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Circular Dichroism: An Alternative Method for Drug Analysis

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ABSTRACT: A case is made for the acceptance of circular dichroism spectropolarimetry as a method for the direct and quantitative analysis of controlled substances in solid dosage forms of drugs. The method is critically described and results are included for the analysis of codeine, heroin, L-cocaine, and D-lysergic acid diethylamide.

KEYWORDS: toxicology, chemical analysis, dichroism

The methods most commonly used in the analysis of drugs include gas chromatography [*1-3*], liquid chromatography [*4,5*], mass spectrometry [*6-8*], infrared and ultraviolet spectrophotometry [*9*], and variations such as fluorescence and radioimmunoassay [*10*]. Simultaneously, alternate methods [*11-13*] and variations of standard procedures are being sought constantly. In almost every instance, recognition of the illicit substance requires its prior separation from either a contrived mixture or a biological fluid. It is unlikely that any one method will be found where separation is totally unnecessary in every case. Reducing the number of instances where separation is necessary is a high-priority item in the development of alternate methods for drug analysis. It should not be accomplished, however, at the expense of speed and simplicity.

A very common property of almost every controlled substance has been virtually ignored in the search for other methods. That property is optical activity. Optical rotations measured at the sodium D line have long been used by criminalists, but the obvious limitation exists that the simple rotation of plane-polarized light to the right or left is not a specific-enough test to identify an anonymous drug. It is, however, sufficient evidence to confirm the presence of a particular isomer, which is important in cases where only one isomer is physiologically active, for example, L-cocaine. Greater discriminatory powers are available from optical rotatory dispersion wherein the optical rotation of plane-polarized light is measured as a function of wavelength [*14*]. Unfortunately, many of the adulterants and diluents added in the trafficking of illicit compounds are themselves optically active and their presence confuses the interpretation of the optical rotatory dispersion spectrum to the point that separation is again necessary for identification.

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Circular Dichroism and Absorption

If both the rotation and optical absorption of an optically active molecule is recorded, then an even greater potential for recognizing a compound becomes available. Where the incident light is circularly polarized, the absorption-rotation phenomenon is referred to as circular dichroism (CD) [*14*]. What is actually measured experimentally is the difference in absorption between the left- and right-rotating circularly polarized components derived instrumentally from an incident source of plane-polarized light according to equation [*1*]:

$$\Delta\epsilon = \epsilon_L - \epsilon_R = 0.3032 \times 10^{-3}[\Theta] \quad (1)$$

where ϵ is the molar absorption coefficient, subscripts L and R refer to left and right components, and $[\Theta]$ is the molar ellipticity coefficient, which is the actual quantity (deg · cm^2/mol) measured instrumentally. The ellipticity describes the polarization of the transmitted beam, which is no longer either plane-polarized or circularly polarized but is the intermediate figure of an ellipse. It is important to emphasize the fact that the molecules which exhibit CD must be chiral and be capable of absorbing electromagnetic radiation. For most drugs the principal chromophore is the aromatic nucleus. Unlike optical rotatory dispersion, CD spectra are obtained only over the wavelength ranges where absorptions occur, which for the aromatic ring is in the ultraviolet range at wavelengths less than 300 nm. It is not a requirement of the method that the chiral center be a part of the chromophore, but it should not be too remote from the chromophore.

A CD spectrum is in itself, therefore, a modification of an absorption spectrum. All of the other standard procedures used to modify an ultraviolet absorption spectrum are equally applicable in modifying a CD spectrum. Consequently, changing solvents, changing pH, and taking first and second derivative spectra [*14–16*] are ways to modify the CD spectrum as an aid to recognizing compounds. New parameters that appear in CD are positive and negative maxima dependent on the relative magnitudes of ϵ_L and ϵ_R. These CD maxima occur at wavelengths close to the absorption maxima. Also observed are wavelengths of zero ellipticity $\lambda°$, that is, where $\epsilon_L = \epsilon_R$. Broad absorption bands are frequently separated in the CD spectra into components of opposite sign differentiated from each other by a unique $\lambda°$. This is indeed common for the benzene chromophore. The $\lambda°$ values are independent of the concentration of the solution [*13*]. Since the only experimental modification is to measure the difference between two *absorbances*, the credibility granted by investigators to the definitive powers of ultraviolet absorption in drug identification should be equally allowed to CD spectropolarimetry.

