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Applications of chiroptical spectroscopy for the characterization of pharmaceutical compounds

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Abstract

Many pharmaceutical compounds contain one or more centers of dissymmetry, thus presenting a unique series of regulatory and compendial requirements. Although most often characterized using chiral chromatography, these molecules can be effectively studied using the various techniques of chiroptical spectroscopy. Techniques which have been found to be very useful for such work include polarimetry, optical rotatory dispersion, circular dichroism, and circularly polarized luminescence. The principles underlying each effect will be briefly outlined, and the application of each illustrated through the inclusion of appropriate examples.

Keywords: Chiroptical spectroscopy; Pharmaceutical compounds; Circular birefringence; Optical rotation; Optical rotatory dispersion; Circular dichroism

1. Introduction

Optical spectroscopy is generally concerned with measurements of the interaction of electromagnetic radiation (whose energy lies between 10 and 50 000 cm^{-1}) with isotropic (materials incapable of influencing the polarization state of light) or anisotropic (materials having the ability to affect the polarization properties of transmitted light) compounds. The various effects which are sensitive to the polarization state of this radiant energy are ignored during the conduct of ordinary spectroscopy, but can be used to study a wide variety of phenomena for molecules lacking certain types of molecular symmetry.

Molecules for which the mirror images cannot be superimposed are denoted as being dissymmetric, or chiral, and these enantiomer structures are capable of being physically separated from each other. The fundamental requirement for the existence of molecular dissymmetry is that the molecule cannot possess an improper axis of rotation. This rule is often trivialized to state that the molecule in question cannot contain either a center of inversion or a reflection plane. The term dissymmetric should be used rather than asymmetric, since it is possible for a molecule to contain proper axes of rotation and still be capable of existing as non-superimposable mirror images.

Chiral molecules interact with electromagnetic radiation in exactly the same fashion as do achiral

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molecules in that they will exhibit optical absorption, have a characteristic refractive index, and can scatter oncoming photons. Dissymmetric compounds will exhibit additional interactions with light whose electric vectors are circularly polarized. For example, the plane of linearly polarized light will undergo an apparent rotation (associated with the circular birefringence of the medium) as it passes through a chiral medium (polarimetry), and the magnitude of this rotation will depend on the wavelength of the light used (optical rotatory dispersion, or ORD). Similarly, left- or right-circularly polarized light will be preferentially absorbed within the electronic transitions of a dissymmetric compound circular dichroism (CD). In addition, the emission of circularly polarized light by a chiral luminescent molecule circularly polarized luminescence (CPL) can be used to evaluate molecular chirality. The optical activity associated with molecular vibrations can also be studied using either vibrational circular dichroism (VCD) or Raman optical activity (ROA), but these techniques lie outside of the scope of this article.

General and readable introductions to molecular optical activity have been published by Charney [1] and Nakanishi [2], and a number of monographs exist which deal with chiroptical issues ranging from the theoretical to the practical [3–11].

2. Circular birefringence (polarimetry) and optical rotatory dispersion

The study of molecular optical activity can be considered as beginning with the work of Biot, who demonstrated that the plane of linearly polarized light would be rotated upon passage through an optically active medium. The method of measurement was improved by Mitscherlich through the use of calcite prisms, and the double-field method of detection was introduced. Since these early developments, many advancements in polarimetry have been made, and a large number of detection schemes are now possible. An extensive summary of polarimetric methodology has been provided by Heller [12].

The experimental measurement of optical rotation is extremely simple. The incident light is collimated and plane-polarized, and then passed through the medium under study. Since the polarization plane of the incident light is fixed, the angle of rotation is defined with respect to this original plane. This is carried out by first determining the orientation of polarizer and analyzer for which no light can be transmitted (the null position), and then finding the new null position after the medium containing the optically active material is introduced between the prisms. The observed angle of rotation is taken as the difference between the two null angles, and a variety of measurement schemes have been developed to obtain the best experimental results. The influence of polarimeter design on the observed signal-to-noise characteristics has been discussed by a number of investigators [13–15].

