

CHAPTER 12

Polarimetry

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12.1 Introduction	425
12.2 Origin of Optical Rotation	426
12.3 Molecular Requirements for Optical Rotatory Power	435
12.4 Instrumentation	438
12.5 Applications	443
Experiments	446
Questions	447
Problems	448
Bibliography	449

12.1 INTRODUCTION

Polarimetry, which may be defined as the measurement of the rotation of polarized light, is a classical method of quantitative analysis and was the first optical property of organic compounds to be interpreted in terms of molecular structure. The measurement of rotation, accomplished with a polarimeter, is a measurement of the change in direction of linearly polarized light after its passage through an anisotropic medium. If the change in direction is caused by anisotropic refraction (circular birefringence), i.e., the refractive indices of right- and left-hand components of linearly polarized light are different, then the change is called "optical rotation" and the medium is said to be "optically active" or, more correctly, to have "optical rotatory power." If the change in rotation is caused by anisotropic absorption or

scattering of linearly polarized light, then the medium is said to exhibit "circular dichroism."

Polarimetry is employed as a method of quantitative analysis of organic compounds that are optically active; notably the sugars, alkaloids, terpenes, and steroids. Optical rotation is used widely as a criterion in the establishment of identity of substances, the characterization of stereoisomers, the elucidation of configuration, and to solve problems involving stereochemical reaction mechanisms and conformational and configurational changes of molecules in solution.

Although the phenomenon of the rotation of polarized light has been known for approximately 150 years, it is only comparatively recently, with the development of commercial spectropolarimeters, that measurements have been extended to wavelengths other than those of the sodium D doublet. The measurement of optical rotatory dispersion i.e., the variation in rotation with wavelength, has become an important tool for the characterization of organic structures. An even more recent development is the availability of instruments which provide measurement of the absorption of circularly polarized light with change in wavelength. Such circular dichroism spectra may have certain advantages for studying optical activity relative to optical rotatory dispersion spectra. These closely related phenomena have become exceedingly important as aids in the solution of complex qualitative problems involving asymmetric molecules.

12.2 ORIGIN OF OPTICAL ROTATION

Light may be described as having a transverse wave motion. The vibrations of the wave motion are at right angles to the direction of propagation and

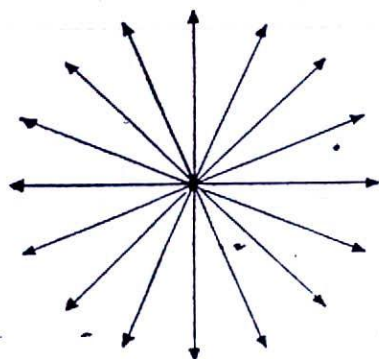


FIGURE 12.1: End view of unpolarized light. The unresolved electric vectors are in a plane perpendicular to the direction of propagation and the equal probability of all directions of vibration is schematically illustrated.

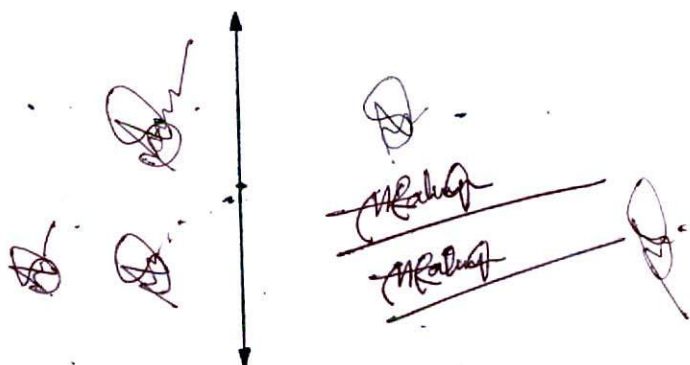


FIGURE 12.2: End view of plane- or linearly polarized light. The resolved electric vector is confined to one plane perpendicular to the direction of propagation.

thus there may or may not be perfect symmetry around the direction of travel. If the light does not have perfect symmetry then, it may be said to be "polarized." Figure 12.1 schematically illustrates an end-on view of unpolarized light. Such a beam of ordinary light may be assumed to consist of millions of waves, each with its own plane of vibration. Since there are waves vibrating in all planes with equal probability, the light beam is said to have "perfect symmetry." If the waves of ordinary light are restricted by some means to vibration in planes parallel to each other, then the light is said to be "plane polarized." Plane or linearly polarized light is a form of electromagnetic radiation in which the energy or electric vector is restricted to vibration in one plane in a sinusoidal manner. See Fig. 12.2 and 12.3.

A plane-polarized light beam may be resolved theoretically and experimentally into two circularly polarized components. The two circular beams

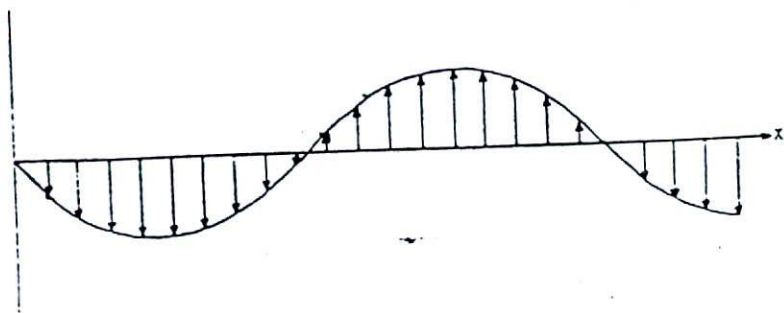


FIGURE 12.3: The electric vector of plane-polarized light at constant time travels in the x direction and vibrates in the plane of the paper.

are of equal magnitude and have opposite senses of rotation. The sinusoidal vibration of the electric vector of right circularly polarized light will describe a right-handed helix in the direction of propagation and similarly a left-handed helix for left circularly polarized light. These two components of plane-polarized light are illustrated in Figs. 12.4 and 12.5.

If in passing through a medium the two circular components of plane-polarized light are propagated with unequal velocities, there is a phase shift between the component beams and the resultant beam of plane-polarized light is rotated in its plane of polarization as it emerges from the medium.

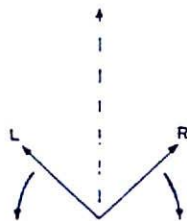


FIGURE 12.4: End view of the resolution of the electric vector of plane-polarized light into right and left circularly polarized light.

