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(54) Title: METHOD AND DEVICE FOR MEASURING CIRCULAR DICHROISM IN REAL TIME

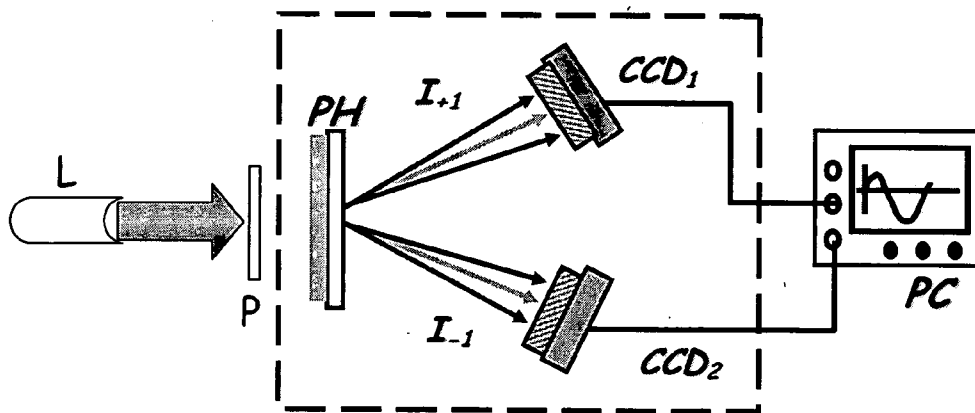


Fig. 3

(57) Abstract: Method and device for measuring the circular dichroism of a sample in which a beam of light passes through the said sample, characterised by the fact that the said beam successively passes through a grating and that the diffracted beams are collected and the signals of the diffracted beams are sent to a computer that through a software calculates the value of the circular dichroism, carrying out the logarithm of the diffracted beams intensities ratio.

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Method and device for measuring circular dichroism in real time

Field of the technique

The present invention concerns a method and a device for measuring circular dichroism using a diffraction grating on a material having an induced linear optical anisotropy.

The above-mentioned grating generates only the fields diffracted to the first order, whose amplitudes are proportional to the ones of the right and left circular components of the light beam impinging onto the grating. The method and the device of the present invention demonstrate that, by using these characteristics of the grating, the circular dichroism of a medium can be easily determined by sending the light beam coming from the medium to be analysed (transmitted or reflected), onto the grating, and measuring the intensity of the beams diffracted by the grating.

State of the art

The systems conventionally used to measure circular dichroism generally require an intense source of light with a wide spectrum, a monochromator, modulators of polarisation, and use phase sensitive detectors (lock-in amplifiers) tuned to the frequency and the phase of the modulator.

They are generally constituted by several optical elements placed before and after the sample to be analysed, and some of these elements are used to alternatively select the right and left circular polarisation of the wave striking the sample, while others are dispersive elements (prisms or gratings) necessary to select the various wavelengths, etc..

The patent application US2004156051A1 describes a method and a device for measuring the birefringence starting from a circularly polarised light beam that

strikes the sample and that uses the Stokes' parameters. In the method and the device described in US2004156051A1, the presence of a polarizer to select the circular polarisation is necessary.

All the systems that are based on conventional methods have moving parts and/or that modulate the light (wavelength and polarisation), therefore limiting their use for dynamic processes studies to the characteristic time of the moving or modulating parts. The method and the device of the present invention propose to overcome the difficulties and disadvantages reported in the state of the art.

The principal aim of the invention is to create a method for measuring the circular dichroism of a sample, in which a beam of light passes through said sample, and said light beam successively passes through an optical element (PH) constituted by a film of material with a spatially modulated linear optical anisotropy. The diffracted beams are detected and the signals of the diffracted beams are sent to a computer which, through a software, calculates the circular dichroism spectrum, performing the intensity ratio of the diffracted beams.

Another important objective is to create a device that permits the method of circular dichroism measurement described above to be realized.

Another characteristic is given by the fact that the optical element with spatially modulated linear optical anisotropy is a diffracting grating.

Another characteristic is given by the fact that the sensors for revealing the beams are multi-channel light detectors.

Another characteristic is given by the fact that the multi-channel detectors can be arrays of photodiodes.

Another characteristic is given by the fact that the multi-channel detectors can be CCDs (Charged coupled devices).

These method and device permit to create a compact spectrograph for measuring circular dichroism in real time.

Said device allows structural information and detailed enantiomeric information about various systems, such as, for example, proteins, carbohydrates, nucleic acids, pharmaceuticals, liquid crystals, etc. to be obtained. The real time measurement of the circular dichroism permits studies of both dynamic processes and kinetics of chiral molecules.

