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Review

A review of the application of chiroptical methods to analytical chemistry

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Abstract: The analytical applications of the two most important chiroptical methods, optical rotatory dispersion (ORD) and circular dichroism (CD), have been surveyed, emphasizing the methods corresponding to the profile of the *Journal of Pharmaceutical and Biomedical Analysis*. After a brief introduction of the ORD and CD methods, the advantages and drawbacks of the application of the two methods have been described, and compared, and the calibration of ORD and CD instruments have been given.

The analytical applications have been divided as follows: the use of ORD and CD in identification studies, the direct determination of optically active substances, the determination of optically active compounds after chromophore group formation, determination of optically inactive substances via their products with optically active compounds. The difference chiroptical methods increasing the selectivity of measurements and the chiroptical titrations have been summarized. Stress has been laid on the applicability of selective chiroptical detectors (based on laser light, too) used in liquid chromatography and the future developments have been mentioned.

Keywords: *Analytical application of chiroptical methods; optical rotatory dispersion (ORD); circular dichroism (CD); determination of optically active and optically inactive compounds; chiroptical methods in liquid chromatographic detection.*

Introduction

Aims and scope

Many of the compounds used therapeutically are chiral in nature. Furthermore it is known that the stereospecificity of drug action is related to the molecular asymmetry of component parts of the human body. As a consequence, the diastereomers and enantiomers of chiral drugs have significantly different biological activities and in certain circumstances, tragic consequences, as in the case of *S*(−)thalidomide. Clearly chiroptical methods* have an important role to play in the quality control of pharmaceutical products.

*Methods based on the optical measurement of chiral molecules are known as chiroptical methods. This paper deals only with the two most important chiroptical methods, optical rotatory dispersion (ORD) and circular dichroism (CD).

Chiroptical methods are widely used as an aid in the elucidation of the structures of chiral molecules. The characterization, determination and control of the optical purity of drugs can be achieved by chiroptical methods, nevertheless they are seldom used in chemical and pharmaceutical analysis. In pharmacopoeias and related texts, the measurement of the optical rotation at the sodium *D*-line, which offers relatively low sensitivity, is generally the only chiroptical method quoted. The aim of the present review is to demonstrate the applicability of the ORD and CD methods, with reference to the more important analytical studies. Besides introducing direct and indirect chiroptical methods (based on chemical reactions), the application of selective chiroptical detectors to liquid chromatography will be discussed.

Basic principles of chiroptical methods

The magnitude of the optical rotatory power varies with radiation wavelength, a phenomenon which is the basis of ORD. The difference in absorption intensity of right and left circularly polarized light at various wavelengths is termed circular dichroism (CD).

For detailed discussions on the theory, instrumentation and applications of ORD–CD, the reader is referred to books by Djerassi [1], Crabbé [2, 3], Velluz *et al.* [4], Snatzke [5], Klyne [6], Ciardelli *et al.* [7], Lambert *et al.* [8] and Charney [9].

The units used most frequently with the ORD method are, optical rotation (α) the specific rotation ($[\alpha]_\lambda^T$) and the molecular rotation ($[\phi]_\lambda^T$); and for the CD method, the ellipticity (ψ), specific ellipticity ($[\psi]_\lambda^T$) and molecular ellipticity ($[\theta]_\lambda^T$); given by the expressions:

$$[\alpha]_\lambda^T = \frac{100 \cdot \alpha}{l \cdot c} \qquad [\phi]_\lambda^T = [\alpha]_\lambda^T \cdot \frac{M_r}{100}$$

$$[\psi]_\lambda^T = \frac{100 \cdot \theta}{l \cdot c} \qquad [\theta]_\lambda^T = [\psi]_\lambda^T \cdot \frac{M_r}{100},$$

where α and θ are in degrees; l in dm, and c in g/100 cm^3. M_r is the relative molecular mass.

The following relationships exist between molecular ellipticity and CD, ($\Delta\epsilon = \epsilon_L - \epsilon_R$) and $[\theta] = 3300\Delta\epsilon$, where ϵ_L and ϵ_R are the molar absorption coefficients for the left and right circularly polarized radiation.

The relationship between CD and ORD may be expressed in terms of an integral transform known as the Kronig–Kramers relation. Thus the corresponding CD curve may be calculated from the experimentally determined ORD spectrum.

Excellent reviews on ORD by Djerassi [1] and on CD by Velluz [4] which discussed the analytical potential of the methods appeared in the early 1960s. Instruments for the measurement of ORD and CD, respectively, became commercially available in the early 1950s and 1960s. Since that time, the technology of the two methods has developed rapidly.

Comparison of the ORD and CD methods

From the aspect of analytical applicability, the two methods are characterized as follows:

(i) The most perceptible specific characteristic in ORD is the background or skeleton effect. The measured optical rotation at a given wavelength is the resultant of the contributions of the short- and long-range Cotton effects. ORD curves provide information on the whole of the molecule and also on the stereochemical environment of

the optically active chromophoric groups. The dispersive nature of ORD can represent an advantage when obtaining information on Cotton effects just beyond the wavelength limits of the instrument.

(ii) A drawback of the ORD method is that the various optically active chromophoric groups generally have overlapping bands, which are difficult to separate, even theoretically.

(iii) The most significant difference between the two techniques is that CD will give a signal only in the vicinity of an optically active absorption band.

In CD studies the individual optically active chromophoric groups do not in general overlap. Accordingly, the CD method can be used to advantage in the analysis of mixtures containing several optically active substances.