The velocity of light (v) passing through a medium is determined by the index of refraction (n) of that medium:

$$v = c/n \tag{1}$$

where c equals the velocity of light in vacuum. For an achiral medium, the refractive index will not exhibit a dependence on the sense of the polarization state of the light. When the medium is chiral, however, the refractive index associated with left-circularly polarized light will not equal the refractive index associated with right-circularly polarized light, and the velocities of left- and right-circularly polarized light will differ on passage through a chiral medium. Since linearly polarized light is merely the vector resultant of two in-phase, oppositely-signed, circularly polarized components, then the differing velocities of the components produces a phase difference as they pass through the chiral medium. Upon leaving the chiral medium, the components are recombined, and linearly polarized light is obtained whose plane is rotated (relative to the original plane) by an angle equal to half the phase angle difference of the circular components. Charney [1] has shown that this phase angle difference is given by:

$$\theta = \frac{2\pi b'}{\lambda_0}(n_L - n_R) \tag{2}$$

In Eq. (2), θ is the phase difference, b' is the medium path length (in cm), λ_0 is the vacuum wavelength of the light used, and n_L and n_R are the refractive indices for left- and right-circularly polarized light, respectively. The quantity $(n_L - n_R)$ defines the circular birefringence of the chiral medium, and this quantity is the origin of what is commonly referred to as optical rotation.

The use of polarimetry data for the definitive probing of chemical problems has been effectively superseded by the use of the spectroscopic techniques yet to be discussed. However, this trend has been resisted somewhat by the pharmaceutical industry, where regulatory agencies normally require measurements of specific rotation as one proof of chiral identity. In fact, the measurement of optical rotation general test (⟨781⟩) is the only chiroptical test quoted in the United States Pharmacopoeia [16], which ensures a continued interest in polarimetry. For example, Chafetz has provided a compilation of the specific rotation values obtained for a very extensive list of steroids [17].

The magnitude of circular birefringence generally increases as the wavelength becomes shorter, and hence specific rotations increase in a regular manner at decreasing wavelengths. This behavior persists until the light is capable of being absorbed by the chiral substance, whereupon the refractive index exhibits anomalous behavior. This variation of circular birefringence as a function of wavelength is termed optical rotatory dispersion (ORD).

The anomalous dispersion observed in ORD spectra arises since the refractive index of a material is the sum of a real and imaginary part:

$$n = n_0 + ik \tag{3}$$

where n is the observed refractive index at some wavelength, n_0 is the refractive index at infinite wavelength, and k is the absorption coefficient of the substance. It has been amply demonstrated that if $(n_L - n_R)$ does not equal zero, then $(k_L - k_R)$ will not equal zero. As an example, the ORD curves measured for tigogenone and deoxytigogenone are shown in Fig. 1. Deoxytigogenone contains no chromophores at wavelengths longer than 250 nm, and its ORD spectrum consists of a plain negative curve. Tigogenone contains a single ketone group, and the ORD within the absorption band of this chromophore is found to exhibit anomalous dispersion. As is evident in Fig. 1, the ORD is positive at long wavelengths and negative at shorter wavelengths.

The earliest work involving chiral organic molecules was entirely based on ORD methods, since little else was available at the time. One of the largest data sets collected to date concerns the chirality of ketone and aldehyde groups [18], which eventually resulted in the deduction of the octant rule [19]. The octant rule was an attempt to relate the absolute stereochemistry within the immediate environment of the chromophore with the sign and intensity of the ORD Cotton effects. To apply the rule, the CD within the $n \rightarrow \pi^*$ transition around 300 nm is obtained, and its sign and intensity noted. The rule developed by Djerassi and coworkers states that the three nodal planes of the n- and π^*-orbitals of the carbonyl group divide the molecular environment into four front octants and four back octants. A group or atom situated in the upper-left or lower-right rear octant (relative to an observer looking at the molecule parallel to the C=O axis) induces a positive Cotton effect in the $n \rightarrow \pi^*$ band. A negative Cotton effect would be produced by substitution