A compact device allows making a portable version of the instrument.

The method and the device proposed permit the following improvements and advantages:

- 1)** the method is based on the use of a single element, other optical elements (prisms, mirrors, wave plates, etc.) are not required, implying a reduction of the light losses;
- 2)** the dispersion properties of the grating allow measurements in real time to be taken, in fact the signal at every wavelength diffracted at different angles can be detected in parallel by a photodiodes array or a CCD;
- 3)** use of polarised light is not necessary;
- 4)** monochromator and phase sensitive detector are not necessary;
- 5)** moving or time-modulated elements are not present;
- 6)** measure of the light beam intensity striking the sample is not necessary, also the method is not sensitive to the intensity fluctuations;
- 7)** linear polarisation, random linear or non-polarised light can be used to measure the circular dichroism;
- 8)** the method does not require complex and repetitive calibration procedures;

- 9) decrease of the production costs because it does not require optical and/or electro-optical elements as the standard systems do;
- 10) performances enhancement, real time measurements due to the absence of moving elements, extremely simplified calibration procedures of the system;
- 11) real time measurement implies the possibility to investigate dynamic processes (new market areas);
- 12) the device can be integrated into multifunctional apparatus and is able for "in situ" measurements.

Synthetic description of the figures

Fig.1 diffraction grating, schematic representation of the spatial modulation of the optical axis in the material;

Fig.2 outline of the method in accordance with the present invention;

Fig.3 schematic view of the device in accordance with the present invention.

Description of the invention

The diffracting grating, on whose properties the proposed method is based, is a film of a material with spatially modulated linear optical anisotropy (dichroism or linear birefringence). The spatially modulated optical anisotropy in the present context indicates that:

- 1) the angle that the optical axis of the material forms with the x-direction indicated in Figure 1, linearly and continuously varies versus the coordinate x.
- 2) the optical axis orientation is uniform along the y- and z-coordinates (Figure 1).

Transmission and diffraction properties of the grating are explained below in order to illustrate the proposed method.

Let us consider a plane monochromatic wave with an arbitrary polarisation that impinges along the normal (axis z , Figure 1) on the grating.

Using the Jones' formalism to describe the polarised light propagation through the above-mentioned grating, the Jones' vector of a light beam with arbitrary polarisation can be written as follows: ^[1]

$$\begin{pmatrix} E_x \\ E_y \exp(i\theta) \end{pmatrix}, \quad (1)$$

where E_x e E_y are the field components along x - and y -axis, while θ represents the phase difference between the two components.

The transmission matrix of the grating can be written in the following way: ^[2]

$$T_r = \begin{vmatrix} a + 2b \cos qx & 2b \sin qx \\ 2b \sin qx & a - 2b \cos qx \end{vmatrix}$$

In the case of a material with induced linear dichroism, a is the average transmission of the grating, and $b = \Delta T / 2$, where ΔT is the linear dichroism. In the case of a material with induced linear birefringence, $a = \cos(\Delta\phi)$ and $b = i(\sin(\Delta\phi)) / 2$, with $\Delta\phi = \pi \Delta n d / \lambda$, Δn is the birefringence, d is the layer thickness of the material and λ is the wavelength of the incoming wave.

Only the first and the zero order diffracted beams are transmitted by this grating, and the three waves fields E_0 (zero order - transmitted beam), E_{+1} and E_{-1} can be written in the following way:

$$\mathbf{E}_0 = a \begin{pmatrix} E_x \\ E_y \exp(i\theta) \end{pmatrix} \quad (2)$$

$$\mathbf{E}_{+1} = b(E_x - iE_y \exp(i\theta)) \begin{pmatrix} 1 \\ -i \end{pmatrix} \quad (3)$$

$$\mathbf{E}_{-1} = b(E_x + iE_y \exp(i\theta)) \begin{pmatrix} 1 \\ i \end{pmatrix} \quad (4)$$

The transmitted wave (equation 2), zero order, has the same polarisation state of the incident wave, but its amplitude is reduced by the factor a . The first order diffracted beam +1 (equation 3) is a wave having left circular polarisation but its amplitude is proportional to the one of the right component of the incident wave. The diffracted beam of the -1 order has a right circular polarisation but its amplitude is proportional to the one of the left component of the incident wave (equation 4).

In any case, the diffraction occurs only onto the first orders +1 and/or -1. If the incoming beam is elliptically polarised, according to both the direction of rotation (right or left helicity) and the ellipticity, energy transfer between the two diffracted beams can be obtained, however the sum of the two beams intensities remains constant. In particular: 1) if the beam striking the grating is linearly polarised, the intensities of the two diffracted beams are equal; 2) conversely, if it is circularly polarised, only the order +1 or -1 is present depending on the helicity.