(iv) The CD method has the disadvantage that it can be applied only in the absorption range, whereas the ORD method is equally well applied to molecules that have no optically active chromophore in the wavelength region to be studied.

Calibration of ORD and CD instruments

Spectropolarimeters may be calibrated with aqueous sucrose solutions or with standardized quartz plates [10, 11].

Inert liquids, e.g. (−)-2-methyl-l-butanol, have been used for the calibration of spectropolarimeters [11].

In contrast to the calibration of the spectropolarimeters used for measuring the absolute values of ORD, the calibration of the apparatus used for measuring CD is a difficult and delicate task.

Basically two methods have been used.

(i) The ellipticity is calculated from the data of ORD curves by means of the theory of Kronig–Kramers. Generally, the ellipticity is calculated from the extreme values of the ORD curves of (+)-camphor-10-sulphonic acid or (+)-camphor [12–15]. D-Pantolactone and D-glucurono-γ-lactone have been applied by Konno *et al.* [16].

(ii) The ellipticity values of different standard substances have been used for the calibration of the apparatus; e.g. (+)-camphor-10-sulphonic acid, ammonium(+) camphor-10-sulphonate, *n*-propylammonium- and *n*-butylammonium salts of camphor-10-sulphonic acid, D-pantolactone, (+)tris(ethylenediamine)cobalt(III)iodide monohydrate [13–19].

Shippers *et al.* have constructed an apparatus which gives absolute CD data directly, based upon calibration at three wavelengths [20].

Analytical Applications

The use of ORD and CD in qualitative analysis

The ORD and CD curves, the ellipticity and the optical rotation measured at different wavelengths all can be used for solute identification.

Specific rotations of the majority of organic compounds have been published in various texts, pharmacopoeias and monographs. Data with reference to the *D*-line of sodium are the most frequently published, but these do not characterize compounds as well as the values measured at the maxima of the ORD curves.

Specific rotation data have been summarized by Mathieu *et al.* for steroids [21], derivatives of terpenes [22], alkaloids [23] and amino acids [24]; and by Sagel *et al.* for antibiotics [25], sugars and sugar derivatives [26].

ORD and CD curves published in various communications and monographs dealing with structure elucidations [1–9] are in many cases useful for identification of compounds.

More characteristic data are obtained if the ratio of the optical rotations measured at different wavelengths is measured [27, 28].

The specific rotations of D-α-tocopherol and DL-α-tocopherol ($[\alpha]^{25}_{546.1}$ = 0.32 and 0°, respectively) are too small to be useful for identification, but the differences in optical rotation of their products of oxidation by alkaline potassium ferricyanide are, however, great enough to distinguish the two forms [29] ($[\alpha]^{25}_{D}$ = 31.5 and 0°, respectively).

Crone and Purdie have successfully applied CD spectroscopy to distinguish between 10 opiates [30] using spectra obtained in aqueous hydrochloric acid, sodium hydroxide and a pH 8.6 buffer which gave the best qualitative results. Opium alkaloids have also been investigated in the solid state by means of CD using KBr pellets [31].

It is well known that cholesteric liquid crystals exhibit enormous optical rotations, of the order of several thousand degrees per millimetre. A significant development has been the discovery that achiral molecules in this medium display Cotton effects. Hence CD measurements may be extended as a general method to achiral molecules (liquid-crystal-induced circular dichroism, LCICD). The UV and LCICD spectra of anthracene in a mixture of cholesteryl nonanoate/cholesteryl chloride, 40:60 (wt %) are shown in Fig. 1 [32].

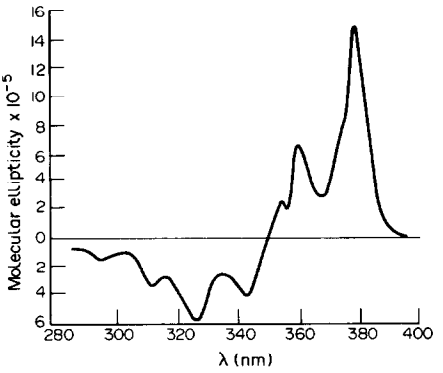


Figure 1
The cholesteric liquid-crystal-induced CD spectra of anthracene in cholesteryl nonanoate/cholesteryl chloride, 40:60 (wt %) (from ref. 32).

Bowen *et al.* [33] have used the same liquid crystalline mixure to induce an extrinsic CD for distinguishing between structurally similar molecules all of which possess the same aromatic chromophore. Morphine, codeine, 3-monoacetylmorphine, 6-monoacetylmorphine, 3,6-diacetylmorphine (heroin), dihydrocodeine, hydrocodone and thebaine can be identified by this technique.

Cycloheptaamylose (β-cyclodextrin) as chiral host can induce chirality into a guest molecule. If the guest contains a chromophore the interaction produces a complex which gives a CD spectrum. This solute-induced CD spectra can be used for drug identification [34–36].

Some drugs including chiral, achiral and racemic compounds have been investigated by this technique; e.g. L-cocaine, L-hyoscyamine, L-isomethadone, phencyclidine, phenobarbital, meperidine, diazepam and DL-methadone, atropine, DL-α-phenethylamine. CD spectra of cocaine and phencyclidine are shown in Fig. 2.

