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Advanced Laser Polarimeter (ALP)

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1 Advanced Laser Polarimeter (ALP)

Why use Advanced Laser Polarimeters?

- Sensitive only to optical activity
- Not affected by absorbance
- Very large linear dynamic range (1 million)
- Long lifetime (10 year MTBF) – no lamp to replace
- Wide variety of HPLC and SFC flow cells
- Ideal for automated applications
  - no user adjustments
  - no stop flow scans
  - data is real time

ALP Applications:

- Confirm Enantiomeric Separation
- Identify the Elution Order of Enantiomers
- Measure "ee" and Specific Rotation
- Control Fraction Cuts in Purification
- Detect Enantiomers without Chromophores
- Monitor changes in Optical Activity during Chemical Reactions
- Monitor Protein/Peptide Conformation

Advanced Laser Polarimeters (ALPs) measure the net optical activity of the contents of their flow cells in real time. The measurement is usually made dynamically as in a flowing HPLC or SFC system but can be made statically with sample stationary in a flow cell. ALPs measure optical activity directly and with proper design exhibit no interferences and no non-linearities across a large dynamic range (one million). These selective and robust characteristics allow exactly the same optical bench technology to be applied from analytical to prep and production level applications with excellent correlation between units operating at drastically different analyte scales from micrograms to tons.

Measured optical activity for a particular analyte depends on a variety of parameters including the interrogation wavelength. Between 200 and 400 nanometers (nm) variations due to wavelength are large and zero crossings (sign inversions) are typical. In most cases variations and zero crossings due to wavelength do not occur much beyond 500 nm. Traditional polarimeters used sodium D line emission at 590 nm and modern laser polarimeters use laser diode emitters at 670 nm (now 635 nm). Measurements at these wavelengths (590, 670, and now 635 nm) have proven to be extremely stable and reproducible with essentially no absorbance-related interferences while shorter wavelengths prove problematic in some cases. Measured optical activity is also affected by solvents and to a lesser extent by temperature.



Figure 1 – Advanced Laser Polarimeter

The large linear dynamic range of ALPs and their ease of operation make them ideal detectors for automated chiral method development and prep purification systems. In method development an ALP will confirm enantiomeric separation by showing the characteristic positive and negative enantiomeric peaks. UV detectors are usually more sensitive than ALPs but UV detectors cannot uniquely identify enantiomeric peaks as compared to peaks from unseparated racemate and achiral compounds.

In prep purification applications the tendency of UV detectors to overload makes ALPs the preferred technology. Overall chiral prep performance is almost always best when an ALP is used to detect and collect enantiomeric peaks and a UV detector is used to track impurities that are to be avoided in collected fractions.

1.1 Theory of Operation

Since high school physics we have thought of light as having properties similar to that of a wave (figure 2A). The wave motion results from vibrations producing crests and troughs. The distance between successive crests or troughs, called the wavelength, corresponds to the light's color. The amplitude of the vibration (figure 2B) corresponds to its intensity. Most light energy that is commonly encountered consists of many waves of light with each wave vibrating in a different plane. "Normal" light vibrates in a number of planes (figure 2C).

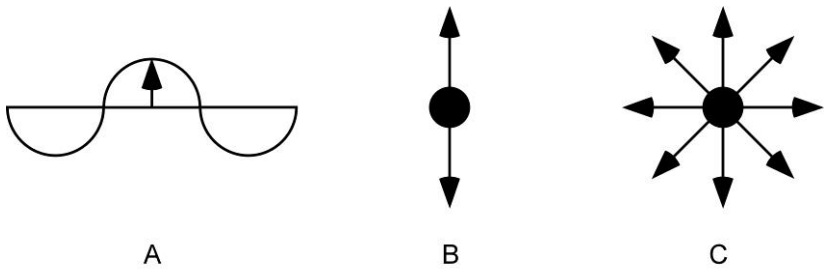


Figure 2 – Wave Nature of Light

If passed through an optical polarizing filter the emerging light will be limited to only the rays vibrating in a single plane (figure 3). The resulting energy is called plane-polarized light because the light is polarized in a single optical plane.

