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AN OPTICAL ROTATION STOPPED FLOW APPARATUS

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A stopped flow apparatus has been proposed for optical rotation changes. This apparatus, even when the signals of optical rotation changes are masked by absorption changes, can detect only the signals of optical rotation changes. The apparatus is examined by several model reactions: The maximum sensitivity is 0.01 degree of rotation, the dead time ca. 80 msec. The 50% change of transmittance does not affect the signals of optical rotation changes.

Since the improvement of stopped flow techniques by Chance,[1,2] various apparatuses have been used for investigating the fast reactions by means of measuring absorption changes. Only a few attempts, however, have been made to design an apparatus for the measurement of optical rotation changes.[3,4] Nevertheless, none of these apparatuses could simultaneously trace both the rapid time courses of absorption and optical rotation changes, which are caused by fast reactions, such as related to the induced Cotton-effect.

In these apparatuses the optical rotation change is measured utilizing a relationship illustrated in Fig. 3. The intensity of the light depends on both absorption and optical rotation changes. It is inevitable that the signals of optical rotation changes are masked or disturbed by the absorption changes, when both the two changes occur by rapid processes. In this case, it is very difficult to distinguish optical rotation changes from absorption changes. This paper has offered a solution for this problem by proposing a stopped flow apparatus which can trace the courses of both absorption and optical rotation changes at the same time. The optical property of a double image prism and operational amplifiers are utilized skillfully for the apparatus.

The details of the apparatus will be reported elsewhere. So the apparatus is simply outlined here.

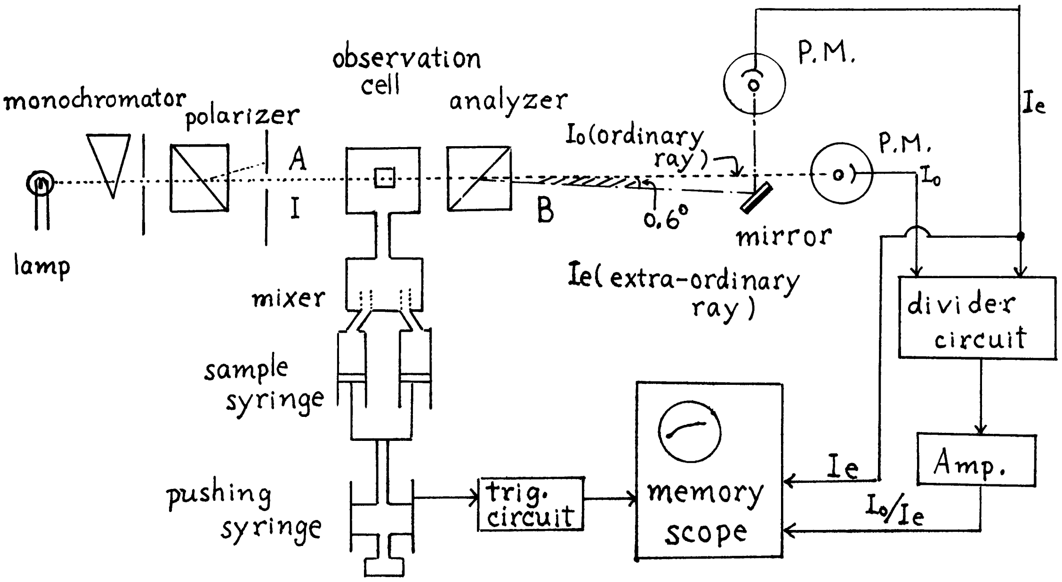

Fig. 1. Scehmatic diagram of the apparatus.

1) Disposition

The schematic diagram of the optical rotation stopped flow apparatus is shown in Fig. 1. The light source is either a tungsten iodide lamp (50 W) or a deutrium lamp (25 W). Prism spectroscope (Czerney-Turner type, focus length 250 mm) is used as the source monochromator.

For the measurement of optical rotation changes, a pair of polarizing prisms (Sénarmont prism) are used. The one is fixed as a polarizer and the other as an analyzer can be rotated around the axis of a light beam (A - B in Fig. 1). The polarized light obtained by the polarizer passes through an observation cell to fall on the double image prism (analyzer). The analyzer divides the beam into two ones, the ordinary ray (I_0) and the extra-ordinary ray (I_e). Each beam is received by the corresponding photomultiplier tubes. The output currents of the photomultipliers are converted into voltages. They are then introduced into an operational amplifier (frequency response 40 kHz) which computes a ratio I_0/I_e.

The observation cell used is of horizontal type,$^{3)}$ 10 mm (optical path) X 5 mm (diameter). Due to the long distance (ca. 70 mm) between the mixer and the cell and due to the large volume of the cell, the dead time obtained is about 80 msec. This dead time can be easily reduced to a few milliseconds by improvement of cell constructions.

