Chiral compound analyses and Faraday polarimetry

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Molecules that make up living things tend to be chiral: they have the property of "handedness" and a preference for one kind of mirror-image isomer, or enantiomer. Molecules that are metabolized by living things are often chiral as well, with specific chiral forms preferred by organisms. In medicine, for example, certain drugs are more effective in a preferred enantiomeric form and may produce fewer side effects. In the food industry, one form of a food supplement may be perceived as sweeter or less bitter, or may be otherwise more nutritious. In agriculture, the preferred chiral form of an insecticide may be more effective at lower application rates or be more specific toward a targeted pest. As the issue of chirality becomes more appreciated in bioscience research, tools to measure it increase in importance in analytical laboratories.

Chiral compounds, also known as optical isomers, are a specific case of stereoisomers. They are distinguished by having enantiomeric counterparts with identical chemical properties in nonchiral environments but differing in the way they rotate a plane of polarized light.[a] As might be expected, polarimetry, the measurement of optical isomers by means of their specific rotations, can be a powerful analytical tool for analyses of chiral compounds. Furthermore, information gained by the use of polarimeters and polarimetric (LC) detectors is key to the full characterization of these compounds.

The property of optical rotation is specific to a compound, and it is related to its concentration in solution.[b] Through the use of polarimetry, one can determine a concentration through a compound's known specific rotation, or vice versa. Using a polarimetric detector in HPLC, one can differentiate between enantiomers, or one may simply find it more appropriate for the detection of certain samples found in nature.

Before discussing how polarimetry can be used in practical applications, it might be well to refresh our knowledge of polarimeters and polarimetric detectors.

When plane polarized light is passed through a solution containing an optically active compound, it will be rotated in a clockwise or counterclockwise direction. In effect, two pieces of information are obtained: a direction (which way it rotates) and a magnitude (how many degrees). This information is unique and important, and translates into practical applications that will be discussed later.

In a simple polarimeter, a solution containing a chiral compound is placed into a measuring cell of a given length, typically, 10 cm. A light beam of the proper wavelength is polarized (via a polarizing lens), passed through the solution cell to a second polarizing lens (typically called an analyzer), and then to an eyepiece. In a standard procedure, the cell is first filled with a blank, i.e., containing everything but the compound to be measured, and the analyzer lens is rotated to obtain a maximum brightness, establishing a zero point or base measuring condition. The cell is then filled with the optically active solution, the analzyer rotated to obtain an equivalent light intensity, and the degrees from the base condition read. In a case in which the solution concentration is known and the specific rotation is not, two measurements of solutions having differing concentrations are necessary to establish the fact of rotation clockwise or counterclockwise.[c]

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In next-generation automated polarimeters, a photocell or photomultiplier replaces the eyepiece and a feedback circuit controls a motor that rotates the analyzer lens. This arrangement is sufficient for a polarimeter, but not for a polarimetric (HPLC) detector, which must respond much more quickly than that permitted by a mechanical system. For this, a Faraday electronics scheme is necessary, which provides a nonmechanical way to turn the analyzer, or, more accurately, to compensate for the rotation from the sample and relate the compensation to a measurement of degree and direction.

Michael Faraday determined that plane polarized light could also be rotated by an electric field as it passed through a transparent medium; further, the strength of the field is (reasonably) proportional to the rotation.[d] A nonmechanical polarimetric detector (or polarimeter) could then be built in which light, rotated by a chiral compound in a cell, could be corrected to a base condition by allowing it to continue through some transparent medium (e.g., air or quartz) around which is a coil. Light then reaching a photocell is transformed to an electrical current. Using a feedback circuit, this current is applied to the coil so that there is a correction, or compensation, to the base condition for a rotation from a sample in the measuring cell. The positive or negative voltage required to effect this compensation is then related to a direction of rotation and a magnitude, in degrees, which is typically displayed on the face of the instrument and/or output to data acquisition elsewhere.

Simplified schematics of a polarimeter and a Faraday polarimetric detector are shown in *Figure 1*. In commercial polarimeters, a wavelength filter is placed in the path of a white light source to yield monochromatic light at the required wavelength; polarimetric detectors use coherent light sources or may simply use white light of a standard spectral pattern.