The extent to which the resultant beam is rotated is quantitatively given by Fresnel's law:

$$\alpha = \frac{\pi}{\lambda}(\eta_L - \eta_R) \quad (12.1)$$

where α is the angle of rotation in radians per centimeter, λ is the wavelength of the incident plane-polarized light beam in centimeters, η_L is the refractive index of the left circular polarized vector, and η_R is the refractive index of the right circularly polarized vector of plane-polarized light. It may be recalled that the refractive index is the ratio of the velocity of light in a vacuum relative to its velocity in a medium, $\eta = c/v$, thus refractive indices serve as a convenient gauge for measuring the unequal velocities of left and right circularly polarized light. The angle of rotation may be converted to the more usual units of degree reciprocal decimeters by multiplying by $1800/\pi$:

$$\alpha(\text{deg dm}^{-1}) = \frac{1800}{\lambda} (\eta_L - \eta_R) \quad (12.2)$$

As can be seen from Eqs. (12.1) and (12.2), the rotation α depends upon a velocity difference (represented by $\eta_L - \eta_R$ or $\Delta\eta$), and the wavelength of the plane-polarized light beam. The angle of rotation is also proportional to the number of molecules in the light beam, which may be given by the product of the density or concentration and the path length. Thus

$$\alpha = [\alpha]_D^T(l\rho) \quad \text{or} \quad \alpha = [\alpha]_D^T(lc) \quad (12.3)$$

where $[\alpha]_D^t$ is a proportionality constant called the "specific rotation," l is the light-path length through the optically active sample in decimeters, and ρ is the density of the pure liquid or c is the concentration of the solute in grams per milliliter. A compound may be reported as having $[\alpha]_D^{25} = +62.5^\circ$ ($c = 26$, H_2O), which means that the compound has a dextro-rotatory specific rotation (the polarization plane is rotated clockwise as one faces the

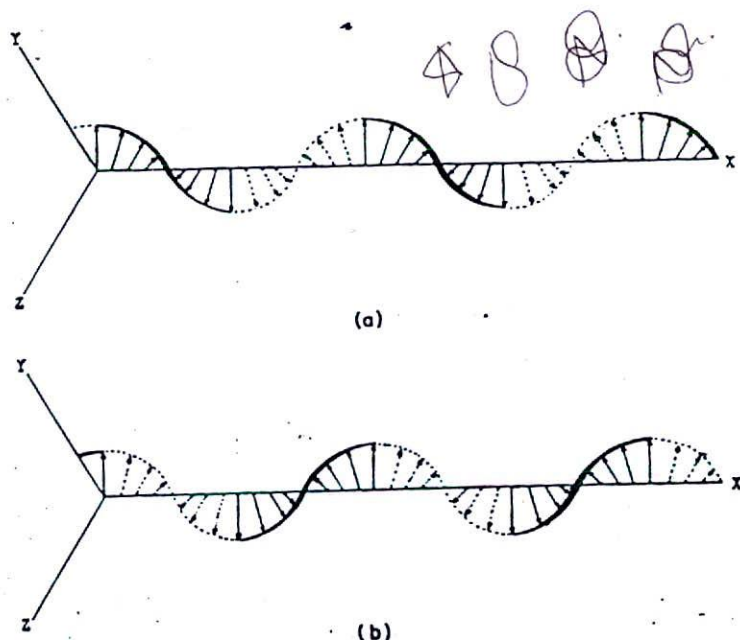


FIGURE 12.5: (a) The electric vector of the right circularly polarized component of plane-polarized light, at constant time. The vector travels in the X direction as a right-handed helix and vibrates above and below the plane on the page. (b) The electric vector of left circularly polarized light.

emerging beam) of 62.5° at a temperature of $25^\circ C$, the rotation being measured with sodium D light in a tube 1 dm long at a concentration of 26 g/100 ml of aqueous solution. When substances of different molecular weight or differing rotation are compared, the most suitable experimental quantity is the molecular rotation ϕ :

$$[\phi]_D^t = \frac{[\alpha]_D^t \cdot M}{100} \quad (12.4)$$

where M is the molecular weight of the optically active substance.

Both α and ϕ are, in principle, constants which are independent of concentration or density and cell path length, however, in some instances,

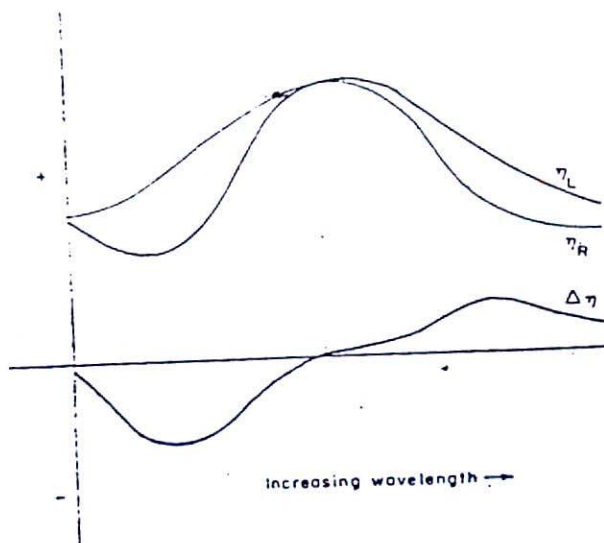


FIGURE 12.6: The change in refractive index associated with rotatory dispersion. $\Delta\eta$ represents the difference in dispersion of left and right circularly polarized light.

when the solute may be involved in some type of associative equilibrium such as hydrogen-bonding with the solvent, these constants will depend on concentration.

The term "rotatory dispersion" arises from the fact that different wavelengths of plane-polarized light are rotated by different amounts. Figure 12.6 illustrates the change in rotation and refractive index associated with rotatory dispersion. Although diagrammatically in Fig. 12.6 a significant curve is shown for the difference $\Delta\eta$, actually it is extremely small. For example at a wavelength of 5893 Å (sodium D doublet) and a cell path length of 1 dm a rotation of 10° corresponds to a $\Delta\eta$ value of approximately 3.27×10^{-8} :

$$\eta_L - \eta_R = \Delta\eta = \frac{\lambda\alpha}{1800}$$

$$\Delta\eta = \frac{10 \times 5893 \times 10^{-9}}{1800} \approx 3.27 \times 10^{-8}$$

Since ordinary refractive indices may be considered to be

$$\frac{\eta_L + \eta_R}{2}$$

and are commonly in the range of 1.3 to 1.7, the difference $\Delta\eta$ is at least one ten-millionth of the refractive index.

An empirical equation developed by Drude quantitatively expresses the variation of α with wavelength for regions of the spectrum where the optically active material does not absorb light:

$$\alpha = \frac{k_1}{\lambda^2 - \lambda_1^2} + \frac{k_2}{\lambda^2 - \lambda_2^2} \dots \sum \frac{k_i}{\lambda^2 - \lambda_i^2} \quad (12.5)$$

In this equation k_i is a constant found by experiment and λ and λ_i are the wavelengths of the incident linearly polarized light and of characteristic active absorption, respectively. Implicit in this equation is the inference that optical rotation is related to an absorption process. The Drude formula may be simplified to a form

$$[\alpha] = \frac{A}{\lambda^2 - \lambda_0^2} \quad (12.6)$$

where $[\alpha]$ is the specific rotation, A is the rotation constant, λ is the measuring wavelength, and λ_0 is the closest wavelength at which the material exhibits