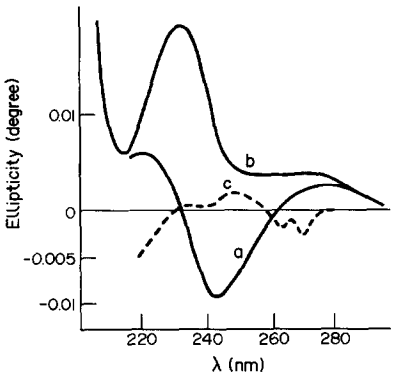


Figure 2
CD spectra for: a, 2.25×10^{-4} M L-cocaine in water; b, 2.25×10^{-4} M L-cocaine in 10^{-2} cycloheptaamylose; and c, 5×10^{-4} M phencyclidine in 10^{-2} M cycloheptaamylose (from ref. 34).

The direct determination of optically active substances

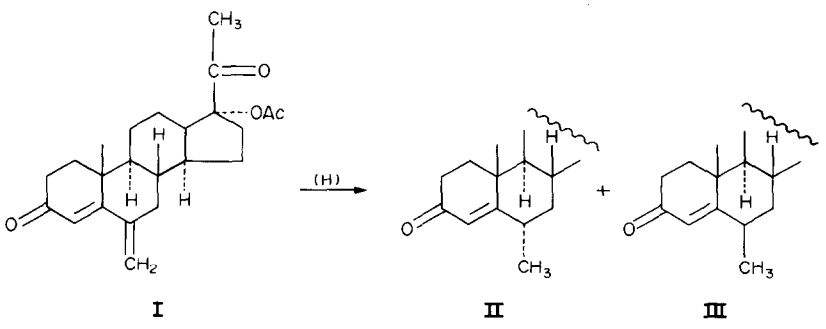
Direct measurements of optical rotation or ellipticity at one wavelength, at several wavelengths for mixtures, are commonly used for quantitative analysis.

In 1960, Djerassi published an excellent review [1], which, though predominantly concerned with structural studies on steroids, also draws attention to the possibilities for quantitative analysis. Hecogenin acetate (an important basic material for the industrial production of steroids) has been determined in the presence of tigonenin acetate, while diosgenin acetate has been determined in the presence of its most frequent impurity cryptogenin acetate.

Two steroid hormones can be determined in the presence of one another via their ORD spectra if the rotations are measured at the maxima of the individual ORD curves and the results calculated by the well-proved principles of spectrophotometry [37]. For testosterone, progesterone, oestrone and androstenolone, the rotation is linearly proportional to the hormone concentration in the range 15 $\mu g\ ml^{-1}$ to 2.5 $mg\ ml^{-1}$.

Often use is made of ketal formation in steroid synthesis. The course of the reaction can easily be followed through observation of the decrease in the Cotton effect curve, which has been studied by chiroptical methods. Zalkow *et al.* [38] have found that the ketone–ketal equilibrium is strongly dependent upon the amount of water present, the structure of the alcohol, as well as on stereochemical factors, such as the size of groups adjacent to carbonyl function.

The enzymatic production of prednisolone from hydrocortisone can be checked very simply after dilution of the reaction mixture. The hydrocortisone may be measured selectively at 326 nm, where prednisolone has no dichroism [39].

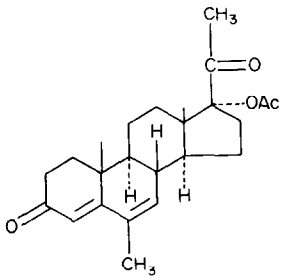


I II III

In the course of the production of medroxyprogesterone acetate (**II**), a drug with a strong progestogenic effect, the stereoisomer (**III**) too is formed as the reduction of (**I**). Potapov *et al.* [40] have established an appreciable difference in the CD spectra of the isomers. At 308 nm, the molecular ellipticity of the β-isomer is 5400°, while that of the α-isomer is zero.

The 4,6-diene derivative megestrol acetate (**IV**) can be prepared by the isomerization of **I**.

At 292 nm, the molar ellipticity of (**I**) is 21,000° while that of (**IV**) is 0°; at 238 nm, the molar ellipticity of (**IV**) is 17,000°, whilst that of (**I**) is 0°. Thus **I** and **IV** can both be determined in the presence of the other [41].



IV

Gergely *et al.* [42] have developed a very fast and selective CD spectroscopic procedure for the determination of Δ^4-3-ketosteroid contamination in drug base substances. The ellipticity is measured at the maximum (342.8 nm) of the multiple Cotton effect curve characteristic of these compounds, with dioxan as solvent. At this wavelength the determination is not disturbed by the base substances or their frequent "diene" or oestrogen contaminants. The norethisterone acetate content of ethynodiol diacetate and the norethisterone content of norethinodrel can be measured quantitatively, even in samples with an impurity level of 0.05%.

Steroids are frequently marketed in the form of liquid injection solutions. Quantitative analysis of certain steroid hormones and vitamin D in liquid dosage forms have been described by spectropolarimetry [43, 44]. Steroid hormones containing the Δ^4-3-keto chromophore group have been investigated by the CD spectroscopic method [45]. It has been established that the various sunflower oils used in the above formulations have no ellipticity at the measurement wavelength; thus, after dilution of the injection solutions with dioxan, the active agent can be determined directly.

In a series of papers, Purdie *et al.* [46–50] have reported the use of the CD method for the determination of narcotic drugs. A procedure has been described for the direct quantitative analysis of L-cocaine in confiscated samples [46]. Among the usual additives present in such samples are lidocaine, procaine and benzocaine. Each of these compounds absorb in the same UV range as the cocaine but all are optically inactive. The normal excipients are starches and simple sugars which are optically active but are CD inactive.