On the basis of the described diffracting grating properties, the method that we propose for measuring circular dichroism can be explained as follows, with the help of Figure 2.

Circular dichroism is generally calculated by measuring the transmitted intensity when a beam with right or left circular polarisation strikes the sample by using the following expression:

$$\Delta A = \log \frac{I_{0L}}{I_{TL}} - \log \frac{I_{0R}}{I_{TR}} \quad (5)$$

where I_{0L} and I_{0R} are the intensities of left and right polarised beams that alternatively strike a sample, while I_{TL} and I_{TR} are the intensities of the left and right circularly polarised transmitted light, respectively.

In the proposed method, the light beam, which strikes the sample to be analysed, can have a linear polarisation or a random linear polarisation or a non-polarised state, when the sample is an isotropic system or a liquid or a solution, etc..

The method requires the use of non-polarised light or random linear polarisation when analysing samples that present linear birefringence (crystals, oriented polymers, liquid crystals, etc.).

The thorough discussion concerns to the use of linearly polarised light; however the extension to non-polarised light will be also considered.

For a linearly polarised wave the incoming beam can be considered as composed of two opposed circularly polarised waves (one right circular and one left circular) of equal amplitude. This allows us to write the Jones' vector of this wave in terms of opposite circularly polarised states, right and left:

$$\mathbf{E}_0 = \frac{E_x}{2} \begin{pmatrix} 1 \\ -i \end{pmatrix} + \frac{E_x}{2} \begin{pmatrix} 1 \\ i \end{pmatrix}$$

If the medium (or, in our case, the sample to be analysed) that is passed through by this wave has circular dichroism, then the right circular polarised component is absorbed differently with respect to the left component, according to the circular dichroism sign. As a consequence, the wave transmitted by the sample will have an elliptic polarisation, whose Jones' vector

$$\mathbf{E}_T = \begin{pmatrix} E_x \\ E_y \exp(i\theta) \end{pmatrix}$$

can be expressed in terms of right and left circular components as follows:

$$\mathbf{E}_T = \frac{1}{2} (E_x - iE_y \exp(i\theta)) \begin{pmatrix} 1 \\ -i \end{pmatrix} + \frac{1}{2} (E_x + iE_y \exp(i\theta)) \begin{pmatrix} 1 \\ i \end{pmatrix}.$$

If the grating is placed immediately after the sample the wave (transmitted or reflected) coming from it and impinging onto the grating will be diffracted in the way described above.

Therefore, using the expressions (3) and (4) it is possible to write the intensities of the beams diffracted from the grating in the following way:

$$I_{+1} = 2b^2 I_{TR} \quad \text{and} \quad I_{-1} = 2b^2 I_{TL}. \quad (6)$$

In the case of linear polarisation of the beam impinging onto the sample, the intensity of the left circular polarised component, I_{0L} , is equal to the intensity of the right circular component, I_{0R} , therefore $I_{0L} = I_0/2$ and $I_{0R} = I_0/2$, can be expressed both in terms of the total intensity, I_0 ; while I_{TL} and I_{TR} can be written in terms of the

intensities of the beams diffracted by the grating, I_{+1} and I_{-1} , by using expressions (6):

The expression (5) becomes

$$\Delta A = \log \frac{I_0 2b^2}{2I_{-1}} - \log \frac{I_0 2b^2}{2I_{+1}} = \log \frac{I_{+1}}{I_{-1}} \quad (7)$$

Equation (7) demonstrates that circular dichroism can be easily calculated by the logarithm of the intensities ratio of the beams diffracted by the grating.

The described method does not necessarily require the use of linearly polarised light to be sent onto the sample, and it is, in fact, possible to use random linear polarised or non-polarised light source. Random linear polarised or non-polarised light is necessary for the analysis of oriented samples that present linear birefringence.

In the more general case of non-polarised light the incoming wave can always be written as being composed of two waves with opposite circular states of polarisation where, however, both the amplitudes of the components waves E_x and E_y and the phase θ randomly vary in time. The wave can be written in the following way:

$$\mathbf{E}_0(t) = \frac{1}{2} (E_x(t) - iE_y(t)\exp(i\theta(t))) \begin{pmatrix} 1 \\ -i \end{pmatrix} + \frac{1}{2} (E_x(t) + iE_y(t)\exp(i\theta(t))) \begin{pmatrix} 1 \\ i \end{pmatrix}.$$

Thus,

$$\mathbf{E}_0(t) = A(t)\exp(i\theta_1(t)) \begin{pmatrix} 1 \\ -i \end{pmatrix} + B(t)\exp(i\theta_2(t)) \begin{pmatrix} 1 \\ i \end{pmatrix}$$

Carrying out a time average ($\langle \dots \rangle$) over a time interval longer than $1/\Delta\nu$, where $\Delta\nu$ is the bandwidth of the radiation itself, it is demonstrated that $\langle A(t) \rangle = \langle B(t) \rangle$.^[1]

Therefore, the grating preserves the same diffraction properties also for non-polarised and for random linearly polarised light.