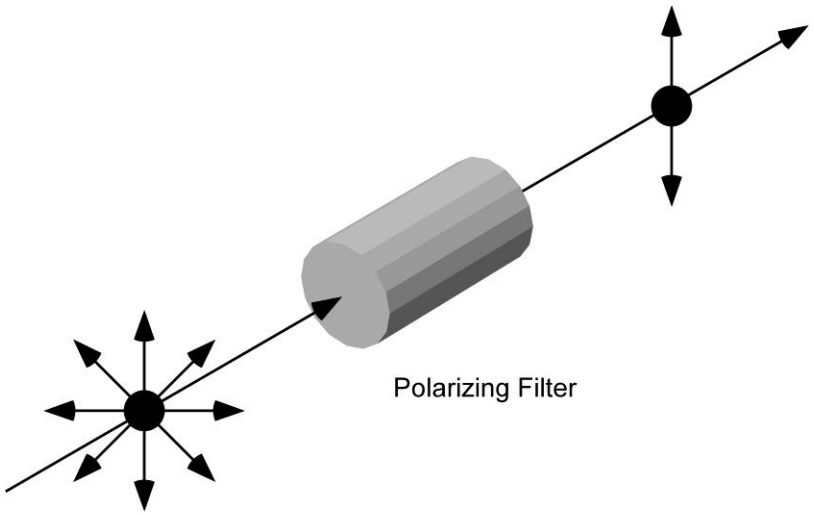


Figure 3 – Plane Polarized Light

So what is optical activity? To answer that question let's shift gears from physics to chemistry.

Certain organic compounds have a special molecular geometry that affects their transmission of plane polarized light. These compounds contain a carbon atom to which is attached four different functional groups. Because a singly bonded carbon atom has a tetrahedral geometry the functional groups can be attached in either of two configurations with one molecule being a

mirror image of the other (similar to your right and left hands). Otherwise the molecules are chemically identical. This special geometry affects the transmission of plane polarized light giving it the property of optical activity and making it unique.

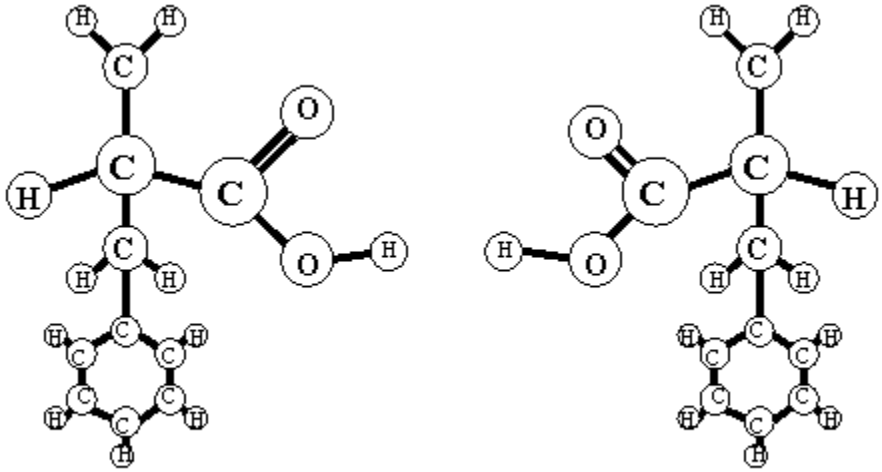


Figure 4 – Phenylalanine Chiral Geometry

Optical activity is defined as the ability of a compound to rotate plane polarized light in one direction or another (positive versus negative rotation). Mirror image, non super imposable isomers are called enantiomers. Enantiomers are chemically identical but contain a difference in 3D molecular geometry that produces an equal but opposite rotation of plane polarized light. Using an ALP we can measure the optical rotation (α) produced by a liquid or gas phase sample.