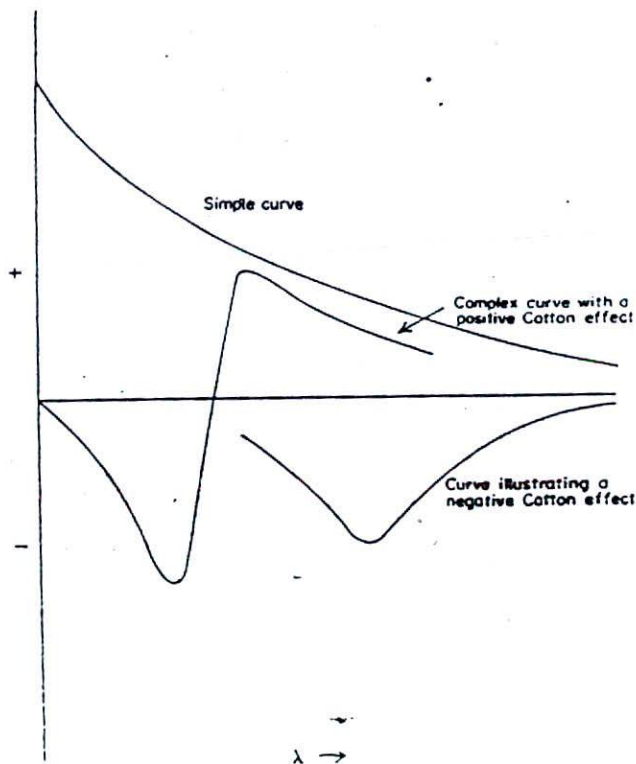


FIGURE 12.7: Types of curves that may be found in optical rotatory dispersion data.

optically active absorption i.e., the nearest absorption band of the molecule contributing to optical activity. The Drude equation is generally applicable to plain optical rotatory dispersion curves (also called "simple curves"), which do not have a chromophore within the range of wavelengths under investigation and thus do not show any maximum and minimum. Other

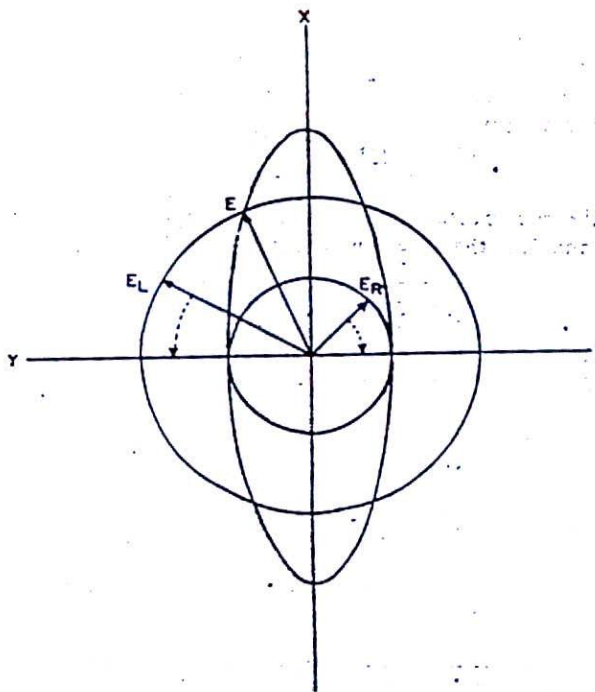


FIGURE 12.8: Elliptically polarized light, which results from unequal absorption of the components of plane or linearly polarized light. E_L represents the electric vector of the left circularly polarized component rotating as a helix counterclockwise away from the viewer. E_R represents the electric vector of the right circularly polarized component. E is the resultant vector $E_L - E_R$ represented as an elliptically shaped helix.

forms of the Drude equation with two or more terms are required for complex rotatory dispersion curves.

In the wavelength region of an optically active absorption maximum, the optical rotation increases, then reverses and becomes zero at or near the wavelength of maximum absorption and then reaches another maximum of opposite sign. This characteristic anomaly, which is associated with circular dichroism in the absorption region, is called the "Cotton effect," after its discoverer. Figure 12.7 illustrates the types of curves that may be found in optical rotatory dispersion data. The Cotton effect arises from the fact that

in the vicinity of an absorption maximum, optically active molecules will absorb the circular components of linearly polarized light to different extents. The origin of the Cotton effect of an absorption band is the vibrating electrical momentum of the band itself. If this vibrating momentum is isotropic i.e., unidirectionally orientated, the molecules will not exhibit circular dichroism. If the vibrating electrical momentum is associated with

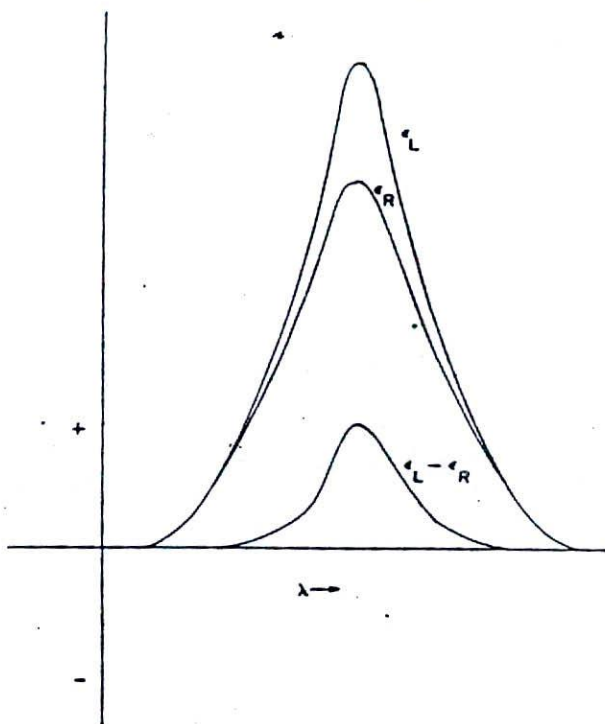


FIGURE 12.9: Schematic diagram showing the origin of a circular dichroism curve ($\epsilon_L - \epsilon_R$).

an electronic polarizability which is not isotropically orientated, then the molecules will respond differently to left and right circularly polarized light and will show circular dichroism and optical rotation. In the absorption process, the light is not only rotated in its plane of polarization but also becomes elliptically polarized. See Fig. 12.8. The resulting angle of ellipticity may be expressed as the molecular ellipticity $[O]_l$, which is proportional to the molecular absorption coefficients, ϵ_L and ϵ_R for the left- and right-hand components of linearly polarized light.

$$[O]_l = 3300(\epsilon_L - \epsilon_R) \quad (12.7)$$

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Just as the optical rotatory dispersion of an optically active material is obtained by measurement of the rotation of linearly polarized light, the circular dichroism of the optically active material is obtained by the measurement of the preferential absorption i.e., the difference in absorption between right and left-hand circularly polarized light. Figure 12.9 illustrates a circular dichroism curve resulting from the difference, $\epsilon_L - \epsilon_R$, of absorption