2) Principle of Detection

Let I represent the intensity for any wavelength of the incident beam. The intensities of the two beams at B, I_o (ordinary ray) and I_e (extra-ordinary ray) are given by eq. (1) $^{5)}$

$$I_o = I \sin^2\theta = I\left(\theta - \frac{1}{6}\theta^3 + \cdots\cdot\right)^2 \simeq I\,\theta^2$$
$$I_e = I \cos^2\theta = I\left(1 - \frac{1}{2}\theta^2 + \cdots\cdot\right)^2 \simeq I \qquad (1)$$

where θ is the relative rotation angle between the two palarizers and fixed about 4 degrees away from the null, i.e. $\theta \ll 1$ radian.

When the optically active substance is present in the cell, the null position will change by $\Delta\theta(t)$. Under the condition that the optical rotation change $\Delta\theta(t)$ is much less than θ and that there is no absorption change, the intensities of the two beams are given by eq. (2)

$$I_o = I \sin^2\{\theta + \Delta\theta(t)\} \simeq I\{\theta + \Delta\theta(t)\}^2$$
$$I_e = I \cos^2\{\theta + \Delta\theta(t)\} \simeq I \cos^2\theta \simeq I \qquad (2)$$

It should be noted that I_o changes with the square of θ, while I_e is constant for $\Delta\theta \ll \theta$.

Therefore, the change of an ordinary light intensity, ΔI_o, produced by $\Delta\theta$, takes the form of eq. (3), provided that $\Delta\theta^2(t)$ is neglected.

$$\Delta I_o = 2I\cdot\theta\cdot\Delta\theta(t) \quad . \qquad (3)$$

In the case that both absorption and optical rotation changes occur due to the reaction in the cell, ΔI_o and I_e are given by eq. (4) and (5) respectively.

$$\Delta I_o = 2\cdot I\cdot\exp\{-2.3A(t)\}\cdot\theta\cdot\Delta\theta(t) \qquad (4)$$
$$I_e = I\cdot\exp\{-2.3A(t)\} \qquad (5)$$

where A(t) represents absorbance as a function of time.

Through the operational amplifier the ratio I_o/I_e is computed. The change of the output voltage produced by the change of rotation, $\Delta(I_o/I_e)$, is propoportional to $\Delta\theta(t)$:

$$\Delta(I_o/I_e) = \Delta I_o/I_e = 2\cdot\theta\cdot\Delta\theta(t) \qquad (6)$$

In this way the term of the absorption change should be eliminated from the signal. On the other hand the absorption change can be observed directly from I_e (eq. (5)).

3) Examples of measurements

Fig. 2. is an example of the measurement of the rotation change for the alkaline catalyzed mutarotation of α-glucose at $\theta = +5^\circ$. It is found that the maximum sensitivity of the apparatus is 0.01° of rotation per cm. of oscilloscope screen height. And even in the case $\Delta\theta = 0.25^\circ$, the optical rotation change does not interfere with the signal of the absorption change.

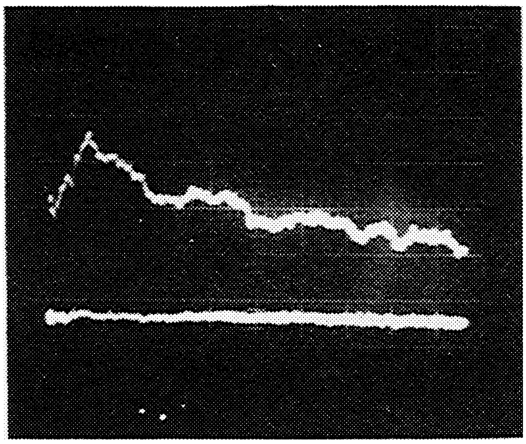

Fig. 2. Time courses of the optical rotation change and the absorption change for the mutarotation of glucose. A-solution: α-glucose 0.8% in water. B-solution: 0.04 M NaOH. Measurement was performed at 590 nm and $\theta = +5^\circ$; at room temperature. Horizontal scale: 0.1 sec/div. Vertical scale: 0.01 degree/div. for the optical rotation signal (upper curve) and 1.4% transmittance/div. for the absorption signal (lower curve).

As Fig. 3. illustrates, when the relative angle between two polarizers changes from $+\theta$ to $-\theta$, the intensity change ΔI_0 is nearly equal in magnitude, but opposed in sign.

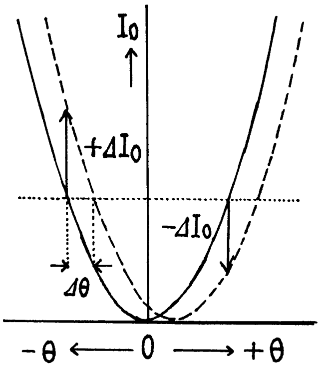

Fig. 3. A relationship between the intensity (I_0) of a transmitted light and the angle (θ) of rotation of the analyzer with respect to the null.

Fig. 4. shows the signals of the same reaction as shown in Fig. 2, but observed at $\theta = -5^\circ$. As is expected, the two signals obtained for the optical rotation change are symmetric with respect to the abscissa. This result justifies that the signals on the oscilloscope screen represent exactly the changes of optical rotation.