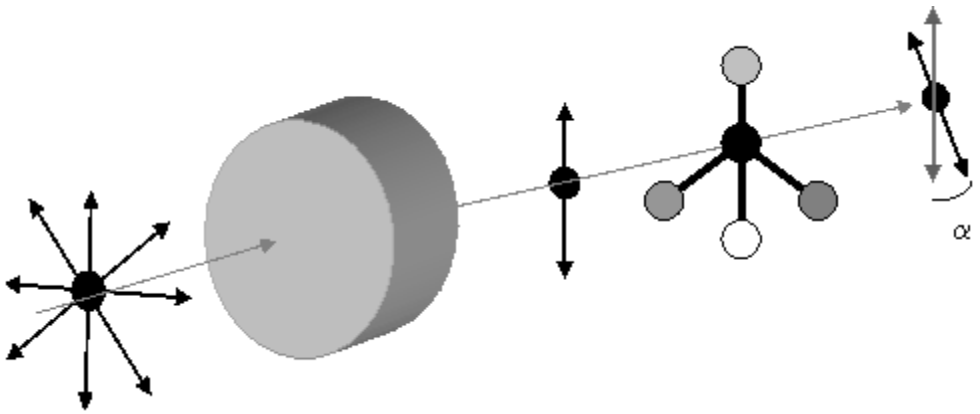


Figure 5 – Rotation of Plane-Polarized Light

The optical system of an ALP is illustrated in figure 6 below and consists of a laser diode, polarizing prism, Faraday rotator, flow cell, analyzing prism, and photodiode.

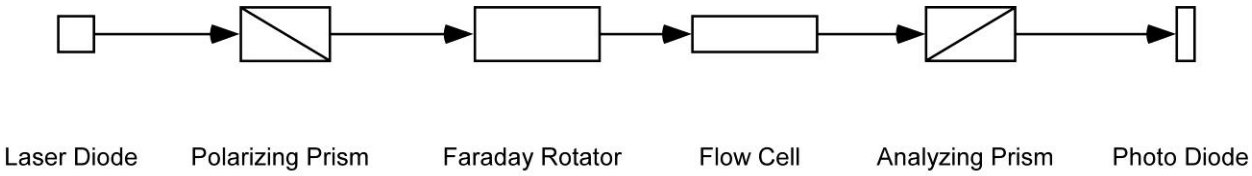


Figure 6 – ALP Block Diagram

Polarized light from a laser diode is filtered with a polarizing prism and this highly polarized light passes through a Faraday rotator and then a flow cell. The Faraday rotator imparts a sinusoidal rotation to the plane of polarization. A sample exhibiting net optical activity flowing through the flow cell will offset the sinusoidal rotation relative to zero rotation. Proper alignment of the analyzing prism and special electronic circuits allow the magnitude and sign of the net optical activity to be extracted from the photodiode signal. Since Faraday oscillation is typically above 500 Hz integration of results is not required and real time values are available continuously. Faraday oscillation is used to reject signal variations (noises) that are not synchronous with the oscillation.

1.2 Comparison to UV and CD Detection

UV (ultra violet absorption) and CD (circular dichroism) are both absorbance based detection schemes and share some characteristics. They require a chromophore in the analyte and excitation with a wavelength in the chromophore's absorbance band. Their linear dynamic range is naturally limited (more so for CD) because as absorbance increases the photon flux at the photodetector is approaching zero and noises increase. In addition to a chromophore, CD also requires a chiral center coupled to that chromophore for detection of optical activity. Both UV and CD are often affected by solvent gradients and CD suffers from other absorbance related interferences.

ALPs do not require chromophores or wavelength tuning to match an absorbance band because they are not absorbance based. ALPs detect optical activity by measuring the angle of rotation of the plane of polarization which is a phase measurement not an amplitude measurement. Large angular rotation only changes the phase angle and does not reduce photon flux at the photodetector.

To compare sensitivity is complicated because of variations in analyte characteristics and chromatographic conditions as well as variations in instruments. For most pharmaceutical compounds, UV is more sensitive than CD or ALP. Exceptions include compounds with no chromophore (sugars, antibiotics, etc.) where only ALPs are sensitive. If CD is first used with an appropriate analyte captive in the flow cell, a stopped flow wavelength scan is performed, and the wavelength of maximum signal is selected then subsequent analysis of that particular analyte by CD will be competitive with ALPs. In automated analytical screening and prep purification applications ALPs almost always perform better because no adjustments or stop flow scans are required and the linear dynamic range is much larger.

1.3 Useful Formulas

Chemical Purity (cp)

Chemical purity (cp) is the relative amount of a compound present in a mixture as a percentage of the total. [cp = CP/MT x 100] where CP is the compound of interest and MT is the mixture total.