The only optical element necessary to the described method is a grating, which possesses spectral selectivity, intending that waves with different wavelengths incident onto the grating are diffracted at different angles. By using two multi-channel detectors, as photodiodes array or CCD (Charged coupled device), the simultaneous acquisition of the diffracted beams intensities at different wavelengths is possible and, therefore, the real-time circular dichroism spectrum, throughout the spectral range of the used light source, can be obtained.

The proposed method does not require any particular procedure for the grating characterisation, such as for example the diffraction efficiency measurement and its determination at each wavelength, since it is simply based on the ratio of the diffracted beams intensities. Films of materials that possess only one or both the indicated anisotropies (linear dichroism or birefringence) can be used to create the grating.

No calibration procedure of the device in the wavelength range of interest is necessary, except for the existence of an optical anisotropy in that range.

Figure 3 schematically illustrates a device according to the invention. A beam of light L passes through the sample S and the diffracting grating PH , which has a spatially modulated linear optical anisotropy. The beams diffracted to the first order I_{+1} and I_{-1} are collected by the multi-channel light sensors $CCd1$ and $CCd2$ that convey the relative signals to the computer PC which, through an appropriate software calculates the circular dichroism spectrum, carrying out the logarithm of the diffracted beams intensities ratio.

The discovery, it should be noted, is not limited to the representations given by the figures, but may be improved and modified by those skilled in the art without, however, departing from the patent framework.

The present invention permits numerous advantages and overcome difficulties that could not be defeated with the systems presently on sale.

Bibliography:

- [1] R.M.A. Azzam and N.M. Bashara, Ellipsometry and Polarized Light, Elsevier B.V (1987)
- [2] L. Nikolova and T. Todorov, Optica Acta, 31 579588 (1984)

CLAIMS

1. Method for measuring the circular dichroism of a sample in which a light beam passes through said sample, characterised by the fact that said beam successively passes through a grating and that the diffracted beams are revealed and the signals of the diffracted beams are sent to a computer which, through a software, calculates the circular dichroism value, carrying out the logarithm of the diffracted beams intensities ratio.
2. Method for measuring circular dichroism according to claim 1, characterised by the fact that the grating has a spatially modulated linear optical anisotropy.
3. Method for measuring circular dichroism according to claim 2, characterised by the fact that the linear optical anisotropy can be birefringence and/or dichroism.
4. Method for measuring circular dichroism according to the preceding claims characterised by the fact that the light beam is linearly polarised or non-polarised or has random linear polarisation.
5. Method for measuring circular dichroism according to the preceding claims, characterised by the fact that the beam that impinges onto the sample is a polychromatic or white light beam.

6. Method for measuring circular dichroism according to the preceding claims, characterised by the fact that it permits the spectral separation of the beam coming from the sample, when the said beam is transmitted by the grating.
7. Method for measuring circular dichroism according to the preceding claims, characterised by the fact that it permits the simultaneous measurement of the circular dichroism on the whole spectral range of the light source and of the detectors sensitivity.
8. Method for measuring circular dichroism according to claim 1, characterised by the fact that the detectors used are multi-channel sensors.
9. Device for measuring the circular dichroism of a sample that presents a beam of light that passes through the said sample, characterised by the fact that the said device has a grating that is successively passed through by the said beam, that detectors collect the diffracted beams and send the relative signals to a computer which, through a software calculates the value of the circular dichroism, carrying out the logarithm of the diffracted beams intensities ratio.
10. Device for measuring circular dichroism according to claim 9, characterised by the fact that the grating has a spatially modulated linear optical anisotropy.
11. Device for measuring circular dichroism according to claim 10, characterised by the fact that the linear optical anisotropy can be birefringence and/or dichroism.

