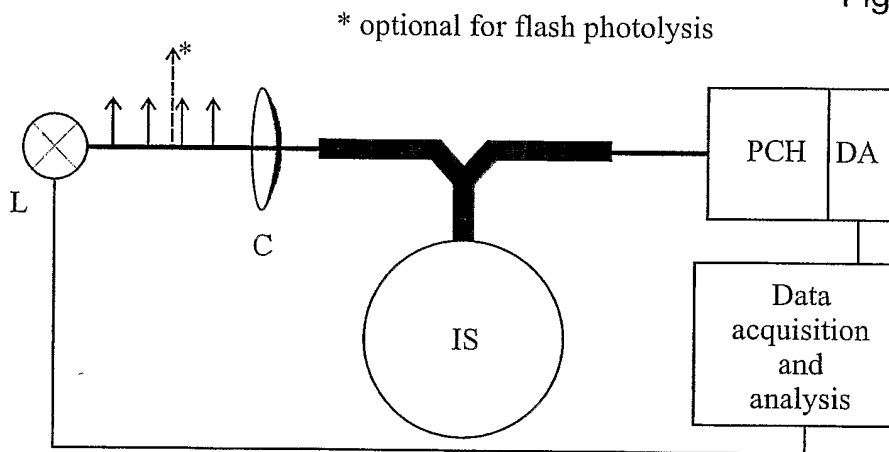


Figure 5B

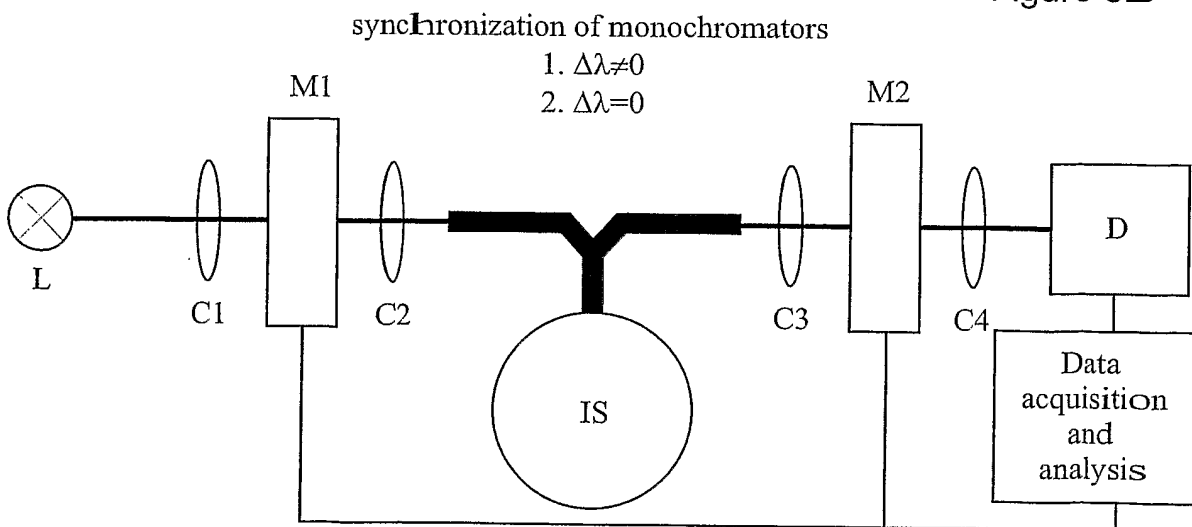


Figure 5C

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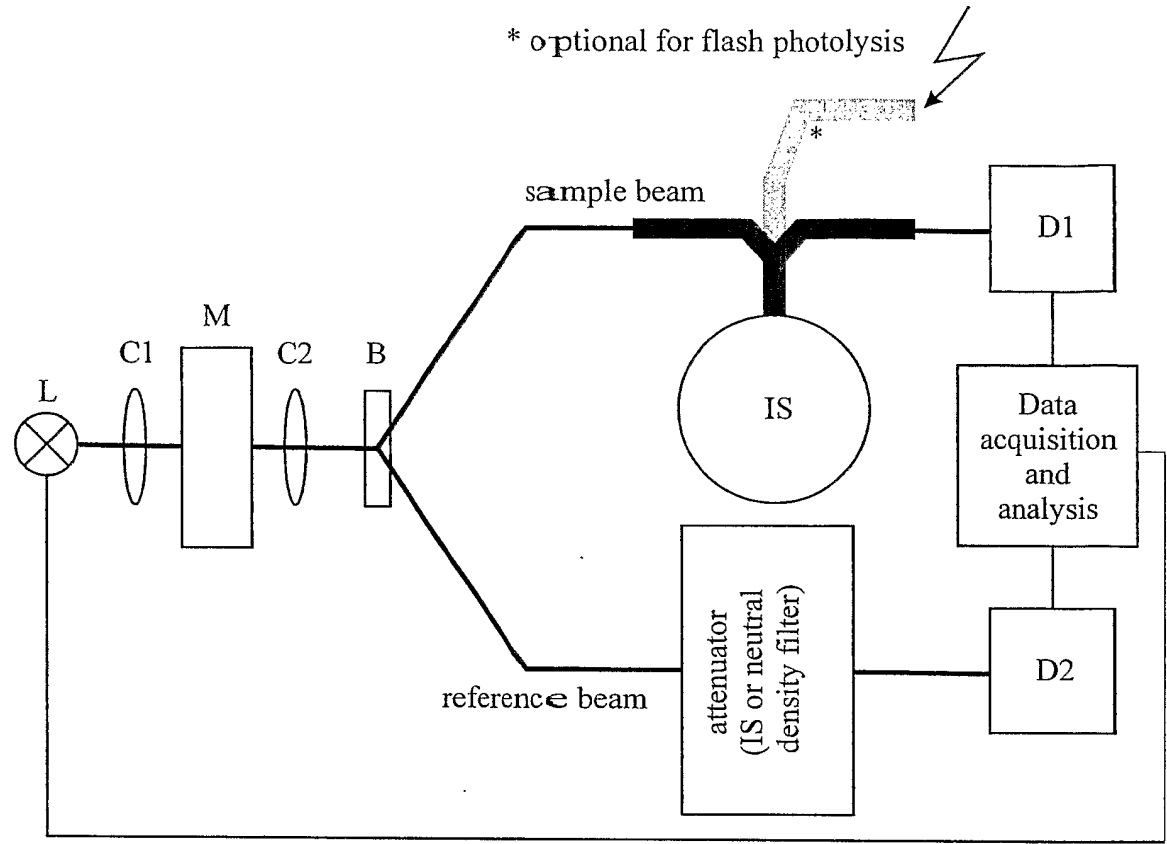