Enantiomeric Purity (ep)

Enantiomeric Purity (ep) is the relative amount of enantiomer present in a mixture relative to the total of both enantiomers. [ep = EA/(EA+EB) x 100] where EA is the enantiomer of interest and EB is the other enantiomer.

Enantiomeric Excess (ee)

Enantiomeric Excess (ee) is the amount of excess or unpaired enantiomer present in a mixture. [ee = (EA-EB)/(EA+EB) x 100] where EA and EB are the enantiomers. Enantiomer Excess is the most popular parameter used in describing chiral mixtures.

Specific Rotation (sr)

Specific Rotation (sr) is a normalized value that is useful in patent applications and in comparing optical activity between different compounds. To be complete sr should specify the solvent, wavelength, and temperature of measurement.

$$[\alpha] = \frac{\alpha}{l \times c}$$

$[\alpha]$ = specific rotation (degrees)
α = measured rotation (degrees)
l = interaction length (decimeters)
c = concentration (grams/milliliter)

1.4 Analytes

This section will briefly discuss analyte classes that are successfully being measured by ALPs.

Small Molecule Pharmaceutical Candidates

The majority of small molecule pharmaceutical candidates are chiral compounds containing one or more chiral centers. ALPs are very useful in many applications during development and production of these compounds. They can be used to properly identify chiral peaks among a number of UV or MS (mass spectrograph) peaks, quantitate enantiomeric separation and purity, and robustly direct prep fraction collectors in enantiomeric purification. ALPs are usually unaffected by gradient elutions and operate reliably for many years without human attention because laser diode emitters have lifetimes of 10+ years.

There are currently a large number of drugs on the market that contain a single chiral center. Many of these compounds are marketed as the pure enantiomers. Over the past decade the ability to separate enantiomer into pure isomers by chiral chromatography has become commonplace. However, as the number of chiral centers in a molecule increase, the isomeric and chromatographic complexity increases rapidly. When a molecule contains two asymmetric centers it may have four isomer; 2 diastereomers, each with 2 enantiomers. (Note: There are 2^n possible isomers where n equals the number of stereogenic centers.) Diastereomers are basically stereoisomers with two or more centers of asymmetry that are not enantiomers of each other. While enantiomers have the same chemical and physical properties, the diastereomers usually do not have the same chemical and physical properties. Thus, in most cases, diastereoisomers can be separated by standard reversed-phase chromatography. However, the pair of enantiomers in each disatereomeric pair cannot be separated in this manner and require chiral chromatographic techniques. For a compound containing two stereogenic centers (2^n =4 possible isomers), it can be difficult to properly identify and pair enantiomers by chiral chromatography with only UV detection, unless all four isomers are available as reference standards. A chiral separation coupled with both a UV detector and polarimetric detector can be very helpful, see chromatograms in Figure 7.

One example of this phenomenon is the antihypertensive labetalol. The chromatograms shown in Figure 7 were obtained on an ASTEC Chirobiotic V chiral column, 4.6 mm x 25 cm, with a mobile phase of MeOH/HOAc/TEA (100/0.15/0.05) at 1 ml/min. The blue chromatogram is a scan from a UV detector; the red chromatogram is the scan from an Advanced Laser Polarimeter (ALP). The two enantiomeric pairs are separated into 4 separate components under these conditions. With the UV scan alone, it is not possible to determine which peaks are from which pair of enantiomers. However, with the ALP in series, additional information is obtained demonstrating that the first and third peaks are from one enantiomeric pair, and the second and fourth peaks comprise the other pair. There is one positive and one negative peak pair with the same area and another positive and negative peak pair with a different area in the ALP plot. This is the result of different specific rotation values for each +/- pair and the same concentration for each +/- pair.

This conclusion is based upon the positive and negative peaks, indicative of the direction of the optical rotation under these conditions, as well as the area under the curve. (Note: The area under the curves for peaks 1 and 3 are smaller than the area under the curves for peaks 2 and 4).

Based on the above example, it is easy to extrapolate to the usefulness of the polarimetric detector for compounds that contain more than two stereogenic centers.

Structures for labetalol isomers are illustrated in Figure 8.