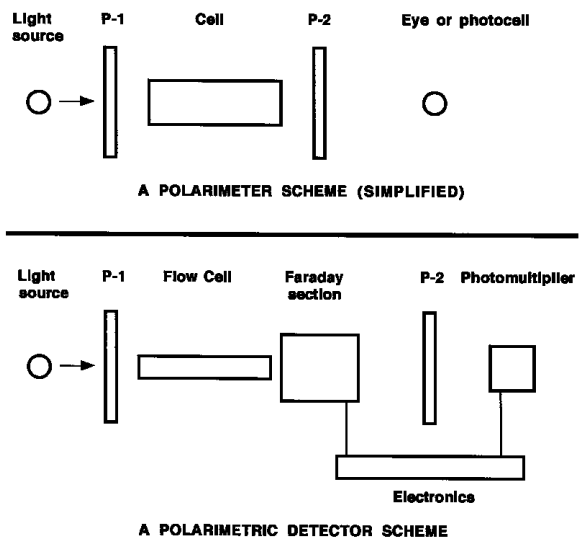


Figure 1 *Early polarimeter and polarimetric detector (simplified diagrams). Plane polarized light is rotated as it passes through a chiral solution. In the simple polarimeter, a second polarizing filter (F-2) is realigned to the first so that the degree of rotation can be determined. Plane polarized light is also rotated as it passes through a transparent medium within an electromagnetic field—the basis for polarimetric detectors and some advanced polarimeters. A photomultiplier provides feedback to the Faraday section, providing a compensating current that is proportional to the required angular correction.*

Figure 2 *CHIRALYSER™ polarimetric detector made by* ***IBZ MESSTECHNIK GMBH*** *(Hannover, Germany); sold in North America by* ***JM Science, Inc.*** *(Grand Island, NY). The detector uses the Faraday modulation/compensation electronic scheme.*

Within the Faraday electronics schemes, there are two variations: one using light intensity as a base condition and the other using an applied electrical phase as a base condition. In the first, in a process known as Faraday compensation, a voltage is applied to the coil that is just sufficient to restore the light to the original (i.e., base) condition of intensity. In the second, in a process known as Faraday modulation/compensation, an alternating current is first applied to the coil to establish a base condition of standard electrical frequency. Rotated light will be manifest as a second electrical phase; further application of a direct voltage to the coil is used to eliminate the phase anomaly, and this voltage is then related to the rotation.[e]

In either case of instruments using Faraday compensation electronics, there are no moving mechanical parts; machines will not wear out or lose performance over time. In the case of the modulation/compensation scheme, measurements are achieved that are independent of light intensity, enabling more field-friendly (nonlaser) light sources, an improved consistency of measurement, and, in the case of polarimetric detectors, fewer complications from coeluting, achiral peaks.

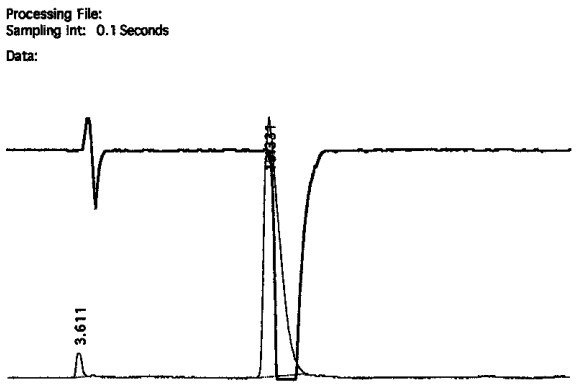
[a] Chiral: containing at least one molecular species that has one or more centers of symmetry, sometimes referenced as stereogenic centers or simply chiral centers. A molecule can generally be said to have a center of symmetry, or be chiral, if a component atom is singly bound only to different and distinct functional groups. Chirality is usually associated with carbon atoms, but other atoms (most notably nitrogen, phosphorus, and sulfur) can also give rise to it. Additionally, chirality can arise through overall molecular structure, as is sometimes the case with multiple fused-ring molecules (which may "pucker" in more than one way).