Drug Analysis

Unless both prerequisites are met, that is, chirality and light absorption, no CD spectrum is obtained. In this regard, diluents such as monosaccharides and disaccharides are chiral, but do not absorb. Commonly encountered dyestuffs are strongly absorbing molecules but are not chiral, since much of their absorption strength is related to extensive conjugation and therefore to planarity in the molecular structure. Neither, if present in a mixture with the suspected compound, should contribute to the overall CD spectrum.

The potential exists therefore for CD to become a very definitive and yet simple alternative method for drug analysis. Separation may not be a prerequisite to identification. Even if this observation is not totally true, at least the number of cases where separation is required will be much reduced. Since absorption is measured, the potential also exists that the method will be quantitative in accordance with the Bouguer-Lambert-Beer law, and we have shown this to be the case.

Difficulties are to be anticipated in three instances: (a) when the drug is not chiral, (b) when two or more compounds in a mixture satisfy both prerequisites for CD, and (c) when the other ingredients are strong ultraviolet absorbers but are not chiral.

Drugs That Are Not Chiral

Three drugs that are not chiral are mescaline and phencyclidine (PCP), which are optically inactive, and the racemic mixture DL-methadone. If CD is to be used to identify these compounds, the only option available is to *induce* chirality. This can be done by using either an anisotropic solvent, such as a cholesteric liquid crystal system [*17*], or an anisotropic solution, where a chiral solute is dissolved in an isotropic solvent [*18*]. Both methods rely on a strong and very specific interaction between the chiral center and the solute. The former option is experimentally cumbersome and unlikely to be adopted for general use. Good candidates for the second option are the crown polyethers and the cycloamylose sugars. Fortunately, molecules that are not chiral are in a small minority among controlled drugs.

Two or More CD-Active Compounds

Compounds that are chiral and that absorb will have theoretically interfering CD spectra when mixed together. Like absorptivities, ellipticities are additive, but due attention must be given to their respective signs. Because the addition is algebraic, the interference is not always as serious as it is for ultraviolet absorption spectra. With CD, a common result is to find that a broad ultraviolet absorption band has been divided into two or more bands of opposite signs, a property of the spectra often used for conformational analysis and in the assignment of electronic transitions [*14*].

Strong Absorbers of Ultraviolet Light

The only solution to the problem of other ingredients that are not chiral but are strong ultraviolet absorbers may be to separate the mixture. The problem is instrumental in origin. If the strong absorber is in large excess, a normal circumstance, then insufficient light is transmitted through the sample cell and there occurs a serious loss in the signal to noise ratio in the recorded spectrum.

Separation

Even when instances are encountered where separation would seem to be necessary, and the choice of CD over more established methods is debatable, one real advantage to CD remains. Once the molar ellipticity coefficient has been determined for a compound in a prescribed solvent, that value does *not* depend on instrument parameters. Therefore it is not essential to run a standard each and every time an unknown is analyzed as is frequently the case for chromatography and mass spectrometry.

Applications to Drug Analysis

Data have been collected for several drug standards and pure compounds and for a variety of mixtures. The pure compounds have been placed in KBr pellets [*12*], in a liquid crystal mixture of cholesteryl chloride and nonanoate [*17*], in ethanol, and in aqueous media [*13*]. The potential of CD to distinguish among controlled substances from different generic classifications is shown in Fig. 1. The solvent is water; the compounds were added as salts and concentrations are on the order of 10^{-4} *M*. Ultraviolet absorption can make the same distinction among families of drugs. However, because of the negative/positive aspects of the CD spectra, distinctions are more obvious. Absorption spectra do fail to differentiate among members of the same family.