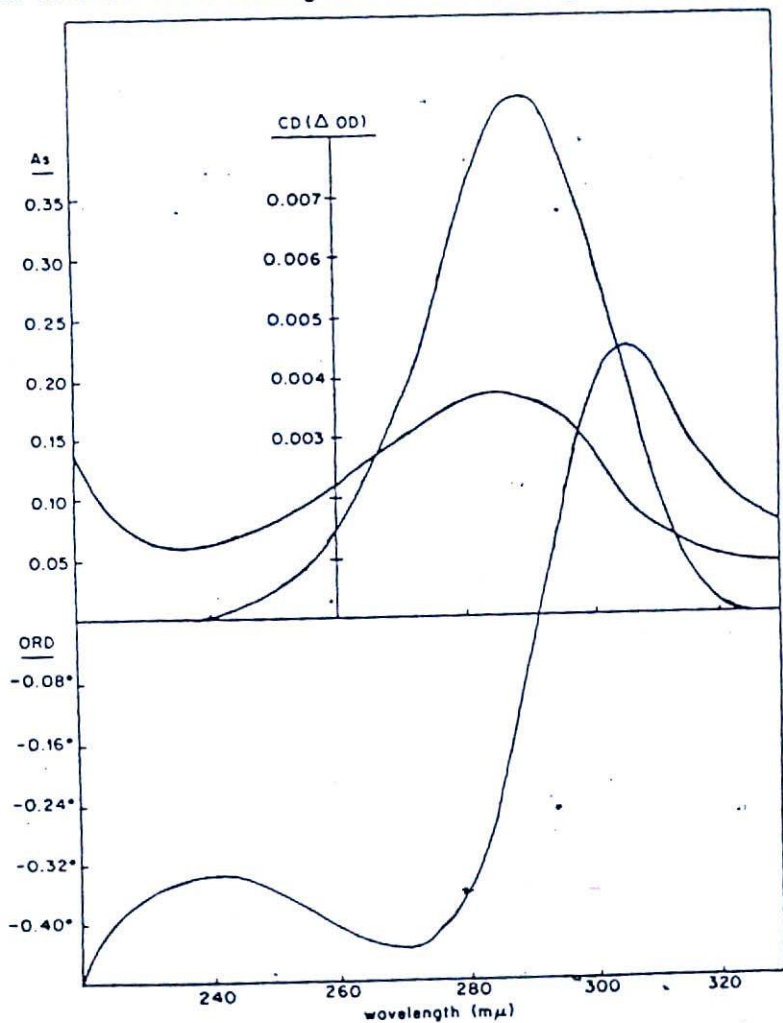


FIGURE 12.10: Absorption, rotatory dispersion, and circular dichroism spectra of a solution of *d*-camphor sulfonic acid in water.

coefficients of left and right circularly polarized light. The relationship between optical rotatory dispersion, circular dichroism and absorption is illustrated in Fig. 12.10 by the spectra of a solution of *d*-camphor sulfonic acid in water.

12.3 MOLECULAR REQUIREMENTS FOR OPTICAL ROTATORY POWER

Substances which refract and/or absorb right and left circularly polarized light to different extents are "optically active" or show optical rotatory power. Such substances usually lack a plane or center of symmetry and thus are

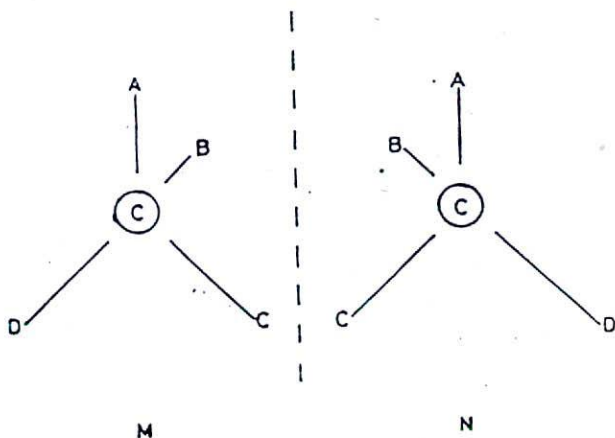


FIGURE 12.11: Simple asymmetric molecules *M* and *N* which are related to one another as object and mirror image.

termed "asymmetric." In general the absence of symmetry is a necessary criterion of optical activity.

A substance will demonstrate optical activity if its mirror image is not superimposable upon the original. This is a fundamental stereochemical requirement which was first elaborated by Pasteur and which led to the concept of the tetrahedral carbon atom. Carbon with four bonds attached to different groups extending to the corners of a regular tetrahedron is asymmetric since it has no plane of symmetry and it cannot be superimposed on its mirror image. Figure 12.11 illustrates this simple case of asymmetry.

Molecules such as *M* and *N* in Fig. 12.11, which are related to one another as objects and nonsuperimposable mirror image, are called "enantiomers" or

"optical antipodes." For every optical active asymmetric molecule there exists one and one only enantiomer, however there may exist several asymmetric spatial arrangements which are not enantiomeric but diastereomeric. The term "diastereoisomerism" is applied to all molecules which may be optically active and are not related to one another as object and mirror image. In contrast to enantiomers, diastereoisomers have different physical properties e.g., spectral properties such as ultraviolet, infrared, nuclear magnetic resonance, and mass spectra; melting points, densities, solubilities, boiling points, refractive indices, viscosities, etc. Enantiomers of the same substance (object and mirror image) have identical physical and chemical properties and differ only in the sign of their optical rotations. A mixture composed of equal molar quantities of two pure enantiomers is called a "racemic modification." Such a modification is optically inactive since it is composed of equal numbers of dextrorotatory (+) and levorotatory (-) molecules and therefore the average rotation is zero.

Frequently, racemic modifications have crystal structures which differ from those of the pure enantiomers. In such instances, a racemic compound or racemate is formed which has the properties of a eutectic mixture and therefore the melting point, density, solubility, and solid-state infrared spectrum differ from the corresponding properties of the pure enantiomer, either (+) or (-). True racemization is the irreversible process of formation of (+)(-) pairs by the reversible interconversion of enantiomers.

Other processes involving asymmetric atoms which are closely related to racemization are mutarotation and epimerization. Mutarotation is the change, with time, in optical rotation of a solution of a pure optically active substance. Such a solution eventually reaches an equilibrium value of optical rotation which is usually not zero. Epimerization is a process involving the change of configuration of one asymmetric atom in a molecule which has more than one asymmetric atom. Epimerization usually involves the interconversion of diastereoisomers, and the diastereoisomers that result differ in configuration at only one asymmetric atom and are called "epimers."

An example of epimerization and mutarotation is the process of mutarotation of $\alpha(+)$ -glucose, which involves epimerization (a change in configuration) at the number 1 carbon atom—the so-called anomeric carbon atom. A solution of $\alpha(+)$ -glucose, $[\alpha]_D^{20} = +113^\circ$, with time undergoes spontaneous mutarotation to an equilibrium value of $[\alpha]_D^{20} = +52.5^\circ$. This change involves the hemiacetal formation at C-1, which opens to the aldehyde form and then may close with a different configuration at C-1—the hemiacetal (anomeric) carbon. Thus the mutarotation of glucose is a spontaneous epimerization since it involves only a change in configuration at the number 1 carbon atom. The epimers that are formed are diastereoisomers and in the special nomenclature of carbohydrate chemistry are called "anomers," since they differ only by the configuration at the anomeric carbon atom C-1.