[b] This relationship is via Biot's formula, $[\alpha]D = \alpha/(l \times C)$, where $[\alpha]D$ = specific rotation of the compound, α is the observed rotation in degrees, l is the light pathlength in dm, and C is the compound's concentration in g/mL, under conditions of designated wavelength and temperature.

[c] For example, a rotation of –270° could otherwise be interpreted as +90°; the analyzer would be turned to the same apparent position. A second concentration, say, 50% of the first one, would indicate which of the two cases is accurate. In the case in which a specific rotation is known and the solution concentration is not, two measurements will also be necessary for the same reason.

[d] This relationship can be represented by the formula, $a \sim d \times V \times H$, where a is the observed rotation, d is the light pathlength, V is the Verdet constant (a physical property of a specific transparent material), and H is the intensity of a magnetic field. The field is generated by a coil, which is defined by its number of windings and the current passing through them. In any particular instrument, the pathlength, Verdet constant, and coil windings are fixed; the observed rotation is therefore proportional to the applied electric current.

[e] A rotation of the light produces a second, overlaid electrical phase having a frequency of twice that of the induced phase. This phase is generated as the ac current is modulated by the rotation.



Analysis: Channel A

Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
1	3.611	N	18267	285617	2.767
2	13.331	N	202027	10029794	97.168
	26.966	N1	431	1138	0.011
	27.146	N2	340	1680	0.016
	27.233	N3	260	557	0.005
	27.525	N4	272	1361	0.013
	27.811	N5	396	1614	0.015
	27.933	N6	342	331	0.003
Total Area				10322092	99.998

Figure 3 *Analysis of a proprietary, pure enantiomer sample showing comparison of a UV and optical rotation chromatographic trace. The optical rotation detector (ORD) is placed downstream from the UV detector. Both outputs are superimposed on this two-channel display, with the signal from the ORD (top signal) offset so that it may display negative peaks. In this run, the combination of the high concentration of sample and its (likely) strong rotation has caused an overrange of the ORD peak—not an unusual occurrence in this preparative HPLC laboratory. Since the purpose of the run was to merely determine the sign of rotation of the sample, the overrange signal was not a problem. The negative ORD peak indicates a negative-rotating enantiomer. In this particular case, the ORD could be used to obtain quantitative information. However, in some cases, the combination of rotation strength and flow cell amount will be inadequate for quantitative analyses compared to the higher signal sensitivities available from UV detectors (assuming samples with chromophores). In such cases, the ORD is used to obtain additional, qualitative information and is considered to be a secondary detector, with the UV detector considered the primary detector. Qualitative information from the ORD could be the indication of the presence of an optical sample (in a "dirty" process sample, for example) and in determining the sign of rotation. Especially if two-channel data acquisition is available, enabling a superimposable display, the ORD trace will point to the peaks of quantitative interest on the UV trace. (Graphic complements of Schering-Plough Research Institute, Kenilworth, NJ.)*

Applications

Applications for polarimeters are well known, with machines in common use in quality control laboratories and process areas as well as the research laboratory. Of significance may be that Faraday-type polarimeters are now commercially available that are equal to the best of the mechanical types in accuracy and precision, but that have no moving parts.

Less well known may be applications for polarimetric detectors; unlike Europe and Japan, these instruments are not yet in wide use in the U.S. Before discussing applications for these detectors, however, we should first understand their limitations. Biot's formula suggests a flow cell with the longest possible light pathlength; however, good chromatography demands the smallest possible cell volume. Compromise cells therefore yield chromatography with relatively wide bandspread.

Perhaps the greatest limiting factor in Faraday compensation systems is the earth's magnetic field, which, at the highest sensitivities of these instruments, interferes with the generated fields of the instruments and influences their output signals. Field instruments, i.e., those used in the typical chemistry laboratory, must be detuned somewhat to prevent them from acting like compasses.[f] As a practical matter, although these detectors may be tuned to levels yielding rotations of 10^{-6}°, 2×10^{-5}° is a more realistic expectation.[g]

A degree of rotation is reached through a combination of the compound's specific rotation and its solution concentration. As a rule of thumb, the following applies to the better of today's machines:

If:

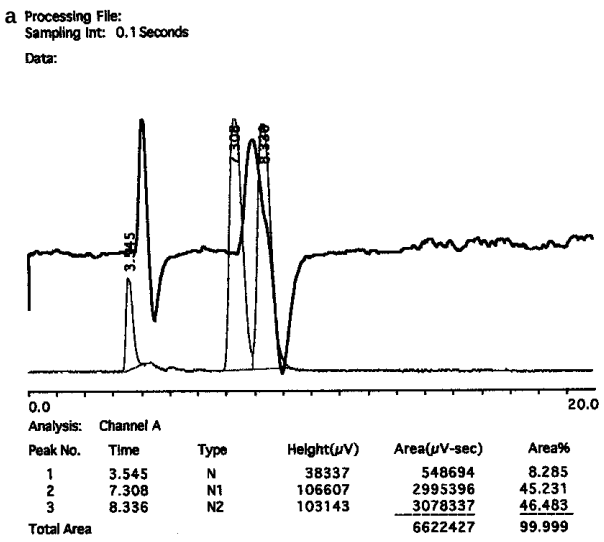
Specific rotation (degrees) × sample concentration (%) × injection volume (μL) ≥ 100,

then:

One will see one's sample above baseline noise.

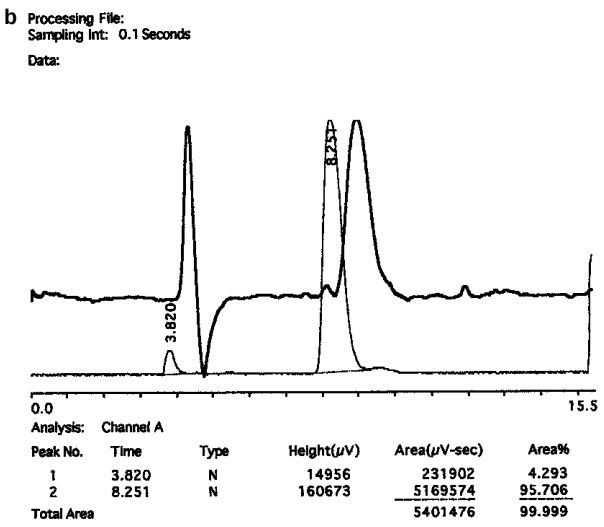
Some chiral compounds may have very small specific rotations and will therefore be below detectable limits. Highly concentrated samples can sometimes produce refractive index (RI) effects and distorted signals; column capacities may limit injection volumes. The presence of microbubbles in the flow cell can also yield RI effects, and degassing, a worthwhile procedure in any HPLC work, is even more important when using polarimetric detection. In the final analysis, even the best of polarimetric detectors will have limits for practical applications.

Despite these limitations, polarimetric detectors are the only instruments that do what they do, and they are destined to come into greater use as their applications become better understood and as general interest in chirality grows.



Analysis: Channel A

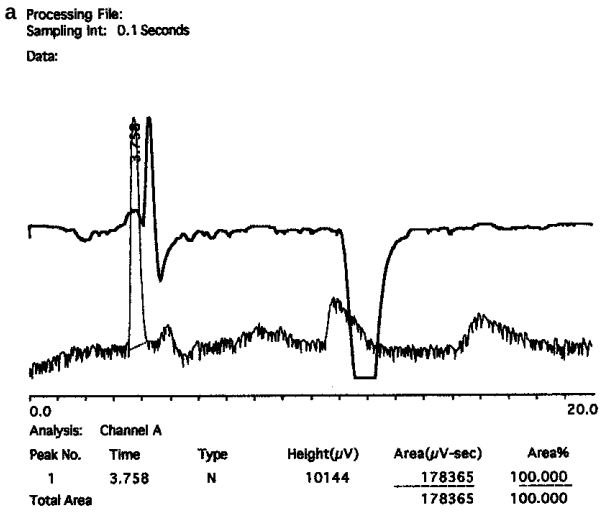
Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
1	3.545	N	38337	548694	8.285
2	7.308	N1	106607	2995396	45.231
3	8.336	N2	103143	3078337	46.483
Total Area				6622427	99.999