The predictions in the early part of this paper are gradually being fulfilled. In KBr, liquid crystal, and aqueous media it is possible to differentiate among eight to ten opiates including morphine, codeine, nalorphine, 3-monoacetylmorphine, 6-monoacetylmorphine, heroin,

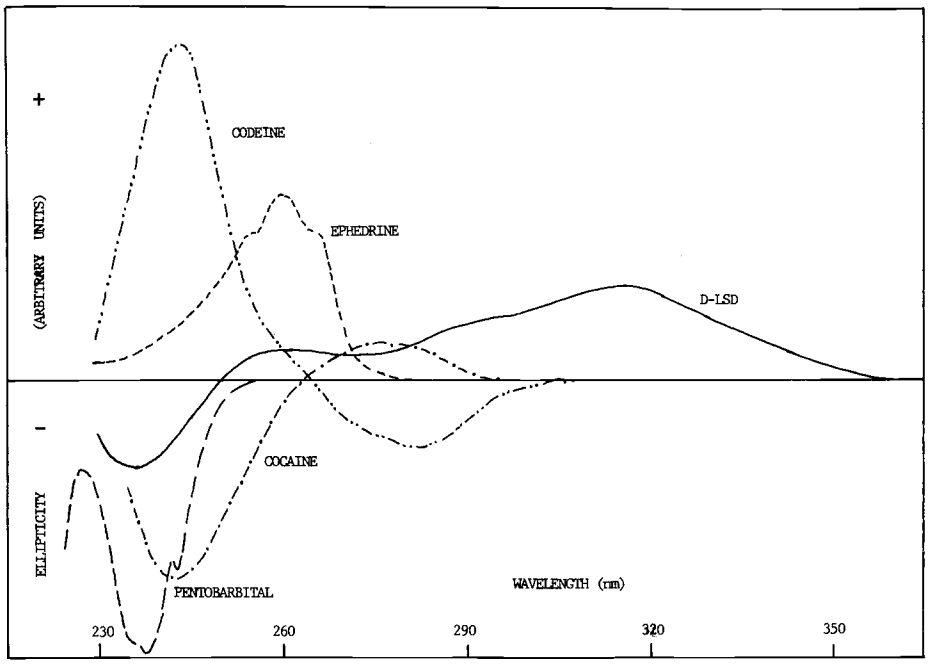

FIG. 1—*CD spectra of representative derivatives of a number of drug families.*

dihydrocodeine, thebaine, hydrocodone, and naloxone [*12,13,17*]. Aqueous media are most amenable to quantitation and molar ellipticities have been obtained [*17*] for these compounds in 0.1*M* hydrochloric acid, in pH 8.6 buffer, and in 0.1*M* sodium hydroxide (except where base hydrolysis has occurred). Those molecules with a 3-OH substituent show a substantial spectral change in aqueous media when the pH of the solution is raised, whereas those with an alkyl ether in Position 3 do not. It is therefore conceivable that a mixture of morphine and codeine can be quantitated by simply changing the pH of the solution and measuring the ellipticity at a wavelength of 300 nm, where codeine shows no signal in 0.1*M* sodium hydroxide (Fig. 2). Opium samples could therefore be quantitated in a single experiment.

The real and indisputable advantage that CD has over ultraviolet spectrophotometry is in the analysis of mixtures. Absorptions are additive and strong absorbers can mask the presence of an illicit substance in a mixture. If, however, the strong absorber is not optically active then it does not contribute to the CD spectrum. In our studies, the investigated mixtures were either prepared in-house or provided to us by the Oklahoma State Bureau of Investigation. Most attention has been given this far to quantifying mixtures that contain codeine, heroin, L-cocaine, and D-lysergic acid diethylamide (LSD). The structural formulas for these compounds are shown in Fig. 3, and the asymmetric centers are identified by an asterisk.