The presence of four different substituents on carbon, creating an asymmetric center, may not be a sufficient condition for the demonstration of optical rotatory power. For example, tartaric acid $\text{COOH}\cdot\text{CHOH}\cdot\text{CHOH}\cdot\text{COOH}$, in which the two central carbon atoms each have four different substituents, exists as (+)-tartaric acid, (-)-tartaric acid, (\pm)-tartaric acid,

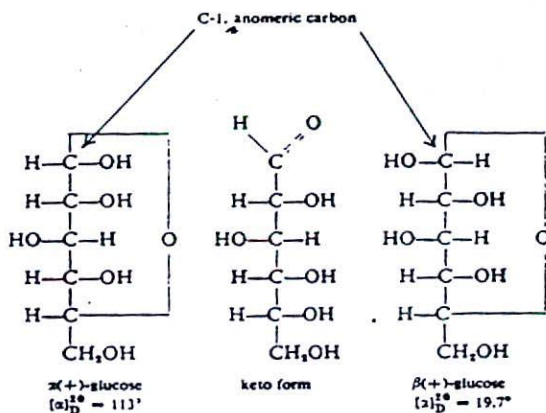


FIGURE 12.12: Formulas for tartaric acid.

and *meso*-tartaric acid. The last two forms of tartaric acid are optically inactive. The (\pm)-tartaric acid is a racemate and the *meso*-tartaric acid is optically inactive due to internal compensation in the molecule, which produces a plane of symmetry. These forms of tartaric acid are illustrated in Fig. 12.12.

Optical activity may exist in carbon compounds which do not have asymmetrically substituted carbon tetrahedra. Typical examples of these types of compounds are shown in Fig. 12.13. Thus optical activity which is often but not always associated with asymmetrically substituted carbon, which may confer a molecular dissymmetry on the molecule, may also be present in certain spiranes, allenes, diaryls, and helical conjugated compounds (e.g., hexahelicene) in which substituents prevent coplanarity. Optical activity is also exhibited by the helical structures of proteins and may be exhibited by compounds containing the elements arsenic, phosphorus, nitrogen, antimony, and sulfur, which like carbon may be asymmetric. In general, molecular dissymmetry is a more basic criterion than asymmetric carbon for optical activity, however, it is probably most accurate to state that molecular symmetry is a condition which does not produce optical activity, rather than that molecular asymmetry is a condition for optical activity. Optical activity, in a broad sense, involves a diastereoisomeric

interaction, since a dissymmetric sample interacts with the enantiomeric circularly polarized components of plane-polarized light.

All substances, regardless of their molecular symmetry or lack of it, may be induced to have optical activity either by an electrical field (the Kerr effect) or by a magnetic field (the Faraday effect). Induced optical activity by these methods has found little direct application to pharmaceutical analysis.

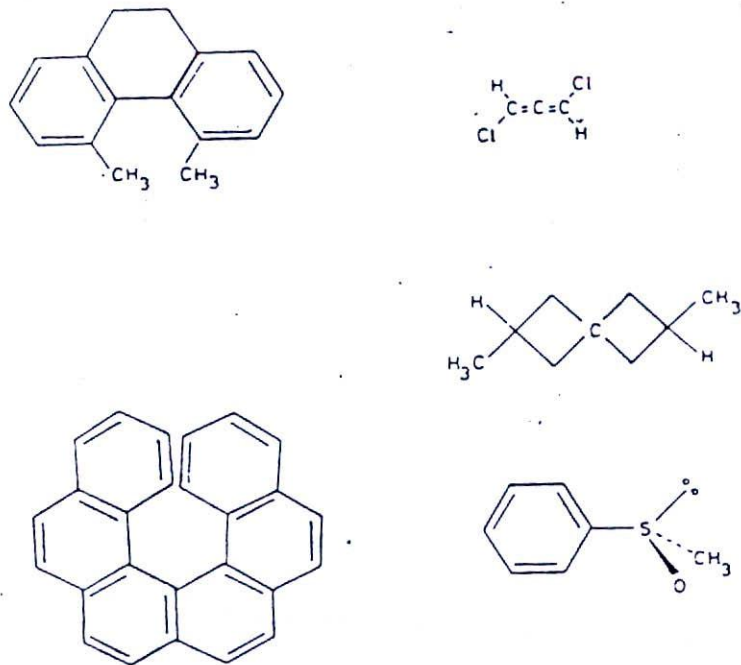


FIGURE 12.13: Some structures which have optical rotatory power, but do not have asymmetrically substituted carbon atoms.

12.4 INSTRUMENTATION

The measurement of optical rotation is the estimation of the angular rotation of a beam of plane-polarized light. The sample under study may be a solid, liquid, or solution, but must be transparent to the incident light. Since many optical materials disperse white light, a monochromatic light source must be used. A simple polarimeter consists of a polarizer, a sample tube, and an analyzer mounted in a graduated circle.

A simple polarimeter such as the instrument schematically illustrated in Fig. 12.14 may be used to estimate the strengths of sugar solutions. Such an instrument, called a "saccharimeter," consists of a polarizer, which produces plane-polarized light, a sample tube or cell for containing the optically active solution, and an analyzer for measuring the rotation, together with the appropriate lenses necessary to allow a parallel beam of monochromatic light (usually the sodium D line) to pass through the solution and enter the

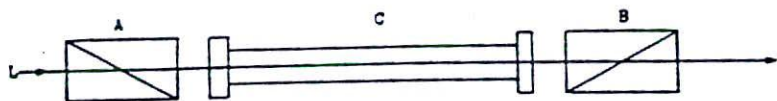


FIGURE 12.14: Schematic diagram of a simple polarimeter: *L*, light source; *A*, polarizing prism; *B*, analyzing prism; *C*, sample tube.

observer's eye. The cell is filled with water and the analyzer is turned until no light passes. The cell is then filled with the sugar solution under test and the analyzer is rotated until the field is dark again. The angle through which the analyzer has been turned is read on a circular scale. Under most practical conditions the angle of rotation ψ is never greater than π and thus $\psi = \alpha$ or $\psi = 180 - \alpha$. To distinguish between these two possibilities the solution is diluted to half-strength and a new value of α is measured.

In its simplest form, the measurement of optical rotation is a differential measurement since the difference produced by the sample is the angular difference between the setting on the circular scale with and without the sample in position. The measurement of optical rotation using the simple polarimeter thus far described involves the detection of a totally dark field where the rate of change of illumination is at a minimum and therefore the accurate location of the angle of zero illumination is quite difficult. Various "half-shadow" devices have been incorporated into visual polarimeters to make the measurement easier by allowing the observer to estimate small differences in the intensity between two halves of a bipartite field. These half-shadow devices alter the plane of polarization of one-half of the beam emerging from the polarizer, creating a split field. A typical example is a small Nicol prism covering one-half of the field from the polarizer. Another design incorporates a pair of Nicol prisms, one of the pair covering one-third of the field emerging from the polarizer and the other prism covering another one-third of the field, thus creating a tripartite field in which the intensity of minimum illumination is matched to make the measurement. The small Nicol prisms, which may be adjusted in some polarimeters, rotate the polarized light beam small amounts in opposite directions and thus introduce a small phase difference usually between

1 and 7° . Another half-shadow device is the Laurent quarter-wave plate—a quartz plate cut so that for a given wavelength of light (usually the sodium D line) the radiation is altered in phase by half a wavelength. In visual polarimeters, the zero position of the instrument is that angle at which the two or three divisions of the observed field are equally dim.