Analysis: Channel A

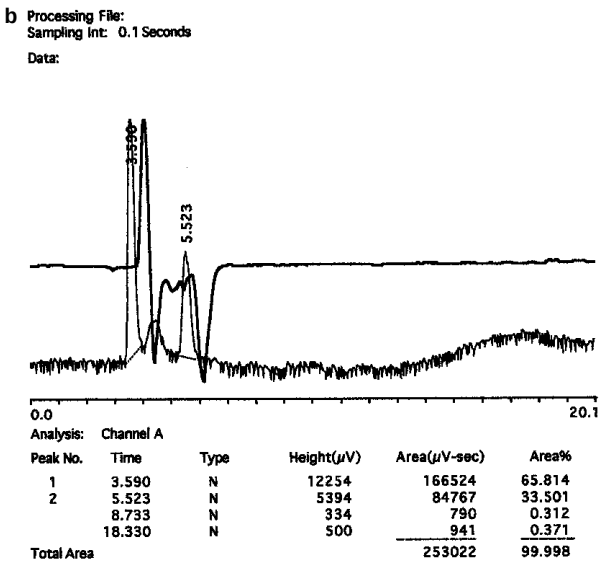
Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
1	3.820	N	14956	231902	4.293
2	8.251	N	160673	5169574	95.706
Total Area				5401476	99.999

Figure 4 *A run to determine elution order of enantiomers in a separation of a racemate (50/50 mixture of two enantiomers), in this case, of chlorpheniramine maleate salt. The ORD (yielding the top signal) is downstream from the UV detector; both outputs are superimposed onto the same display by using two of the available channels. As may be seen by the signal-to-noise ratios of the two, the UV trace will yield the best quantitative information; thus it is used for this purpose. However, the ORD has established that the positive rotator has eluted first by this method. As the method is refined to suit the purposes of the chromatographer, the ORD will be used to track the elution order. For standard HPLC preparation of a preferred enantiomer from a racemate, it is desirable to have the preferred enantiomer elute first; thus a method is developed to accomplish this. Once the preparative separation to the preferred enantiomer is accomplished, however, it is necessary to determine its chiral purity, i.e., the ratio of the preferred enantiomer to the other one. In this case, the opposite situation of elution order will be sought, with the lesser peak eluted first, so that it is not lost in the tail of the dominant peak. An ORD is used to obtain elution order in the development of both methods, i.e., the preparative method and the analytical method. a) The object of the analysis was only to confirm that the separated product was indeed the (+) enantiomer. (Graphics complements of Schering-Plough Research Institute.)*



Analysis: Channel A

Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
1	3.758	N	10144	178365	100.000
Total Area				178365	100.000



Analysis: Channel A

Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
1	3.590	N	12254	166524	65.814
2	5.523	N	5394	84767	33.501
	8.733	N	334	790	0.312
	18.330	N	500	941	0.371
Total Area				253022	99.998

Figure 5 *Quantitative analysis of a proprietary, pure enantiomer sample having no UV chromophore. The sample has a strong enough rotation to be detectable via the ORD; in this case, the amount of sample used was too much, since its signal (top trace) has overranged. If a chiral sample has too weak a chromophore for UV detection, it may be detected via ORD if its combination of rotation strength and amount in the flow cell is adequate. The ORD is in the role of primary detector. a) Related (proprietary) compound having a very weak chromophore (at ~5.5 min). The ORD trace might yield better quantitative information in this case also. (Graphics compliments of Schering-Plough Research Institute.)*

How can these instruments be used? Applications for polarimetric detectors arise as a result of the information they provide as discussed earlier: They tell the direction as well as the magnitude of rotation. Also, a built-in deficiency, viz., their nonresponse to nonoptical compounds, yields others.

Obviously, they are used to characterize optically active samples, but they are also used to differentiate optically active compounds from nonoptically active compounds in mixed samples. In a typical arrangement, the polarimetric detector is placed immediately downstream from a UV detector. Both traces are output simultaneously, and the peaks generated via the polarimetric detector point to the chiral compounds in the UV display.

Probably the biggest application area is the characterization of separated enantiomers, most useful in chiral HPLC methods development. A polarimetric detector will show elution order, i.e., which enantiomer elutes first,[h] which is of consid-

[f] An overly tuned polarimetric detector could be used for navigation. Such a detector may yield a different result when its orientation on the laboratory bench is changed; further, metal masses, such as belt buckles or cars passing on the roadway, could also affect measurements.