Heroin [47] and D-lysergic acid diethylamide [48] have been determined without separation in confiscated samples, and L-cocaine has been analysed in binary mixtures [49] with L-morphine or (±)methadone. The heroin was determined either directly in 0.1 M hydrochloric acid or as morphine after complete hydrolysis of the hydrochloric

acid on the addition of a pellet of sodium hydroxide [47]. Four chiral alkaloids of opium, namely morphine, codeine, thebaine and noscapine have been successfully determined in opium powders by means of a simple microcomputer-aided method [50].

Cannabinoids in chloroform extracts of marihuana have been analysed spectroscopically using CD [51]. Cannabis is easily distinguished from other green plant materials by its intense negative Cotton band with a wavelength maximum at 310 nm. Direct determination of Δ^9-tetrahydrocannabinol and cannabinidiol is possible in the residual organic solution following extraction of acid analogues into 1 M sodium hydroxide.

Palma *et al.* [52] have analysed directly, cocaine hydrochloride and codeine tablets using spectropolarimetry. Codeine samples were freed from optically active sugars by extraction with chloroform at pH 8–9. The procedure is as accurate, faster and more specific than the USP–NF XX method.

CD spectroscopy can be used very effectively for the determination of the nicotine content of chopped tobacco leaves, after a simple and direct single extraction into methanolic potassium hydroxide [53]. A detection limit of 0.16 $\mu g\ ml^{-1}$ is claimed. However, the method does not distinguish between nicotine and its structural analogues so that results need to be reported as total nicotine.

Tetracyclines readily undergo epimerization to 4-epitetracycline, and this process results in a considerable loss in physiological activity. Mixtures of tetracycline and 4-epitetracycline were assayed by CD, utilizing the large difference in the spectra when measured in acidic solutions at 262 nm [54]. The assay has been applied to the analysis of commercial tetracycline hydrochloride capsules. Direct analysis of tetracycline in human urine also has been described using CD [55]. The limit of detection was found to be 1.8 $\mu g\ ml^{-1}$. Dissolved sugars, proteins and glucuronides do not interfere.

Neomycin *B* and Neomycin *C*, the main components of aminoglycoside antibiotic Neomycin, differ with respect to the configuration of one asymmetric centre. The quantity of Neomycin *B* and Neomycin *C* in the sample can be calculated from the experimentally determined optical rotations, the values of specific optical rotations and the overall concentration [56].

Glenn's orthogonal function method has been extended to correct for interferences in spectropolarimetric analysis [57], and has been applied to the determination of some penicillins in the presence of the corresponding degradation products, mainly penicilloic acid. A spectropolarimetric method has been developed for the analysis of ephedrine and pseudoephedrine in their mixtures [58]. Depending on the solvent, both compounds give different positive plain rotational dispersion curves in the examined wavelength range (589–313 nm). Through variation of the solvent, a wavelength has been found where ephedrine has no optical rotation and pseudophedrine can be determined without interference.

α-Amino acids have been determined using ORD [59]. L-Acids show a positive Cotton effect whilst the D-isomers show a negative Cotton effect in the range of 220–230 nm. The method is 100–700 times more sensitive than polarimetric measurements, with respect to the *D*-line of sodium.

Direct determination of pantothenol has been performed using solutions and emulsions [60]. Polarimetry, as a general method, has been suggested for enzymes catalysing a variety of reaction types, e.g. intestinal peptidases, glutamate decarboxylase, phosphohexose isomerase, glutaminase and glutamate-alanine transaminase [61]. Hassan [62] has described the microdetermination of lysozyme enzyme based on the optical rotation of the molecule.

Han and Purdie showed that stereoisomers can be determined by CD [63]. Binary mixtures of the quinine and cinchonine alkaloids, of pilocarpine and isopilocarpine and of mixtures of atropine with hyoscyamine have been investigated without performing any form of chromatographic separation.

The water contents of various solvents can be determined sensitively by polarimetric methods based on measurement of the optical activity of D-tartaric acid dissolved in the solvents [64].

The determination of optically active compounds after chromophoric group formation

The introduction of chromophoric groups into an analyte has often been used for chiroptical testing [1–9]. Application of this method is advantageous when:

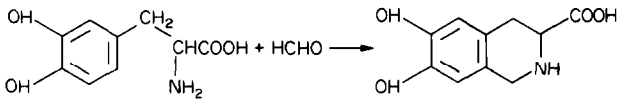
(a) an optically active absorption band results from the introduction of the chromophoric group;
(b) a more selective determination is possible via the chromophoric group;
(c) there is an improvement in sensitivity;

and

(d) the signal-to-noise ratio is enhanced.

Weber has described the reaction of dextroamphetamine with 1-fluoro-2,4-dinitrobenzene to form the 2,4-dinitrophenyl derivative [65]. The optical rotation of the derivative is about 30 times greater than that of dextroamphetamine. Both the content and optical purity of the drug at single-dose levels may then be determined.

The optical rotation of laevodopa is an important criterion of purity. The specific rotation $[\alpha]_D^{25}$ of laevodopa is only about $-12°$, which is insufficient to control optical purity. Laevodopa, however, can be cyclized with formaldehyde, resulting in a more than 10-fold increase in specific rotation [66].