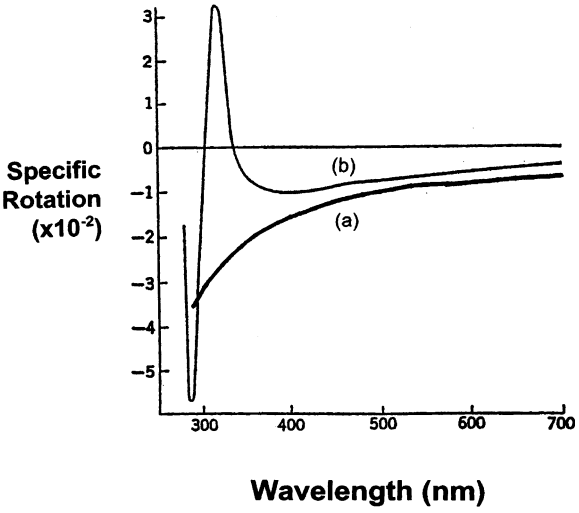

Fig. 1. Optical rotatory dispersion spectra obtained for methanolic solutions of (a) deoxytigogenin and (b) tigogenin.

within the upper-right or lower-left back octant. Although exceptions to the octant rule have been shown, the wide applicability of the octant rule has remained established. The ability to deduce molecular conformations in solution on the basis of ORD spectra data has proven to be extremely valuable to synthetic and physical organic chemists, and enabled investigators of the time to develop their work without requiring the use of more heroic methods.

3. Circular dichroism

As just discussed, circular birefringence and optical rotatory dispersion are easily interpretable outside regions of electronic absorption, but exhibit anomalous properties within absorption bands. This effect arises since the refractive index also contains a contribution related to molecular absorptivity, as discussed earlier. Not only will the phase angle between the projections of the two circularly polarized components be altered by passage through the chiral medium, but the amplitudes of these components will be modified by the degree of absorption experienced by each component. This differential absorption of left- and right-circularly polarized light is termed circular dichroism (CD), and is given by $(k_L - k_R)$.

The effect of one circularly polarized component being more strongly absorbed than the other is that when the projections are re-combined after leaving the chiral medium, they no longer produce plane polarized light. Instead the resulting components describe an ellipse, whose major axis lies along the angle of rotation. The measure of the eccentricity of the ellipse which results from the differential absorption is termed the ellipticity, ψ. It is not difficult to show that [1]:

$$\psi = \frac{\pi z}{\lambda}(k_L - k_R) \quad (4)$$

where z is the path length in cm, and λ is the wavelength of the light.

The ORD and CD spectra obtained for *N*-5,5-dimethyl-2-cyclohexen-1-on-3-yl)-gitingensine are shown in Fig. 2, where the existence of a positive Cotton effect in the ketone band is evident. Clearly, the two bands of the CD spectrum represent a more simple interpretative possibility than does the ORD spectrum. Once the spectral advantages of CD spectroscopy were realized, this technique became the method of choice for the conduct of chiroptical investigations.

It would be impossible to summarize the utility of CD spectroscopy for the study of molecular stereochemistry, since entire monographs have been written on the subject [4–11]. However, the technique can be used in exactly the same manner as is conventional absorption spectroscopy, with the differential absorption following its own analog of Beer's Law. The CD spectrum of a given compound can be used for diagnostic purposes, as was demonstrated in the identification of morphine, codeine, thebaine, or noscapine [20]. Being proportional to concentration, CD intensities can be used to evaluate the concentration of drug substances in pharmaceutical dosage forms [21].

The circular dichroism spectra of highly purified captopril (1-[2*S*)-3-mercapto-2-methylpropionyl]-(*S*)-proline), its component fragments, and its three other diastereomers have been reported [22]. The UV absorption spectrum of captopril consists of a single band maximum at 200 nm, while the CD spectrum consists of a single negative peak located at 210 nm. The CD spectra obtained for captopril and its three diastereomers are illustrated in Fig. 3, and can be explained largely in terms of a summation of the chirality of the individual components, (*S*)-proline and (2*S*)-3-mercapto-2-methylpropionic acid. However, this summation was found to be only approximate since the individual chiralities were found to be increased in the diastereomer compounds.