The quantitation of codeine in in-house codeine-lactose mixtures is easily done to better than 0.5% without separation. Analysis of Tylenol® 3 and 4 for codeine is complicated because of the excessive absorption of acetaminophen (which is not chiral) ($\epsilon = 13\,000$) and related compounds, but it is made quantitative after separation with diethyl ether. Before separation, results differ from the prescribed formula by $\pm 2\%$ and after separation the agreement is better than $\pm 0.5\%$. Similar results are found for the analyses of cough syrups Phenaphen®, Fiorinal® #3, and Phenergan VC. An in-house mixture of codeine and quinine

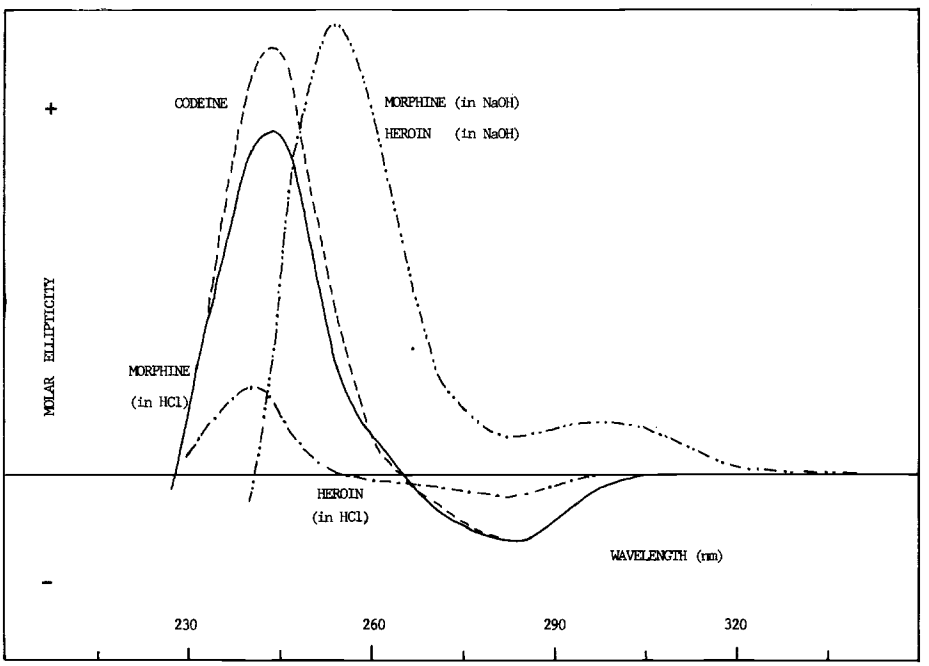

FIG. 2—*The effect of pH on the CD spectra of codeine, morphine, and heroin.*

can be quantitated without separation because the CD bands are quite distinct from each other.

Heroin is best quantitated either in an aqueous hydrochloric acid solution or as morphine after rapid hydrolysis in solutions of 0.1*M* sodium hydroxide. The latter is preferred because the molar ellipticity value is greater for morphine and the hydrolysis is quantitative. Case work samples from the Oklahoma State Bureau of Investigation were readily quantitated without prior separation. Composition of heroin in these samples varied from 0.5 to 76% by weight. The CD results were in excellent agreement with independent analyses and with our own in-house gas chromatographic analyses. The additives did not interfere in our determinations and were not specifically identified. With gas chromatography we found evidence for the presence of 6-monoacetylmorphine in two samples, probably a remnant from the synthesis. In 0.1*M* sodium hydroxide, this too is hydrolyzed to morphine and does not complicate the CD analysis.

L-Cocaine is readily recognizable by its CD spectrum (Fig. 1). In methanol the 278-nm band is split into two positive maxima (Fig. 4a). The racemic mixture DL-cocaine would have no CD spectrum when dissolved in an isotropic solvent because the signals from the D- and L-isomers are equal and opposite to each other and cancel in the same way that a DL-racemate shows no preferential rotation at the sodium D line. The evidence should be defensible in establishing the presence of the illicit L-isomer and help bring to an end the "cocaine controversy." Evidence from this laboratory was presented as a confirmatory test for the possession of L-cocaine in a drug-related manslaughter case tried in Oklahoma County Court. A number of case work samples have also been quantitated. Solutions are prepared by simply adding 2 to 4 mg to a 25-mL aqueous aliquot. Insoluble materials are discarded after centrifugation. Again, additives offered no interference and were not identified. It is significant to note that the additives might have included the usual adulterants such as procaine, lidocaine, and benzocaine, all of which absorb but none of which are optically active.