Most small molecule pharmaceutical candidates exhibit specific rotation values between 10 and 40 degrees.

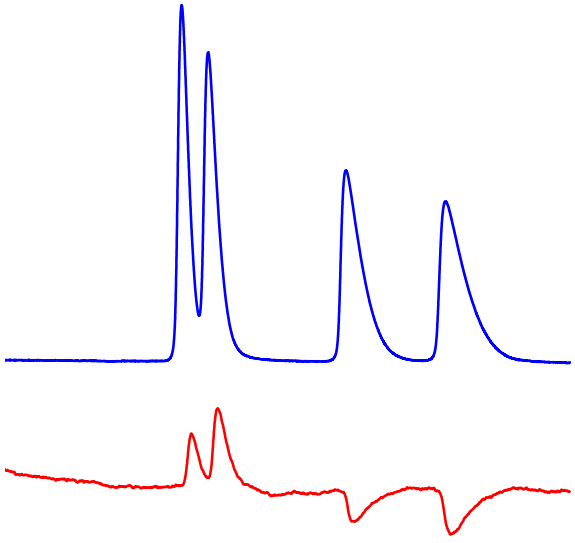


Figure 7 – Labetalol, a diastereomer

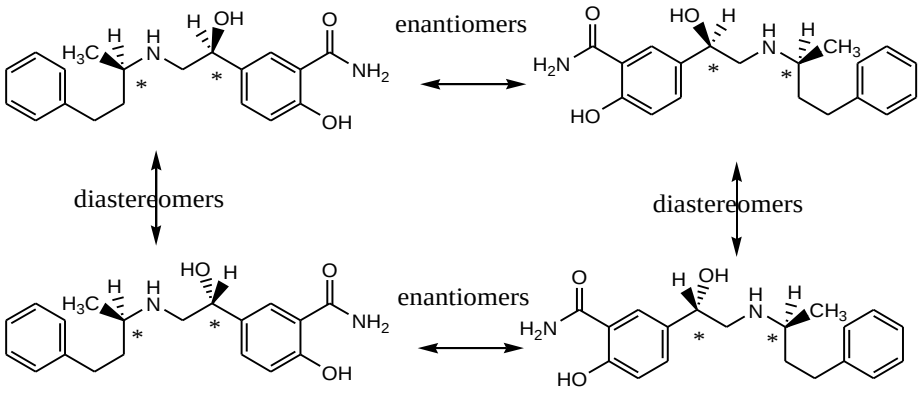
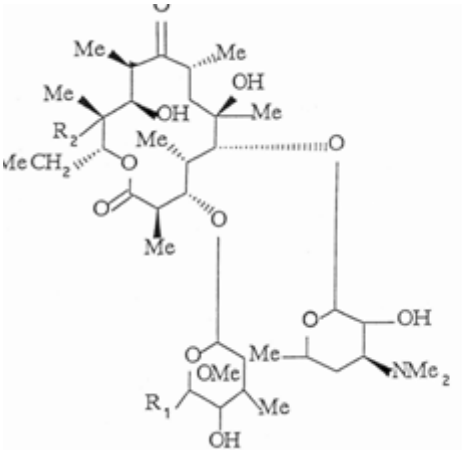


Figure 8 – Labetalol Structures

Antibiotics and Sugars: Compounds without Chromophores

Most antibiotics and sugars have no useful chromophore but exhibit specific rotation values above 100 degrees. This means that absorbance based detectors (e.g. UV and CD) are not useful and ALPs enjoy increased sensitivity as compared to small molecule pharmaceutical candidates.



Form	-R1	-R2
A	-OCH3	-OH
B	-OCH3	-H
C	-OH	-OH

Figure 9 – Structure of Erythromycins

Antibiotics like gentamicin and erythromycin occur in multiple structurally similar analogs. Mass differences between the analogs are usually less than 2% and UV detection is usually not possible above 220 nm. There are large differences in specific rotation for these analogs even though structural differences are small. Thus ALPs can not only easily detect but often fingerprint antibiotics because the ratio of analogs is usually process specific.