Figure 6

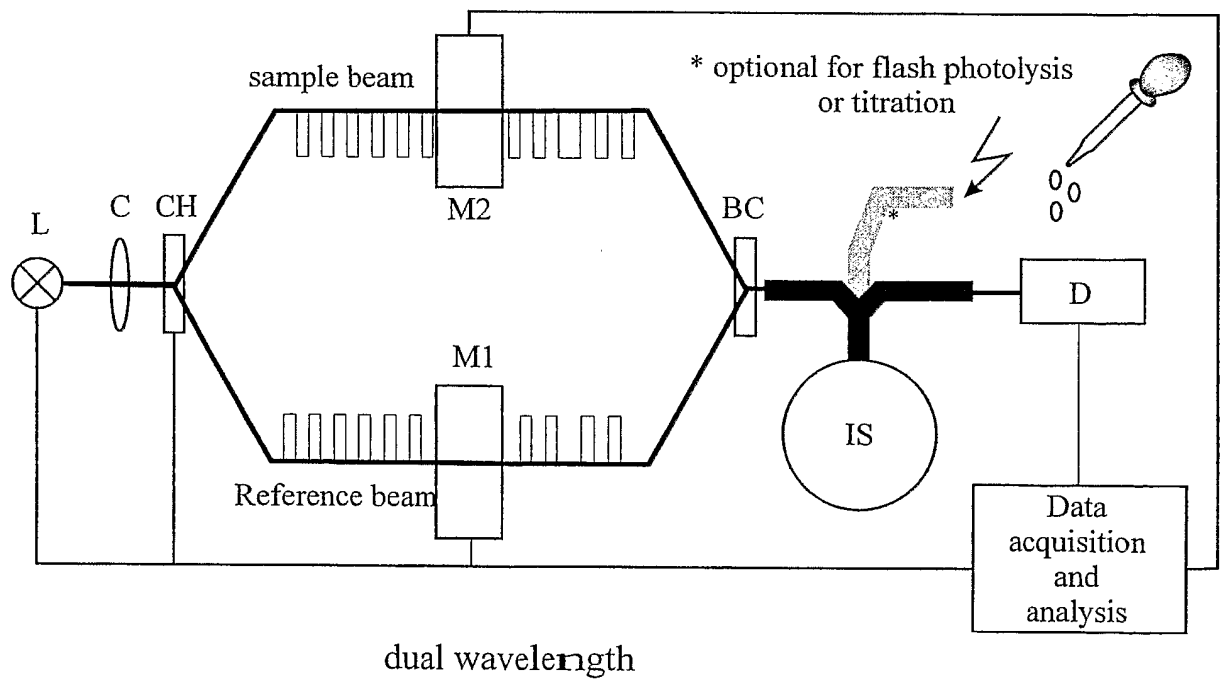


Figure 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/HU 2005/000038

A. CLASSIFICATION OF SUBJECT MATTER
G01N 21/17, G01J 3/42

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 21/00-21/59, 2 1/84-21/958, 35/00-35/10, 37/00, G01J 3/00-3/10, 3/28-3/457

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 practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	GB 2394542 A (MARK JOHNSON) 28. 04. 2004, p. 3-5, fig. 7	1, 3-9
A	US 4645340 A (BOSTON UNIVERSITY) 24. 02. 1987	1-9
A	SU 1191784 A (ИНСТИТУТ МЕДИКО-БИОЛОГИЧЕСКИХ ПРОБЛЕМ) 15. 11. 1985	1-9
A	RU 2125267 C1 (ДАНФОСС А/С) 20. 01. 1999	1-9

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