Complex formation can also be used as an aid to the determination of optical purity of compounds; for instance, ethambutol can be determined sensitively via its complex with copper [67]. The optical purity of amino acids also can be tested by means of ORD and CD methods [68, 69]. Optically active amino acids when dissolved in an alkaline solution of the potassium salt of the optically inactive CO^{III}-*NN'*-ethylenebis(acetylacetonimine)–glycine complex show multiple Cotton effect curves in the visible region. The optical purity may then be determined by measurements at 475 nm (CD) and 500 nm (ORD), respectively.

Sugars and their derivatives may be investigated as well, in the presence of each other, on the basis of the chelates formed with molybdates at different pH values [70–73].

Difference ORD and CD methods

Difference chiroptical methods (ΔORD, ΔCD) are generally used to increase the assay selectivity. The compound to be determined is maintained at the same concentration in both the test and reference solution. The ΔORD or ΔCD curves result exclusively from the change in the spectrum of the compound in question in response to chemical or physical effects in one of the solutions. This technique is particularly important as it

concurs with methods based on the measurement of optical rotation, for the ORD curves display practically no wavelength where selective measurement is possible (skeleton effect).

In a series of papers, Gergely *et al.* [74–78] have reported the use of the ΔORD and ΔCD methods for the determination of steroids.

The ethynyl steroids are prepared from the corresponding 17-keto compounds by ethynylation. The contaminating 17-keto compounds exhibit a sharp rotatory peak, which may be explained by the n–π^* electron transfer of the carbonyl chromophore. The 17-keto compounds are reduced with sodium borohydride to 17-ol derivatives. In the ORD spectrum of the reduced solution, the sharp rotation peak, characteristic of the 17-ketosteroids, is absent [74]. Since the optical rotation of the ethynylsteroids is not affected by the sodium borohydride, the significant difference between the optical rotation of the reduced and unreduced solutions may be used as the basis for a determination. It is found that impurity levels of 17-ketosteroids in ethynylsteroids in the range 0.05–3%, thus may be determined.

17-Ethynylsteroids in the presence of a large excess of sodium alkoxides at high temperature can be converted to the corresponding 17-keto compounds.

The maximum in the Cotton effect curves of these latter compounds can be measured sensitively [75]. By application of the ΔORD method, the greater selectivity can eliminate the interference by manufacturing impurities in the 17-ketosteroid. ORD curves of ethynylandrostendiol are given in Fig. 3.

The long-wavelength absorption band of α, β unsaturated ketones varies considerably with change in the polarity of the solvent. This solvent effect has been utilized as the basis of a difference CD spectroscopic method for the determination of Δ^4-3-ketosteroid present in pharmaceutical formulations [76]. The ellipticity of a cyclohexane solution of these compounds is measured at 353.1 nm against a methanolic solution with the same concentration. A difference CD curve obtained in this way for testosterone phenylpropionate is depicted in Fig. 4.

The method has been used successfully for the determination of Δ^4-3-ketosteroid content of oily injections with dioxane-isopropanol as solvent pair [77].

21-Hydroxy- and 21-acetoxycorticosteroids have been determined by means of ΔCD spectroscopy. With periodic acid, 21-hydroxycorticosteroid compounds can be transformed to a 17β-carboxylic acid and formaldehyde.

The 20-keto group of the 17β-keto side chain has an optically active absorbance band between 270 and 300 nm, and an intensive positive Cotton effect is observed in the CD

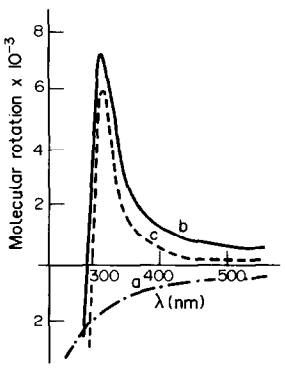


Figure 3
ORD spectra of ethynylandrostendiol. a, Before reaction; b, after reaction; c, difference spectrum (from ref. 75).

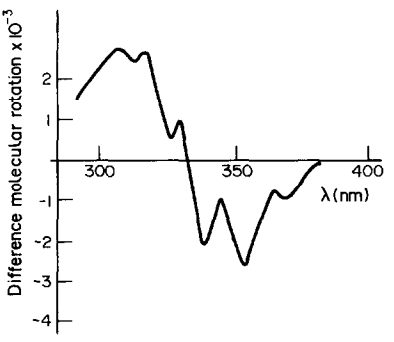
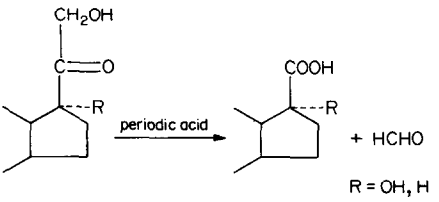


Figure 4
Difference CD curve of testosterone phenylpropionate (the cyclohexane solution was recorded against the methanol solution); concentration: 0.5 mg cm^{-3} (from ref. 76).



spectra. Following periodate oxidation, this band is no longer present [78]. The quantitative determination is based on the considerable difference between the ellipticities before and after oxidation.

The corticosteroid C_{21} esters react with periodic acid only after alkaline hydrolysis. The ΔCD method selectively measures the quantity of corticosteroid ester in the presence of 21-hydroxycorticosteroids, as the latter are oxidized in both the unknown and the reference solution.

Davidson [79] has described an ingenious difference spectropolarimetric procedure for the assay of (−)-adrenaline in formulations containing adrenaline hydrogen tartrate. The concentration of (−)-adrenaline was determined on the basis of the optical rotation measured at 249 nm corrected for the optical activity of tartaric acid. The latter being determined by measuring the difference in rotation of solutions at pH 1.1 and 5.6, respectively. The total concentration of adrenaline may be determined spectrofluorimetrically.