Gentamicin is an antibiotic administered to patients suffering from potentially life-threatening bacterial infections. It has a narrow therapeutic range and constant monitoring is necessary due to the fact that excess dosage can cause kidney and auditory nerve damage. Chemically, gentamicin is exceptionally stable, and is used extensively in animal husbandry. It can be stored at elevated temperatures for extended periods-of-time without loss of biological activity.

Amino Acids

ALPs give consistent measurements (sign and amplitude of rotation) from chiral materials and are very useful in differentiating and quantitating amino acids without the constant use of external standards and reliance on retention time to indicate sign.

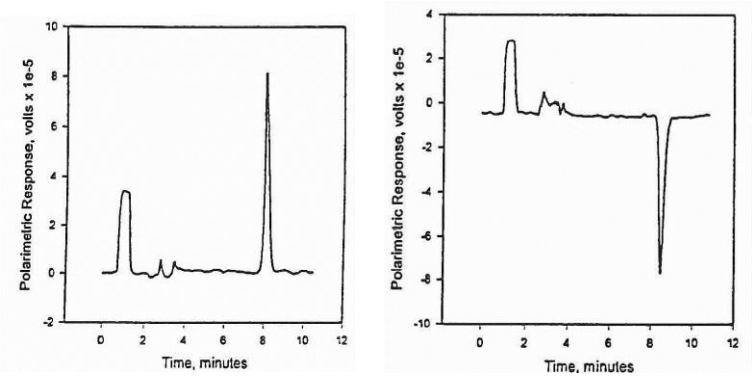


Figure 10 – DNS-Phenylalanine

Natural Products

Nature is chiral and so natural products exhibit optical activity. Many compounds only appear naturally in one enantiomeric form. Some compounds like limonene, an oily compound found in citrus fruit, caraway, dill and bergamot, occurs in both *d* and *l* forms. The specific rotation of limonene is approximately 101^{o}.

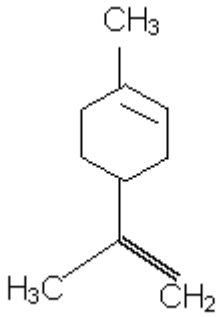


Figure 11 – Structure of Limonene

Foods, Flavors, and Fragrances

Because taste and smell are chiral chemical effects, foods, flavors, and fragrances can be successfully analyzed and processes controlled by ALPs.

Fertilizers and Pesticides

Many new and developing fertilizers and pesticides are chiral for the same reasons that most small molecule pharmaceuticals are chiral -- improved specificity and efficacy with reduced toxicity. ALPs are very useful in analyzing and controlling the processing of these compounds.

Two notorious pesticides, *p,p'*-DDT and *p,p'*-DDD (see figure 12) are of significant concern to environmental chemists due to the fact that they are biologically active and non-degradable. This biological activity goes beyond their toxicity as an insecticide and unfortunately includes

toxicological activity towards vertebrates. Additionally, these molecules are of a class of compounds that has been implicated as endocrine disrupters. Therefore, it is of considerable value to be able to monitor levels of DDT and DDD in soil, surface water and ground water.

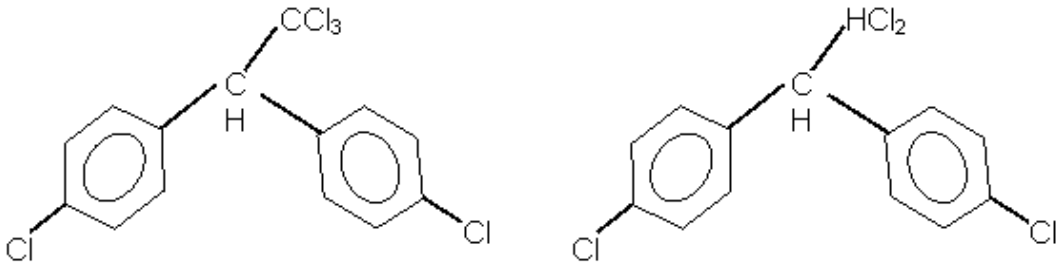


Figure 12 – Structure of p,p'-DDT and p,p'-DDD

Although both insecticides are achiral (i.e. exhibit no optical activity), commercial formulations typically contain *o,p'* isomeric analogs (~10 to 20%) which are optically active (see figure 13). By monitoring environmental samples for the presence of these analogs, the highly probable presence of the achiral, toxic components may be indicated.