A variety of pharmaceutically active compounds have been characterized by means of CD spectroscopy. These include penicillin [23], tetracycline [24], anhydrotetracycline [25], nicotine [26], various cannabinoids [27], various corticosteroids [28], various β-lactam antibiotics [29], reserpine [30], ampicillin [31], naproxen [32], various cephalosporins [33], and amphotericin B [34].

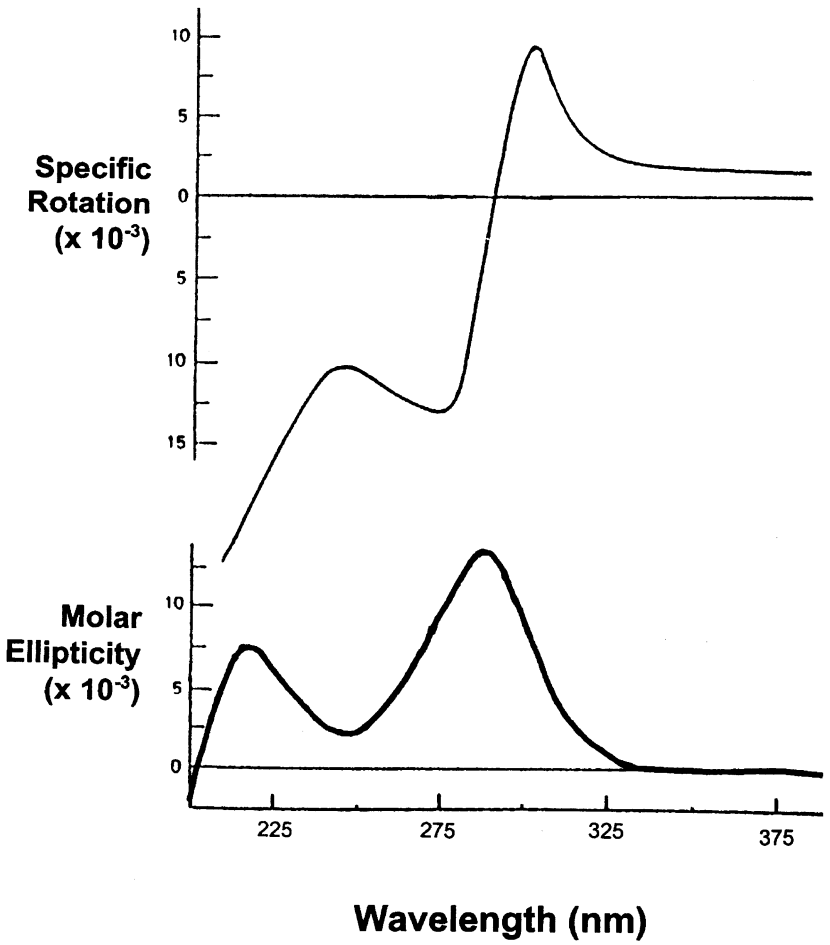

Fig. 2. Optical rotatory dispersion (upper trace) and circular dichroism (lower trace) spectra obtained for *N*-(5,5-dimethyl-2-cyclohexen-1-on-3-yl)-gitingensine.

4. Circularly polarized luminescence

One of the additional chiroptical techniques which has received a significant amount of attention is that of circularly polarized luminescence (CPL) spectroscopy [35–39]. CPL is the spontaneous emission of left- or right-circularly polarized light by a chiral luminescent species. In the CPL experiment, one simultaneously measures both the total luminescence (TL) intensity:

$$I = (I_L + I_R)/2 \quad (5)$$

as well as the differential emission:

$$\Delta I = I_L - I_R \quad (6)$$

Any dependence upon the arbitrary units by which I and ΔI are reported is removed by taking the ratio of these quantities, thus obtaining the luminescence dissymmetry factor:

$$g_{lum} = \Delta I/I \quad (7)$$

CPL spectroscopy can be thought of as the luminescence analog of CD spectroscopy, since each technique depends on the same general aspects of molecular structure. However, the CD spectrum will be dictated by the ground electronic state of the system under investigation, while the CPL spectrum reflects the properties of the luminescent excited state from which the emission originates. Should the geometry of a molecule remain un-