[g] 2×10^{-5}° corresponds to an arc of about 7 ft on a circle the size of the earth.

[h] Specifically, the identification of the levo (–) or the dextro (+) forms. Note that these are directional designations and have no relation to absolute conformation—designated by "R" and "S." The relation of absolute conformation to direction may be determined through the use of circular dichromism instruments or other standard instrumental means. In most cases, however, these relationships will be found in the literature.

erable importance to chiral HPLC. Consider methods development for a preparative separation. If the object of the separation is to prepare the maximum amount of a preferred enantiomer, it is important to discover a method in which the desired enantiomer elutes first, since efficiency can be several times greater. Once this enantiomer has been isolated, however, it is usually necessary to then determine its chiral purity,[i] i.e., that portion not contaminated by the undesired enantiomer. In this case, there will be a very large peak and a very small one, perhaps less than 1%. If the smaller peak elutes second, there is a likelihood that it could become lost in the tail of the dominant peak. For this reason, a separation method resulting in the elution of the smaller peak first is desirable. Identification of the enantiomers through the use of the polarimetric detector can therefore guide methods development.

Another feature of a polarimetric detector can be exploited. If there is an enantiomeric mixture in its flow cell, the detector will show a net signal that is proportional to the component enantiomers of the mixture. For example, a mixture of 25% of one enantiomer and 75% of its coenantiomer will result in a net signal of 50% of the coenantiomer.[j] Therefore, against a reference, pure enantiomer standard, it is possible to obtain a rough approximation of chiral purity using nonchiral HPLC columns.

In most cases in which adequate separations of enantiomers are found for chiral columns, an absorbance detector will yield better quantitative measurements, simply because of its greater sensitivity. In some cases in which resolutions are poor, however, a polarimetric detector may yield more accurate data.[1]

Examples of real world chromatography using a chiral detector (*Figure 2*) placed just downstream from an absorbance detector are shown in *Figures 3*, *4*, and *5*. Chromatographic traces for both detectors are displayed simultaneously.

Perhaps the greatest limiting factor in Faraday compensation systems is the earth's magnetic field, which, at the highest sensitivities of these instruments, interferes with the generated fields of the instruments and influences their output signals.

In certain preparative or even process chiral chromatography situations, use of a polarimetric detector could enable a signal-based, rather than a time-based, automation scheme, with the possibility of long-term, unattended operation. The positive and negative voltage outputs from the detector (resulting from the detector's seeing positive and negative peaks) afford the opportunity for definitive switching. Provided a test run ensures that there are no artifacts from RI effects, the detector will ignore any nonchiral species that may be present. Simulated moving bed adsorption (SMB) preparative LC—used to make large-scale separations of two-component systems (such as the chiral separation of enantiomers)—could be especially suited to the definitive switching of valves.

In an application perhaps more theoretical than practical, a polarimetric detector could even serve as a kind of universal detector, detecting nonchiral as well as chiral compounds in a procedure sometimes called indirect polarimetry. This technique utilizes a chiral mobile phase, chosen not to interfere with the species being measured. In effect, an artificial baseline is established, either above or below the real baseline. A chiral species will show in its usual fashion relative to the new baseline; nonchiral species will be displayed as displacement peaks, as a depression of the signal from the chiral mobile phase.

Polarimetry is an obvious complement for work on chiral compounds. Instruments using Faraday electronics have made no-moving-part polarimeters and polarimetric LC detectors possible. New generation machines have increased the range of applications as well as the percentages of samples that can be seen. These instruments are beginning to claim their rightful place in North American laboratories.

[i] Determinations of this type are often designated by the desired enantiomer's enantiomeric excess, expressed as a percentage, a.k.a. percent e.e.

[j] A 50–50 enantiomeric mixture, i.e., a racemate, will have a net signal of zero—no deviation from the baseline.

Reference

1. Mannschrect A, Mintas M, Becher G, Stühler G. Liquid chromatography of enantiomers: determination of enantiomeric purity in spite of extensive peak overlap. Angewandte Chemie 1980; 19(6):459–70.

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