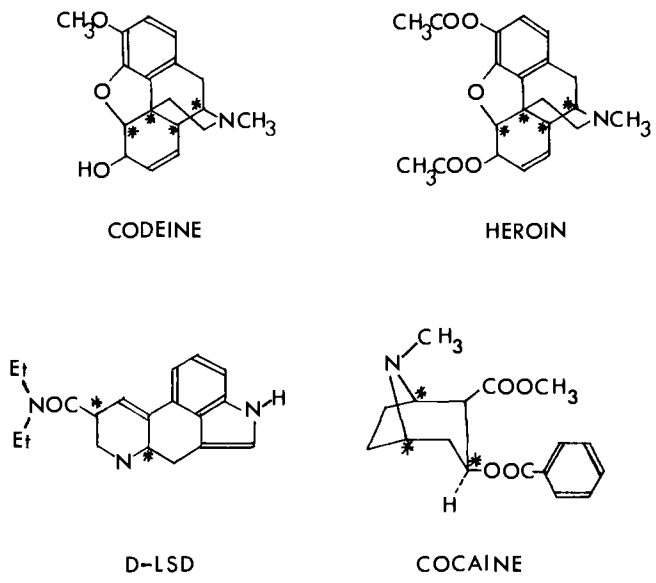

FIG. 3—*Molecular structures of codeine, heroin,* D-*LSD, and cocaine. Asterisks identify the asymmetric carbon atoms.*

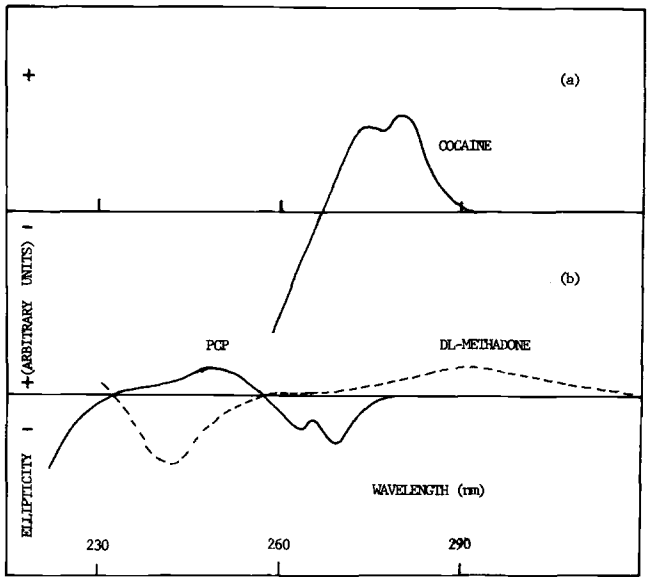

FIG. 4—(a) *CD spectrum of cocaine in methanol;* (b) *induced CD spectra of PCP and* DL-*methadone in aqueous solution with cycloheptaamylose.*

D-LSD in aqueous solution has a large molar ellipticity coefficient $[\Theta]_{310}$, which lowers the level of detection. Quantitation of samples from case work is in progress. The samples contain a variety of dyestuffs in what are frequently referred to as microdots. The insoluble material is presumably starch. Other samples are blotter papers impregnated with the com-

pound. Preliminary analyses of the soluble components show the presence of 5 to 34 μg D-LSD in a total sample size of about 10 mg. No separation steps were taken in the sample preparation. The analysis time was on the order of 15 min.

Also shown in Fig. 4 are the CD spectra for the optically inactive compounds PCP and DL-methadone to demonstrate that chirality can be induced. The chiral induction agent is cycloheptaamylose in water. The sugar is in excess of the drug by 5:1 in molar concentration. Quantitation experiments have not yet been begun.

In closing we would emphasize one thing. The CD technique is as simple to learn as is ultraviolet absorption spectrophotometry. Instruments are commercially available from JASCO Inc., Easton, Md. The work on heroin and on LSD was done by undergraduate student assistants (R. K. K. and H. A. M.). Details of the experimental results and procedural details will be made available in later publications as each project is completed, but suffice it to say, at this time, that the usual experimental methods used in ultraviolet absorption are applicable [*13*].

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