In a series of papers, Berglöf *et al.* [80] have described the determination of unconjugated bilirubin in human serum. Bilirubin is inherently optically inactive, however, but when it is bound to albumin, very strong optical activity is induced. Bilirubin can be oxidized and thereby transformed into optically inactive derivatives. The difference in rotation measured for serum samples before and after oxidation yields the bilirubin concentration.

The glucose content in its mixture with saccharose has been determined after reduction to optically inactive sorbate; the difference between the optical activities of the solutions before and after the reaction is proportional to the glucose content [81]. The method of Lipták and Paál [82], based on the complexation between glucose and borate, is applicable for the selective determination of glucose in pharmaceutical solutions containing optically active lactate.

Determination of optically inactive substances via their products with optically active compounds

The chiroptical methods have in some cases been successfully employed for the determination of optically inactive compounds.

β-cyclodextrin (chiral host) can induce chirality into an achiral guest molecule. If the latter contains a chromophore, the interaction produces a complex which gives a CD spectrum. Commercially available Demerol tablets have been assayed directly for pethidine using induced CD spectral data obtained for solutions resulting from the dissolution of tablets in an alkaline solution of β-cyclodextrin [35]. The inclusion of barbiturates within the cavity of cycloheptaamylose results in extrinsic optical activity. The induced Cotton effects have been used to assay commercial seconal sodium suppositories without prior separation [83].

Meta- and *ortho*-nitrobenzaldehyde have been determined in mixtures by measuring the optical rotations of their Schiff bases with an optically active compound (α-phenylethylamine) at three different wavelengths [84]. Benzaldehyde [85] and *p*-methoxybenzaldehyde [86] have been determined similarly.

Aromatic amines have been determined spectropolarimetrically by treating them with optically active dibenzoyltartaric anhydride [87].

A spectropolarimetric method has been proposed for the determination of methanol in ethanol by means of (+)1-α-phenethyl-3-phenyl-2-thiourea [88].

The method often has been used to determine different metals with optically active ligands (most frequently D-tartaric acid) through the chiroptical properties of the resulting complexes [89–95].

Chiroptical titrations

Assay by chiroptical titration is possible when the chiroptical properties of the compound being determined, or of the "compound" formed between the titrant and the test material, or possibly the indicator, undergo change.

The titration is usually carried out in large volume with a concentrated titrant, as in the case of conductimetric analysis. A method has been developed for the determination of acids and bases, using D-tartaric acid as indicator [135]. This indicator has also been used for the determination of a range of metals [136, 137].

The scope of the method has been extended by the use of two chiral and stereospecific aminopolycarboxylic acids, namely D-(−)-1,2 propylenediaaminetetraacetic acid and D-(−)-*trans*-1,2-cyclohexanediaminetetraacetic acid, as titrants, thereby permitting the determination of up to 39 metals [96–104, 138, 139].

A combined coulometric, spectropolarimetric method has been applied to the microdetermination of certain metal ions [105].

The use of chiroptical methods in liquid chromatographic (LC) detection

The LC detectors based on optical activity are potentially advantageous in a number of difficult areas of organic analysis. Particularly as most chromatographic eluents are not optically active, therefore the analyst is not restrained by the choice of eluents or gradients.

One major advantage of optical activity detection is its inherent selectivity [106, 107]. Compounds that do not possess optical activity simply will not interfere with analysis, even if they co-elute with analytes. This is especially useful when physiological fluids are being analysed.

The unique selectivity of the polarimetric detector for LC is due to the fact that optical activity is frequently associated with biological activity. The ability to differentiate between optical isomers is important, however, these isomeric compounds will generally co-elute on most conventional stationary phases currently available [108]. Therefore, the possibility of using a selective detector which can differentiate between optical isomers, even if they are not, or only partially, separated, is attractive.

The sensitivity of polarimetric detectors has been discussed from a theoretical point of view by Baumann and Cox in separate articles [109, 110]. In practice it is found that with high sensitivity polarimeters it is possible to measure 10^{-6}–10^{-7} radians, whilst with laser beam instruments it is possible to get down to 10^{-10}–10^{-11} radians [111].

Polarimetry has been applied to solute detection with conventional column chromatography. Thus Rossi [112] has separated the aminoglycoside antibiotics, neomycin *B* and *C*. By means of a specially constructed micro-cell (110 μl) it was possible to record the optical activity continuously, with an automatic polarimeter.

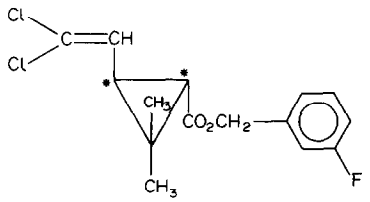
A number of successful assays have been reported with low-pressure LC systems using stationary phases such as starch and cellulose derivatives [113]. More recently, chiral detectors have been applied to high-performance liquid chromatography (HPLC), using spectropolarimeters equipped with a micro flow cell with typically 10-cm optical pathlengths and internal volumes of up to 33 μl [106, 114–116]. With such a system, optically active (−)α-bisabolol has been determined in ethanolic camomile extracts [114] using reversed-phase columns and aqueous acetonitrile gradient elution.