Plane-polarized light may be produced by reflection, by transmission through a pile of plates, or by absorption of the monochromatic light through Polaroid film [an iodine poly(vinyl alcohol) complex which is made

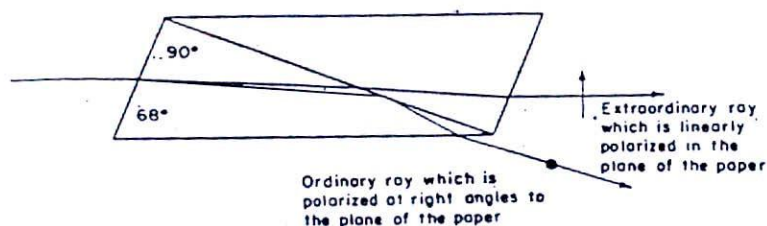


FIGURE 12.15: The Nicol prism.

dichroic by stretching the film). These methods either involve great loss of energy or do not give complete polarization and most polarimeters use prisms for the production of polarized light.

Prisms of the Rochon and Wollaston type, which are usually made of either quartz or calcite, produce two beams of light polarized in mutually perpendicular planes and traveling in different directions. One of the two beams is termed the "ordinary ray" and the other the "extraordinary ray." If one ray is removed by inserting a stop, the other may be used as the incident plane-polarized light of the polarizer. In the Nicol, Foucault, and Glan-Thompson prism designs the difference in the angles of refraction of the ordinary and extraordinary rays of plane-polarized light allow separation of one light ray by total reflection. See Fig. 12.15.

In most visual polarimeters a pair of Nicol prisms is used. One prism serves as the polarizing prism, which produces linearly polarized light, and the other Nicol prism, located after the sample cell, is used as an analyzing prism which is rotated to the angle of extinction. If the two prisms are oriented identically with respect to their optic axes, the radiation is "uncrossed" and maximum light is transmitted. If the analyzing prism is situated so that its optic axis is at an angle of 90° (crossed position) to the optic axis of the polarizing prism the intensity of the light is a minimum. According to the law of Malus, the energy of the linearly polarized light beam transmitted by the analyzer varies as the square of the cosine of the angle between the optic axes of the polarizing and analyzing prism. This is written

$$E = E_0 \cos^2 \theta \quad (12.8)$$

where E_0 is the energy of the beam as it leaves the polarizing prism, E is the energy of the beam as it leaves the analyzing prism, and θ is the angle between the optic axis of the polarizer and the optic axis of the analyzer. An optically active substance placed in the sample cell will change the angle of extinction to which the analyzer must be set to obtain minimum intensity. The law of Malus then becomes

$$E = E_0 e^{-Klc} \cos^2(\theta + \alpha) \quad (12.9)$$

where K is the absorption coefficient of the sample, l is the path length of the sample cell, c is the concentration of the sample, and α is the optical rotation introduced by the sample. If the sample does not absorb light and a minimum of reflected and unpolarized light is produced, then Eq. (12.9) becomes

$$E = E_0 \cos^2(\theta + \alpha) \quad (12.10)$$

The change introduced by the optically active sample (α) is observed on a large circular scale which is graduated in angular degrees and usually may be read with a precision of approximately 0.005 – 0.010° .

Since visual polarimeters use the human eye as the sensing device, most accurate determinations must be made with the polarimeter in a darkened room where the operator's eye is dark adapted. Repeated routine measurements of an almost extinct field may become less precise due to fatigue and thus polarimeters employing a photoelectric sensing element have been designed. The substitution of photoelectric devices for the end-point determination has allowed the use of visual instruments for automatic recording and has facilitated the design of spectropolarimeters.

A simple photoelectric polarimeter may employ a chopper, which allows alternative transmission of the two areas of a conventional bipartite field of a visual polarimeter to a photomultiplier detector. The electrical signal of the photomultiplier which is generated by oscillation of the plane of polarization when the two halves of the bipartite field differ in intensity is amplified and fed to a servomotor mechanism, which rotates the analyzing prism to the balance point and thus automatically compensates for intensity differences and the angular rotation may be read on a mechanically coupled digital indicator.

Several modifications in the design of the simple photoelectric polarimeter just described have been designed. These instruments eliminate the conventional half-shadow device of the visual polarimeter and differ mainly in the method by which the plane of polarization is caused to oscillate. This oscillation may be achieved by mechanically rotating the polarizing prism over a small adjustable "symmetrical" angle. Balance is obtained by manually rotating the analyzing prism until a galvanometer attached to the photomultiplier tube gives equal readings for the two positions of the oscillating prism and the angle of rotation may be read conventionally. Another modulator which produces oscillation of the plane-polarized light is a

rotating "half-shadow" disk, which consists of two semicircular pieces of D and L quartz. The plane of polarization may also be caused to oscillate by means of a Faraday cell, which consists of an electromagnet surrounding a rod of transparent material such as dense flint glass. When current flows in the solenoid magnet, the rod becomes optically active and the optical rotation of the rod varies in direct proportion to the magnetic field. Reversal of current to the magnet causes reversal of rotation and thus alternating current will cause an alternating rotation of optical activity of the rod. The oscillating polarized light beam which is produced by the polarizing device and the alternating-current Faraday cell pass through to an analyzer, which consists of a polarizer in crossed orientation to the initial polarizer. At balance, a uniform light wave strikes the photomultiplier. An optically active

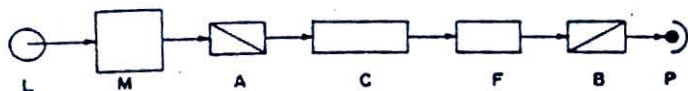


FIGURE 12.16: Schematic diagram of photoelectric polarimeter: *A*, polarizing prism; *B*, analyzing prism; *C*, sample tube; *F*, Faraday cell or half-shadow prism or half-shadow disk; *L*, light source; *M*, monochromator; *P*, photomultiplier tube.

sample will distort this wave pattern and the distortion may be analyzed by a phase-sensitive detector which then supplies direct current to another Faraday cell that acts as a null device and compensates for the optical rotation of the sample. The current supplied to the second Faraday cell is proportional to the optical rotation of the sample and may be measured by a galvanometer or by various read-out devices such as a chart or digital meter. A schematic diagram of the photoelectric polarimeters just described is given in Fig. 12.16. Most designs of the photoelectric polarimeter may be altered to produce spectropolarimeters (which measure the change in optical rotation with change in wavelength of plane-polarized light) simply by incorporating a monochromator between the energy source and the polarizing device.