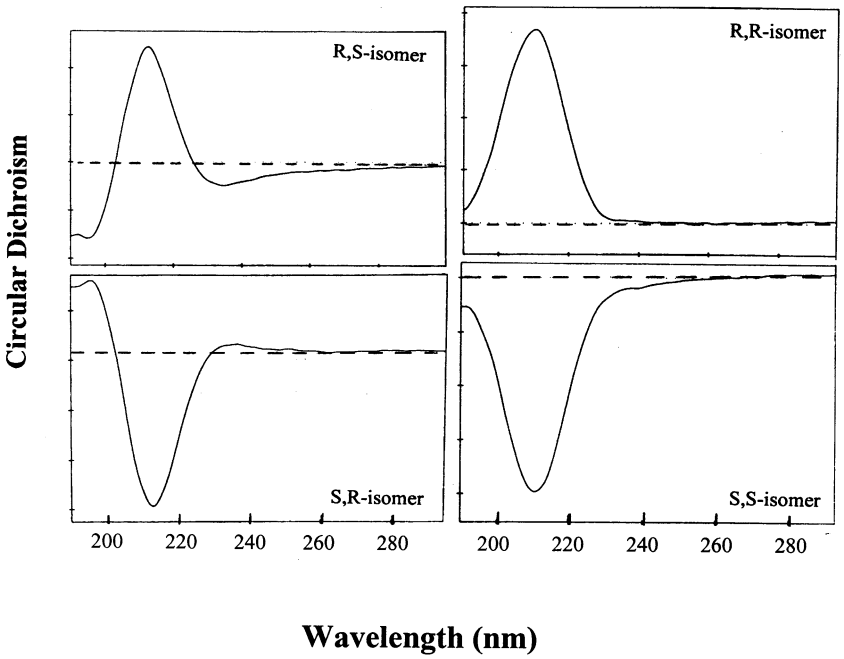

Fig. 3. Circular dichroism spectra obtained on the four diastereomers of captopril, 1-[(2*S*) 3-mercapto-2-methylpropionyl]-(*S*)-proline. The diastereomers are identified first by the absolute configuration of the side chain, followed by the absolute configuration of the proline group.

changed during the excitation process, then its characterization by either CD or CPL spectroscopies would yield equivalent information. The presence of a structural change for a molecule in its photoluminescent state is indicated by a non-correspondence of the CD and CPL spectra obtained for transitions between identical energy levels.

No commercial instrumentation is yet available for the detection of CPL, and therefore all spectrometers have been built by their users. Detection schemes using either analogue or digital techniques have been developed, and compelling reasons have been provided for the detection of CPL by differential photon-counting [40].

Owing to the favorable spectroscopic characteristics associated with the energy levels of Tb(III), CPL spectroscopy has proven to be the method of choice for studying the coordination chemistry of chiral lanthanide complexes [41]. These properties may be exploited for the use of Tb(III) complexes as reagents in CPL investigations of molecular chirality. For instance, CPL spectroscopy has been used to develop a chiroptical test for the enantiomeric identity of cinchona alkaloids [42]. The chief attributes of the lanthanide reagents are their high degree of luminescence in fluid solution, and the particularly simple sequence of energy levels which yield extremely sharp bands which are not shifted by crystal field effects and which are located in the visible region of the spectrum. In practice, the CPL induced in the $^5D_4 \rightarrow {}^7F_5$ emissive Tb(III) transition has been found to be of the greatest utility [41].

In one type of investigative mode, coordinatively unsaturated TbIII) compounds containing replaceable water molecules may form a ternary complex with an additional complexing substrate (*S*) ligand:

$$Tb(APC)(H_2O)_3 + S \Leftrightarrow Tb(APC)(S) + 3H_2O \quad (8)$$

In Eq. (8), APC represents an aminopolycarboxylate ligand (such as EDTA). When *S* is chiral, then the CPL induced in the Tb(III) emission bands can be used for determinations of enantiomeric identity or enantiomeric purity. In addi-