Polarimetric detectors are particularly well equipped for sugar analysis [106, 116] despite inherent lower sensitivity. For instance, when a mixture containing 200-μg quantities of xylose, arabinose, fructose, glucose, sucrose, maltose and lactose was examined with a multidetector system, comparable peak areas were observed for the UV and refractive index detectors, whilst the chiral detector gave peaks reduced by a factor of two. Accordingly, detection limits were estimated to be 10 μg for UV and refractive index (RI) detection, and 20 μg for the polarimeter. However, the UV and RI detectors lack the selectivity of the chiral system.

Individual amino acid enantiomers have been assayed, resolved by HPLC using optically active supports complexed with metallic ions, together with polarimetric detector (λ, 254 nm) [117]. Enantiomers of valine, threonine, isoleucine, serine, phenylalanine, tyrosine, tryptophan and asparagine are completely resolved in less than 1 h. Even if the enantiomers are not totally resolved, the detector reveals evidence of their partial separation and gives the elution order.

It is frequently very useful to apply chiroptical and conventional LC detectors in series, in particular UV-vis and RI. One of the first applications of such a system was by Boehme [115] who used LC with UV and polarimetric detection for the analysis of chiral compounds in mixtures containing non-chiral compounds. Since *R*- and *S*-isomers do not show any difference in their absorption characteristics, only the total amount of *R*- and *S*-enantiomer can be determined with the UV detector, whilst the response of the polarimetric detector depends on the actual quantitative ratio of the enantiomers.

Permethrinic acid pentafluorobenzyl ester (PBE) is a compound which has two asymmetric carbons in a cyclopropane ring [115]. The two pairs of enantiomers have different insecticidal activities. The *cis*- and *trans*-isomers are readily separated by LC, whilst the *R*- and *S*-enantiomer ratio may be determined from the UV and polarimeter responses. The UV absorption corresponding to the *trans*-PBE peak is proportional to



the total amount of enantiomer present, whilst the response of the polarimetric detector is dependent upon the ratio of enantiomers.

L-Epinephrine can be specifically analysed in ophthalmic formulations by HPLC using a UV detector in series before a polarimetric detector [118]. Expressions have been developed using the optical rotation–UV detector response ratio of an L-epinephrine standard that enables determination of the concentration of each enantiomer in an unknown mixture. The method requires minimal sample preparation and 12–15 samples can be analysed per hour.

D- and L-penicillamine cannot be separated chromatographically on a conventional stationary phase. As a consequence, when using an RI or UV detector, a single peak corresponding to the total amount of the two optical isomers is observed, however, with a polarimetric detector the percentage of each isomer in the peak may be determined [108].

DiCesare effectively separated carbohydrate mixtures, using the polarimeter and the RI detector in series [108].

Sensitivity in ORD or in CD may be enhanced by lasers. Yeung, Kuo and co-workers have demonstrated the feasibility of using a sensitive laser based optical activity detector [107, 119–125]. Optical rotations of 1×10^{-5} degree and detection of biological compounds at the 100 ng level have been repeatedly reported.

Six naturally occurring carbohydrates have been separated in 100 μl samples of human urine [119]. The laser was operated at 488 nm to better match the spectral response of the photomultiplier tube. The results of the analysis of human urine are shown in Fig. 5. Applications to the HPLC of untreated human urine are also presented [107].

Another important clinical application is the determination of free and esterified cholesterol in human serum using reversed-phase HPLC [120]. In Fig. 6, free cholesterol (peak A) is clearly separated from the esters (peak B–E).

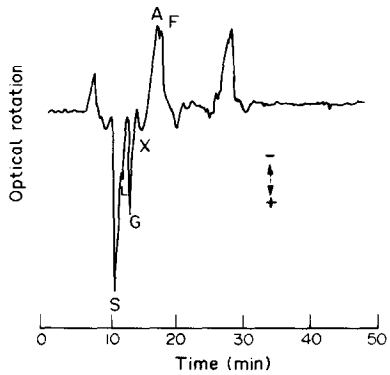


Figure 5
Chromatograms of components in human urine by optical rotation: S, sucrose; L, lactose; G, glucose; X, xylose; A, arabinose; F, fructose (from ref. 119).

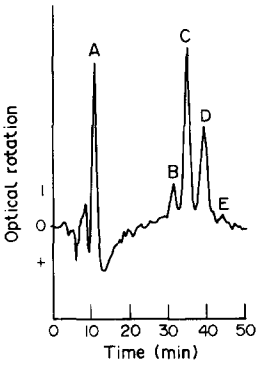


Figure 6
Chromatogram of components in human serum by optical rotation. A, cholesterol and cholestanol; B, cholesteryl linolenate and arachidonate; C, cholesteryl palmitoleate and linoleate; D, cholesteryl palmitate and oleate; E, cholesteryl stearate (from ref. 120).

A particularly interesting problem is the search for biological markers in fossil fuels. The fossil fuels originate from biological material, and it is known that some optically active compounds are retained within the matrix. Therefore, chromatograms obtained using chiroptical detection can serve as fingerprints of individual fossil fuel reserves, such as shale oils [121].

Recent advances in HPLC technology, using very efficient small diameter microbore and capillary columns, require development of low volume detectors. One of the promising detector concepts involves the application of a laser with an optical activity detector [122–124]. The microcell has a volume of 1 μl for a 1-cm optical path. The detectability of the system was 1.5×10^{-5}°, and a detection limit for fructose of 11 ng has been realized [122]. This can be compared with the conventional laser-based optical activity detection system which has a limit of detection for fructose of 100 ng [119].

The selective polarimetric detector is also capable of conversion to a universal LC detector as an alternative to refractive index detection.