Spectrophotometers may be converted into photoelectric polarimeters and spectropolarimeters by inserting polarizing and analyzing prisms into the light path of these instruments. In single beam spectrophotometers, the prisms are arranged so that the analyzer can be moved from $+\theta$ to $-\theta$. A solution which is not optically active transmits the same amount of light at both settings, whereas an optically active solution produces two different intensities for each position of the analyzer. The ratio of these two intensities is then related to the angular rotation of the sample α by the following equation

$$R = \frac{E_2}{E_1} = \frac{\cos^2(-\theta + \alpha)}{\cos^2(\theta + \alpha)} \quad (12.11)$$

where R is the ratio of the two intensities, E_1 at position $+\theta$ of the analyzer, and E_2 at position $-\theta$ of the analyzer. R values are converted to optical rotation through the use of tables or may be approximated to angular rotation α by the following equation

$$\tan \alpha = \frac{(1 - \sqrt{R})}{(1 + \sqrt{R})} \cot \theta \quad (12.12)$$

Double-beam spectrophotometers compare the energy of two identical units, each consisting of a polarizer and an analyzer and constructed to fit the sample and reference compartments of the spectrophotometer. The polarizer and the analyzer are crossed in each of the two compartments except for the small angle of $+\theta$ and $-\theta$ in the two units, respectively. When an optically active solution is substituted for the solvent, $+\theta$ and $-\theta$ become $\theta \pm \alpha$ and $-\theta \pm \alpha$, depending on the sign of the optical rotation of the solution. The intensities of the transmitted plane-polarized light in the two beams are no longer equal, and as before with the single-beam spectrophotometer the ratio of the two intensities R , which is directly determined, is related to the optical rotation.

The major disadvantage of this method for determination of optical rotation and optical rotatory dispersion is that the recorded curve is in terms of R values or transmittance values rather than optical rotation, and the values of the ratio is not always directly proportional to the angle of rotation, particularly when $|\alpha| \geq |\theta|$ and/or the solute exhibits circular dichroism at the wavelengths used.

To record the circular dichroism of an optically active solution, linearly polarized light must be resolved into two circularly polarized components. Passage of the monochromatic polarized beam through an appropriate quarter-wave plate, a Babinet-Soleil compensator, the use of a Fresnel rhomb, and a Billings' cell (Pockels' effect modulator) are four methods currently available for providing this resolution in commercial circular dichroism instruments. Such instruments directly measure the difference in absorption for the two circular components, and this difference is converted to an extinction coefficient, which is related to the molecular ellipticity by Eq. (12.7). The theory and design of circular dichroism instruments is beyond the scope of this present chapter.

12.5 APPLICATIONS

Measurement of the optical rotation of a compound may be used as one of the physical constants of a pure liquid or solute. Optical activity is the only physical property that will distinguish (+) and (-) isomers. The specific rotation is a physical constant, but, as noted previously, the concentration, temperature, solvent, and wavelength of plane-polarized light must always be stated when the specific rotation is reported.

Optical rotation may be used as a quantitative method for the determination of optically active compounds. An optically active substance may be determined in the presence of inactive compounds. It may be possible to analyze two component mixtures at a single wavelength in which both substances are optically active since optical activities may be additive, and therefore there is only a single composition possible for any value of rotation between that of the pure compounds. In most instances, the optical rotation is not strictly linear with concentration and a calibration curve must be constructed in which the observed rotation is plotted as a function of concentration with the other specified variables such as cell path length, temperature, and wavelength of plane-polarized light kept constant.

The determination of optical rotation finds its greatest application in the qualitative identification of organic compounds. Several methods have been developed which empirically correlate rotational data usually obtained at the sodium D line with the structure of organic compounds. Two examples follow. A method of molecular rotational differences based upon the empirical correlation of differences in molecular rotation of a series of known compounds may be applied to an unknown compound by carrying out a transformation on the unknown and assigning structural requirements to this unknown based on a correlation of the differences in rotation with known molecules. This approach to structure determination has been used successfully in steroid structure determination. Optical rotation is also used in carbohydrate structure determination in which empirical rules such as Hudson's rules may be widely applied to carbohydrate molecules.

Optical rotatory dispersion data have found most use in the determination of conformational and configurational information about known organic structures. Measurements of optical rotatory dispersion provide a means of distinguishing between random-coil and regular helical arrangements in polypeptides and proteins. Such optical rotatory dispersion spectra are dependent on conformation and disappear when the macromolecule assumes a random-coil arrangement.

The optical rotatory dispersion method for low molecular weight compounds involves study of the Cotton-effect curves which result when there is absorption of plane-polarized light by a chromophore, usually the carbonyl group, which has some sort of asymmetric environment. The underlying principle of the method is the octant rule, which qualitatively predicts the contribution of the substituents to rotatory dispersion when a substituent is introduced into a carbonyl-containing compound at a specific site. To apply the rule, a structure such as cyclohexanone in the chair configuration is divided into eight octants by three mutually perpendicular planes. One plane passes through the carbonyl carbon atom and carbons 2 and 6; a second plane passes through oxygen of the carbonyl and carbon 4; the third plane bisects the carbonyl group and is perpendicular to the other two planes. See Fig. 12.17. If a substituent is placed at any position other than on one

of these planes it destroys the symmetry of the system and the orbitals involved in the $\eta \rightarrow \pi^*$ transition of the carbonyl group may interact with the substituent. Therefore axial or equatorial substituents at carbon 4 do not contribute to the Cotton effect. Equatorial substituents at carbon 2 or carbon 6 have almost no effect on the sign of the Cotton effect. Axial or equatorial substituents at carbon 5 and axial substituents at carbon 2 have

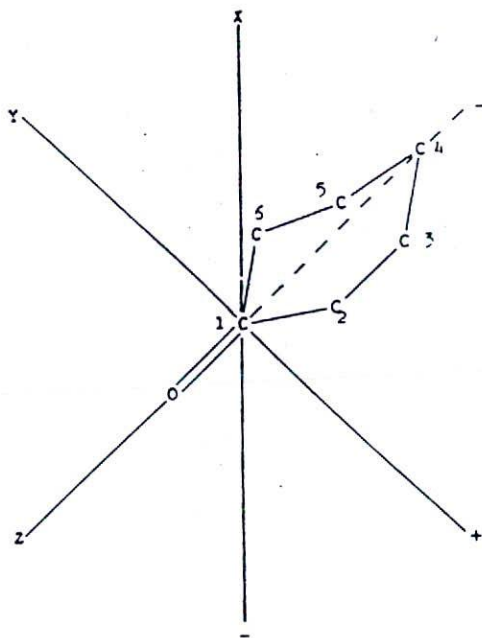


FIGURE 12.17: The basis for the octant rule.

a positive contribution to the Cotton effect and substituents at all other positions of the back four octants contribute negatively. In the example of cyclohexanone, the front four octants may be ignored. The octant rule allows prediction of the sign of the $\eta - \pi^*$ transition of the asymmetrically perturbed carbonyl group and allows certain conclusions about configuration and conformation of its environment to be made which have been experimentally substantiated for a large number of simple ketones.

Circular dichroism curves, for the most part, yield the same type of qualitative information as optical rotatory dispersion curves, and theoretically at least one type of curve may be converted to the other. Circular dichroism curves may be more useful for quantitative measurement of the Cotton effect, since the transitions which are observed are much sharper, like

ultraviolet absorption spectra, and do not tail off as do optical rotatory dispersion curves. (See Fig. 12.10.) Circular dichroism curves, therefore, may facilitate quantitative study of isolated asymmetrically perturbed chromophores which, in optical rotatory dispersion data, would have a continuous background due to chromophores which absorb at more distant wavelengths superimposed on the Cotton effect of a chromophore.