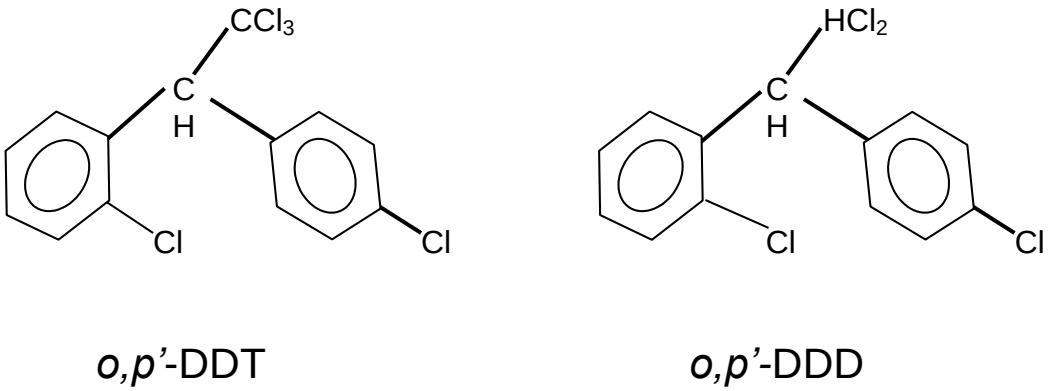


Figure 13 – Structure of o,p'-DDT and o,p'-DDD

1.5 Applications

Most ALPs are used in quantitation, method development, prep purification and process control as described below. In other applications the unique features of being sensitive only to optical activity and free of absorbance effects supports an elegant solution to an analytical or process problem. For example ALPs usually simplify the analysis and monitoring of chiral compounds occurring in complex organic mixtures by reducing or eliminating separation and standards requirements.

Analysis & QA/QC

ALPs measure both the magnitude and direction of the net optical activity (rotation) of the contents of their flow cells. When installed after a UV detector in an HPLC or SFC system an ALP indicates net optical activity (rotation) while the UV detector indicates absorbance which is related to sample mass. If the Relative Response Factor (RRF) between a UV and ALP is available, then it is possible to deconvolve overlapping enantiomeric peaks and display enantiomeric excess in real time. Calculation of specific rotation is even easier.

Example: QA/QC of Antibiotic Residues in Milk – Gentamicin[1]

Gentamicin is an antibiotic administered to patients suffering from potentially life-threatening bacterial infections. It has a narrow therapeutic range and constant monitoring is necessary due to the fact that excess dosage can cause kidney and auditory nerve damage. Chemically, gentamicin is exceptionally stable, and is used extensively in animal husbandry. It can be stored at elevated temperatures for extended periods-of-time without loss of biological activity. Gentamicin occurs in four optically active analogs as pictured in figure 14.

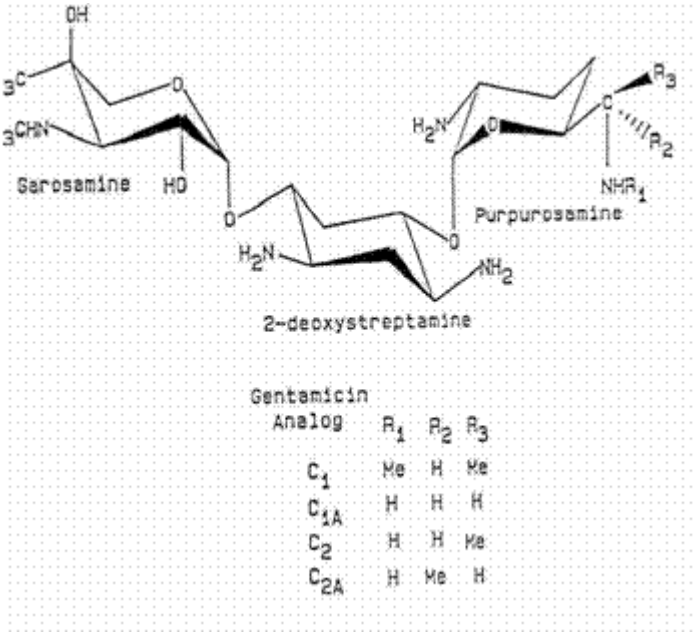


Figure 14 – Structure of Gentamicin Analogs

Because of its stability and antibiotic activity, it is used in dairy cattle, but treated animals should be held from production until the antibiotic clears their system (~ 2 weeks). Unfortunately, this has not always been the case and gentamicin residues have been detected in commercially produced-milk and milk products. It would obviously be useful to develop a sensitive method for detecting these materials, allowing subsequent identification of the source of the contamination in order to minimize public exposure.