tion, the CPL bandshape will contain details which can be used to identify the coordinative mode of the chiral *S* ligand. For instance, the CPL obtained after Tb(EDTA) was allowed to form complexes with 25 simple amino acids enabled deductions to be made about the solution-phase coordination chemistry [43]. As evident in Fig. 4, a variety of CPL lineshapes have been found to be associated with different substrate functional groups. For example, L-alanine binds to Tb(III) using the α-amino carboxylic acid functionality, L-aspartic acid bindings using the two terminal carboxylic acid groups, L-cysteine binds using the carboxylate and sulfhydryl groups, and L-histidine bindings using the carboxylic acid and imidazole functional groups. Now that the basic system has been described, it is not difficult to extend the technique to permit the use of TbEDTA) complexes as reagents for determination of the enantiomeric identity or purity of any simple amino acid.

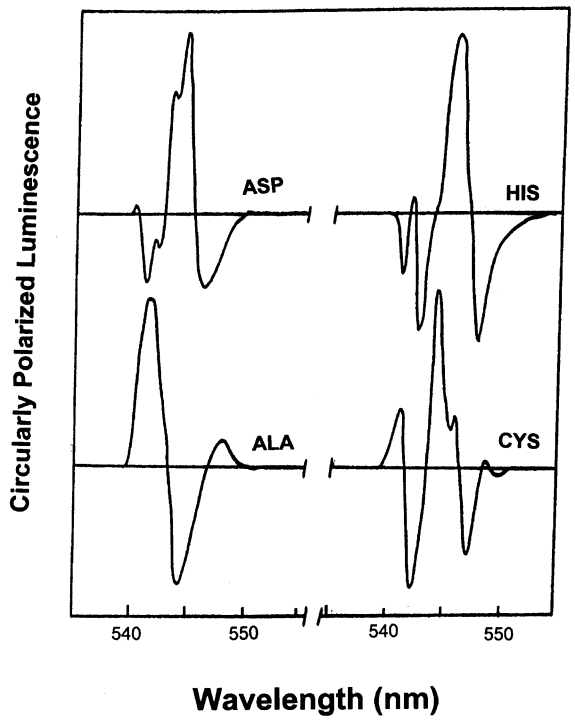

Fig. 4. Circularly polarized luminescence spectra obtained within the $^5D_4 \rightarrow {}^7F_5$ transition of Tb(EDTA) after complexation with L-alanine (ALA), L-aspartic acid (ASP), L-cysteine (CYS), and L-histidine (HIS).

Optical activity may also be induced in racemic mixtures of trigonal TbIII) compounds if these form outer-sphere complexes with an associating substrate ligand:

$$Tb(DPA)_3 + S \Leftrightarrow [Tb(DPA)_3](S) \quad (9)$$

In Eq. (9), DPA signifies the anion of pyridine-2,6-dicarboxylic acid. When *S* in chiral, the formation of the outer sphere complex generates two diastereomers of unequal stability. CPL will be observed in the Tb(III) emission bands as soon as one diastereomer is present in excess over the other. The highly labile nature of the $Tb(DPA)_3$ complex ensures that the equilibrium described by Eq. (9) is reached rapidly. The $Tb(DPA)_3$ reagent has been found to form outer-sphere complexes with hydroxycarboxylic acids, amino acids, amines, amino alcohols, amino acids, tartrate substrates, sugars, and resolved transition metal complexes [44]. It may easily be envisioned that the CPL obtained after formation of an outer-sphere Tb(III) complex could be used for determination of the enantiomeric identity of dissymmetric centers in the bound substrates, or evaluation of the enantiomeric purity of these substrates.

5. Concluding remarks

The majority of pharmaceutically active compounds produced to date have usually been small molecules, and many of these contain centers of dissymmetry. In present practice, the chirality of these agents is evaluated using polarimetry, where the sign and magnitude of the specific rotation is used to confirm the enantiomeric identity and/or purity of the analyse. However, it should be recognized that the range of techniques suitable for the study of molecular optical activity is extensive, and that molecular chirality can also be studied by ORD, CD or CPL. Since the development of dissymmetric chemistry is currently an extremely active research area in pharmaceutics, and one of great interest to regulatory bodies, it is anticipated that the development of chiroptical methods and applications will have to proceed at an equivalent pace.

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