EXPERIMENTS

- E12.1. Specific rotation of camphor. Fill a polarimeter tube with ethanol and determine the zero reading of the polarimeter using a sodium-arc light source. Note the temperature at which the measurement is made. The half-shade angle of the instrument may be adjusted to obtain maximum sensitivity of the zero setting.

Accurately make-up a solution containing approximately 10% w/v camphor in ethanol. Fill the prepared polarimeter tube, note the temperature of the solution, and measure its rotation. Calculate the specific rotation $[\alpha]_D$ of camphor, $[\alpha]_D = \alpha/lc$ where α is the observed rotation in degrees, l is the polarimeter tube length in decimeters and c is the concentration in grams per milliliter.

Determine the rotation of a camphor solution supplied by the instructor and using your calculated specific rotation calculate the concentration of camphor in this unknown solution. Is the unknown sample material natural or synthetic camphor?

Explain any deviation between the concentration determined by the measurement of optical rotation and the actual concentration of the unknown. What improvements may be suggested to make your determination more accurate?

- E12.2. Determine the fructose content of fructose and sodium chloride injection NF supplied by the instructor, according to the procedure of the *National Formulary*, twelfth edition, p. 171.
- E12.3. Determine the epinephrine content of epinephrine solution USP according to the procedure of the *U.S. Pharmacopeia*, XVII, p. 226.
- E12.4. Acid hydrolysis of sucrose.* The rate of hydrolysis of sucrose may be followed by measuring the decrease of optical rotation with time. The complete hydrolysis of sucrose, $[\alpha]_D^{20} = +66.5$ ($c = 26$, H_2O), yields equimolar quantities of glucose $[\alpha]_D^{20} = +52.5-53.0$ ($c = 10$, H_2O), and fructose $[\alpha]_D^{20} = -92$ ($c = 2$, H_2O). During the process of hydrolysis the sign of the observed rotation decreases because of the high negative rotation of fructose, which more than balances the positive rotatory contribution of glucose. This hydrolysis reaction has been called "inversion" because of the change in sign of the rotation, and the reaction products are known as "invert sugar."

Prepare six solutions containing 10.0 g of sucrose per 100 ml of solution of the following molarities of hydrochloric acid; 0.25, 0.50, 1.00, 1.50, 2.00, and 3.00 *M*. Measure the optical rotation α_0 immediately after mixing

* Dawber, J. G., D. R. Brown, and R. A. Reed, *J. Chem. Educ.*, 43, 34 (1966).

the solutions, i.e., at time zero, and repeat the observations of the rotation of each solution at suitable time intervals α_t to observe the initial stages of the hydrolysis. Measure the optical rotation after approximately 1 week, α_x , to estimate the rotation of the completed reaction. Close control of temperature is necessary in this experiment. The solutions should be stored and the measurements should be made at a constant temperature.

The rate constant of the reaction k is given by the following equation:

$$k = \frac{2.303}{t} \log_{10} \left[\frac{\alpha_0 - \alpha_x}{\alpha_t - \alpha_x} \right]$$

For each solution plot a graph of t against $\log_{10} [\alpha_t - \alpha_x]$ and evaluate k (-slope). Plot the rate constant values vs. the molarity of the acid solutions and also $\log_{10} k$ values against the Hammett acidity functions H_0 for the various molarities of HCl.

H_0 values for the HCl solutions are; 0.25 M = 0.55, 0.5 M = 0.20, 1.00 M = -0.18, 1.50 M = -0.47, 2.0 M = -0.67, 3.0 M = -1.05, where

$$H_0 = pK_{BH^+} - \log_{10} \frac{[BH^+]}{[B]}$$

which is derived from the protonation of a neutral base B.

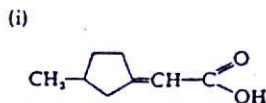
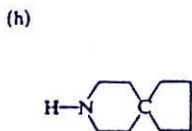
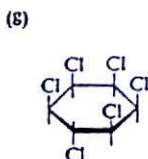
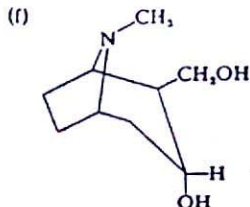
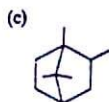
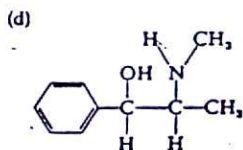
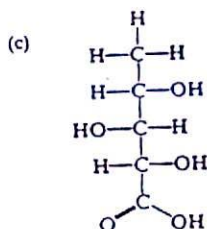
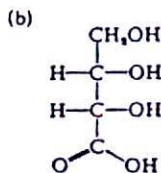
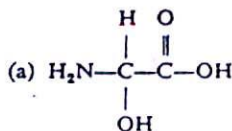


If the acid-catalyzed hydrolysis of sucrose is a unimolecular process, a graph of $\log_{10} k$ against H_0 should be linear and have a slope of 1.0. If the graph of k vs. acid molarity is linear, then the reaction mechanism is bimolecular.

QUESTIONS

- Q12.1. Describe the relationship between refractive index and optical rotatory power.
- Q12.2. Explain the following terms:
- enantiomer
 - diastereoisomer
 - racemate
 - mutarotation
 - inversion of configuration
 - elliptically polarized light
- Q12.3. Discuss the following statements:
- Optical rotation is not a universal property of all substances.
 - The measurement of optical activity is inherently a measurement of molecular dissymmetry.
 - The optical rotation of a solid, liquid, or pure compound in solution serves as a characteristic physical property of the substance and often will give a more specific identification than will the determination of other physical constants such as melting or boiling points.
 - The octant rule relates the sign of the rotatory power of an optically active ketone in the 300-m μ region to the substituents creating dissymmetry in the molecule.

Q12.4. Which of the following structures are optically active? Draw all isomers showing enantiomeric relationships.



PROBLEMS

- P12.1. The specific rotation, $[\alpha]_D^{20}$, of scopolamine is -28° . If a 1-dm tube is used and an optical rotation of -0.69° is observed, what is the concentration of scopolamine in this solution?
- P12.2. The specific rotation of quinidine sulfate, $[\alpha]_D^{25}$, in ethanol is 212 . What is the concentration of a solution of this salt which gives an observed rotation of 25.05° in a 2-dm tube?
- P12.3. A solution of chloramphenicol containing 1 g/100 ml of ethanol was placed in a 20-cm tube and had an optical rotation of $+0.38^\circ$ at 25°C . Calculate the specific rotation of chloramphenicol.

- P12.4. Chlortetracycline has a specific rotation of -275.0° . Calculate the expected optical rotation of a methanol solution containing 250 mg in 50 ml using a 0.5-dm tube.
- P12.5. The specific rotation, $[\alpha]_D^{20}$, of α -D-glucose is $+113^\circ$ and the specific rotation of the corresponding β epimer, β -D-glucose, is $+19.7^\circ$. A solution of α -D-glucose undergoes spontaneous epimerization to an equilibrium mixture of the α and β forms (mutarotation) which has a specific rotation of $+52.5^\circ$. Calculate the percentage of the α and β forms in the equilibrium mixture.

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