12. Device for measuring circular dichroism according to claim 11, characterised by the fact that the light beam is linearly polarised or non-polarised or has random linear polarisation.
13. Device for measuring circular dichroism according to the preceding claims, characterised by the fact that the beam that strikes the sample is a polychromatic or a white light beam.
14. Device for measuring circular dichroism according to the preceding claims, characterised by the fact that it permits the spectral separation of the beam coming from the sample when the said beam is transmitted by the grating.
15. Device for measuring circular dichroism according to the preceding claims, characterised by the fact that it permits the simultaneous measurement of the circular dichroism on the whole spectral range of the light source and of the detectors sensitivity.
16. Device for measuring circular dichroism according to claim 9, characterised by the fact that the detectors used are multi-channel sensors.

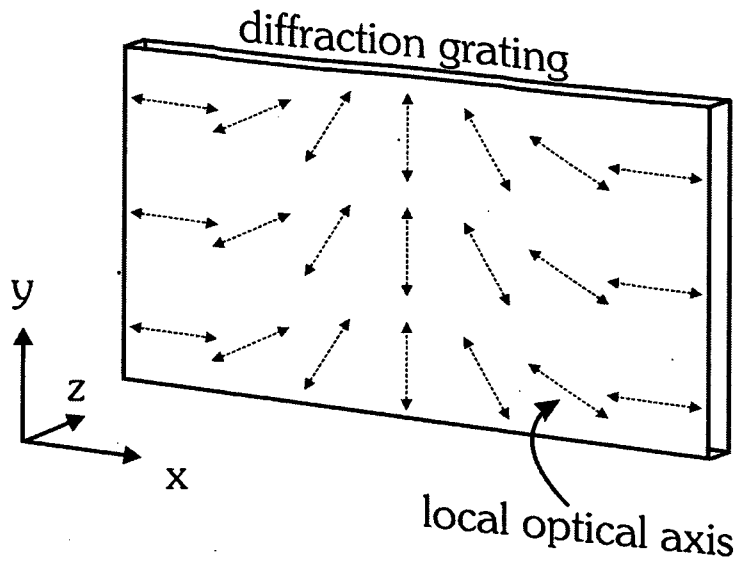


Fig. 1

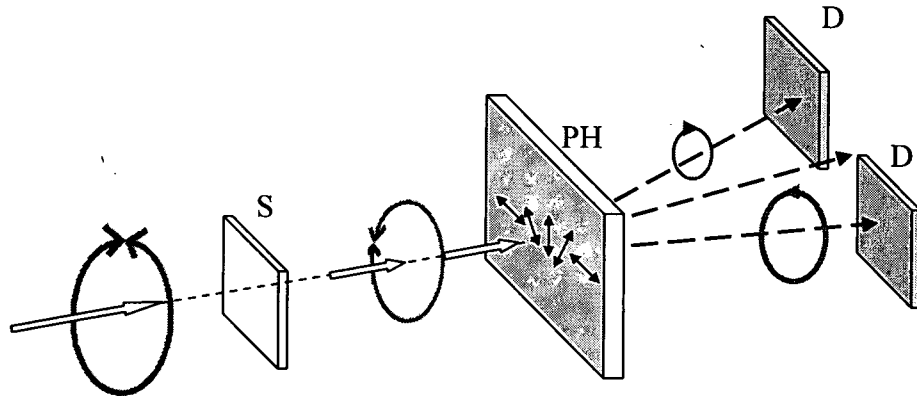


Fig. 2

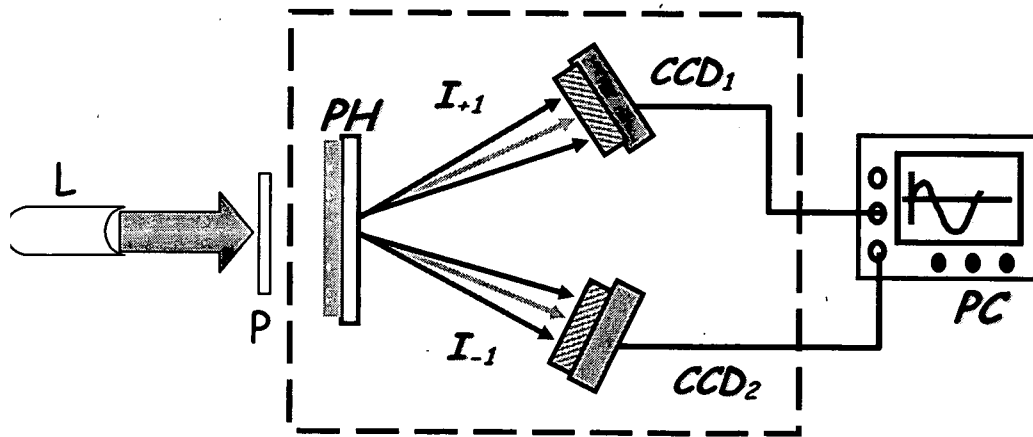


Fig. 3