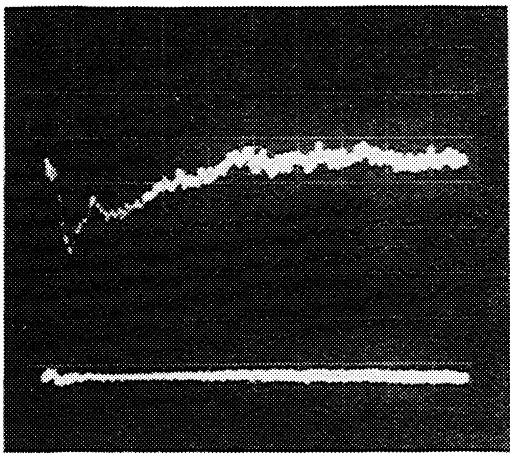

Fig. 4. Time courses of the optical rotation change (upper curve) and the absorption change (lower curve). Measurement was performed under the same conditions as in Fig. 2. except for at $\theta = -5^{\circ}$. Horizontal scale: 0.2 sec/div.

In order to make sure that the change of absorption does not affect the signal of the optical rotation change, the hydrolysis of optical inactive Murexide was observed.

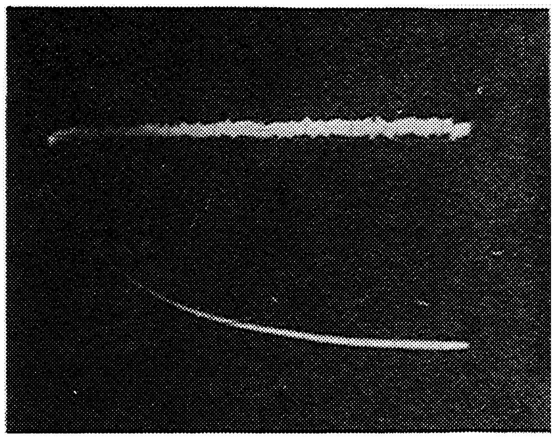

Fig. 5. Absorption and optical rotation changes for the hydrolysis of Murexide. A-solution: 0.5 M HNO_3. B-solution: 1.1×10^{-4} M Murexide in water. λ = 500 nm and $\theta = +5^{\circ}$; at room temperature. Horizontal scale: 2.0 sec/div. Vertical scale: 0.014 degree/div. for the optical rotation signal (upper curve) and 14.7% transmittance/div. for the absorption signal (lower curve).

This result proves that even the 50% change of transmittance affects the signal of the optical rotation change by less than 0.01 degree.

In order to compare the relaxation times obtained from the optical rotation change with that from the absorption change, the sulfitolysis of Cystine is used. Fig. 6. reveals that the two relaxation times obtained by two different methods are exactly identical.

These experimental data confirm that the assumptions for deriving eq. (4), such as I_e is nearly constant, are all satisfied with sufficient accuracy, and that the apparatus detects the rapid change of the optical rotation even in the case of the large absorption changes.

The apparatus described here may lead to important advances in understanding chemical reactions, particularly reactions accompanying with conformational changes, for which the kinetic measurements using only absorption changes have rarely given important informations.

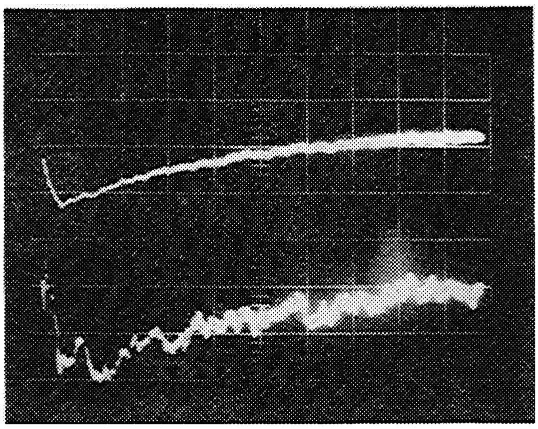

Fig. 6. An example of the simultaneous measurement of a relaxation time by absorption and optical rotation changes. A-solution: 1.0 M Na_2SO_3 in water. B-solution: 4% Cystine in 2 N HCl. Measurement was performed at 300 nm and $\theta = +5^\circ$; at room temperature. Horizontal scale: 0.2 sec/div. Vertical scale: 0.21 degree/div. for the optical rotation signal (upper curve) and 0.5% transmittance/div. for the absorption change signal (lower curve).

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REFERENCES

1) B. Chance, in A. Weissberger (ed.); Technique of Organic Chemistry Vol. VIII, Pt. II. p. 728, Wiley (Interscience), New York 1963.
2) B. Chance, J. Franklin Inst., 229, 455 (1940).
3) K. Hiromi, S. Ono, S. Itoh,and T. Nagamura, J. Biochem., 64, 897 (1968).
4) R. Lumry, R. Legare,and W. G. Miller, Biopolymers, 2, 489 (1964).
5) L. Ingersoll, Phys. Rev., 23,439 (1906).

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