Bobbitt and Yeung [122] developed a new method in which an optically active eluent was used with an optical activity detector to detect optically inactive components eluting from a chromatographic column. The signal due to the eluent can be nulled optically by rotating the analyser an amount equivalent to the rotation of the eluent. As an optically inactive compound elutes, it replaces an equal amount of the eluent in the detector cell so that a decrease in optical rotation is observed. In such a manner universal response is obtained as a result of indirect polarimetry [122–124].

The practical use of chiral solvent systems is realised in microbore LC, with eluent additives such as, (−)-2-methyl-1-butanol, (*R*)-(+)-limonene and α-pinene. Optically inactive analytes such as dibutyl phthalate and hydrocarbons could be detected at the 12-ng level. Figure 7 shows the detection of a phthalate mixture using an eluent composed of limonene/isooctane, 95:5.

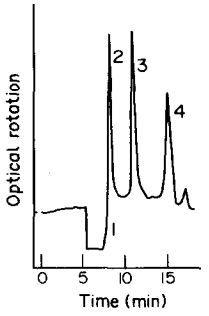


Figure 7
Detection by indirect polarimetry: eluent, (*R*)-(+)-limonene/isooctane, 95:5. 1, Injection peak; 2, dioctyl phthalate; 3, dibutyl phthalate; 4, diethyl phthalate (from ref. 123).

Using indirect polarimetry, by proper choice of two eluents with differing optical activity and the application of a simple equation, quantitative information can be obtained without the need for analyte identification [122].

When an absorbing analyte elutes, the heat generated in the detector causes a reduction of the density of the eluent present, so that a decrease of the optical rotation is observed. This is the principle underlying absorption detection via indirect polarimetry. The method is very sensitive and an absorbing analyte such as *N*-methyl-2-nitroaniline has been detected at the 36-pg level [123].

In some cases, CD spectrophotometers have been coupled to a high-performance liquid chromatograph. An advantage of using a circular dichrograph is that it enables selective detection of optically active compounds with suitable chromophores [126, 127].

The CD active compounds can be detected in a complex matrix of other compounds such as is encountered in natural-product extracts, or biological fluids. Natural pyrethrins, tryptophan, rotenoids and amaryllidacae alkaloids have been used to evaluate the system [126]. Monitoring at selected wavelengths has enabled detection levels in the low microgram range to be realized. By using stopped-flow techniques, full CD spectra can be obtained.

Drake *et al.* [127] developed a detection system for the simultaneous monitoring of the light-absorption and CD of the eluent from an LC column. It has been shown to be capable of recording simultaneously the concentration, as the absorbance and the optical activity, on the basis of difference absorbance for left- and right-circularly polarized light of a given fraction with a single detection system, and also, of measuring the enantiomeric purity of that solute in the fraction.

The optical resolution of pavine (obtained by synthesis from papaverine) on a triacetyl cellulose column has been reported [127].

Miscellaneous methods

Interesting and specific spectropolarimetric and CD assays have been suggested for the determination of the activity of penicillins [128]. The methods are based on optical rotatory or ellipticity changes resulting from the addition of penicillinase enzyme, which causes hydrolysis of the β-lactam ring. Examinations were carried out at about 250 nm, where the change of optical rotation is maximal and where the error arising from the mutarotation of the final product is of less significance than in the visible region. The method is sufficiently sensitive to detect losses in drug activity occurring in solution at room temperature in periods <1 h.

Simultaneous quantitative determination of the four optical isomers of lisergic acid has been described [129]. The method involves a combination of quantitative thin-layer chromatography for the separation of non-enantiomorphous forms and of polarimetric measurements. A computer-aided procedure is used to evaluate the experimental results.

The stability and degradation of levamisole has been studied by means of a column chromatographic, polarimetric method [130].

The concentrations of pilocarpine and isopilocarpine have been determined by a combination of UV spectrophotometric and polarimetric measurements [131].

The kinetics of the hydrolysis of pilocarpine in aqueous solution were investigated utilizing polarimetry [132].

The effects of antioxidants in ophthalmic solutions containing adrenaline [133] and physostigmine [134] have been studied by means of an ORD method.

Conclusions

Optical activity is a particularly interesting molecular property because of its relation to biological activity.

In this review an account has been presented of the analytical applicability of the more important methods, based on the measurement of optical activity, namely, ORD and CD. Such methods are known by the generic term chiroptical. These methods are an essential part of the analyst's armoury, and often provide the simplest solution to a given analytical problem. The greatest common advantage of ORD and CD methods is that optically inactive substances do not interfere with the determinations, so that the chiral compounds often can be measured directly.

The ORD method frequently can be applied to the determination of molecules possessing no chromophores for which spectrophotometric measurements are inappropriate. A further advantage of ORD and CD is that in many cases, for instance optically active saturated and unsaturated ketones, sensitive measurements can be at higher wavelengths.

Chiroptical methods are equally well applied to the determination of optically inactive substances that induce optical activity via chemical or physical interactions.

Important steps have recently been taken in the analytical applications of chiroptical spectroscopy, primarily by the teams of Purdie and Yeung. The sensitivity of the apparatus for CD and ORD determinations has been improved and the measurable wavelength range has been extended in both the UV and the IR directions.

In the future the apparatus will be fitted with new facilities (e.g. difference CD or ORD, and derivative spectrum accessories) facilitating in the analysis of multicomponent chiral systems. The selective chiroptical detectors based on laser radiation and the diode-array detection systems, alone or in series with other detectors, can be applied to HPLC so leading to widespread use of chiroptical methods.

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