The problem of detecting gentamicin is compounded by the fact that gentamicin does not absorb in the UV or visible region of the spectrum. Unfortunately, derivitization to enhance detection eliminates the subtle chemical differences between the four analogs making chromatographic separation impractical. However, gentamicin's optical activity not only offers an opportunity to differentiate between the four analogs but also overcomes the absence of a good chromophore.

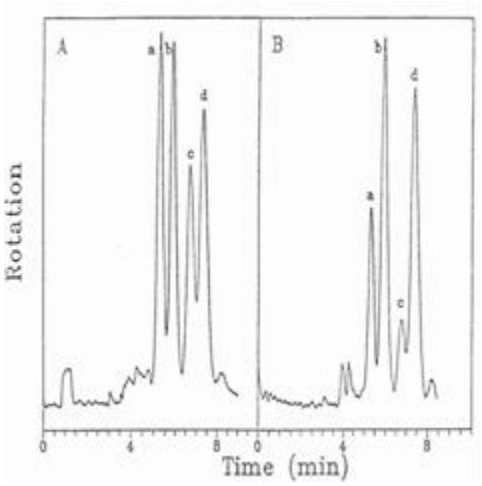


Figure 15 – Gentamicin from 2 different Suppliers

The above figure shows an analysis of the gentamicin analogs from two different commercial suppliers using ALP detection. The separation was by reverse phase, ion pair chromatography. The eluent consisted of methanol and 0.4 M TFA/H_2O (80:20, v/v) with a flow rate of 0.75 mL/min. The total injected amount was 10 μg.

This is the first demonstrated separation of the four-gentamicin analogs in a single separation system. The ALP can detect gentamicin below the 50-ng level without requiring derivitization. Previous work used UV/Vis detection with derivitization but the added moiety dominated the chromatographic properties of the derivatives making it impossible to separate all four analogs. Note that with all four analogs separated, differences are clearly observed in the distribution of the four components from samples obtained from the two commercial suppliers. Identification of the source of gentamicin involved in a contamination is now possible.

Previously, only the specific rotation for the enantiomeric mixture was reported due to the difficulty in detecting and separating all four analogs. With all four gentamicin analogs separated, the ALP can be used to determine the specific rotation for each of the four species. First, the relative masses for each enantiomer were apportioned in a separate experiment using a refractive index detector. Using the measured optical rotations and the relative abundance of

each of the four analogs, a calculated value for the mixture can be obtained. This calculated value agrees with the accepted value found in the literature. Structurally, the four are virtually identical. However, the specific rotations are clearly distinct, underscoring both the selectivity of the ALP detector and the sensitivity of specific rotation to the arrangement of atoms at or near the chiral center.

Determination of specific rotations for the four gentamicin analogs; C_1, C_{1a}, C_2 and C_{2a}.

Compound	Concentration (wt%)	$[\alpha]^a$
Mixture	100	116.8[b]
Gentamicin C_1	22.4	132.8 ± 0.9
Gentamicin C_{1A}	29.8	106.9 ± 1.8
Gentamicin C_2	16.1	143.5 ± 3.1
Gentamicin C_{2A}	31.7	98.4 ± 1.8

[a] Units $^{o}\,dm^{-1}\,(gm/mL)^{-1}$; 632.8 nm at 25° C. [b] Direct Measure

Table 1 – Specific Rotation of Gentamicin Analogs

In order to screen a large quantity of milk samples for possible contamination, a method must be both sensitive and rapid. The selectivity of the ALP is a distinct advantage in such applications. For this protocol, the milk sample was acidified with 0.1M trifluoracetic acid, centrifuged for 5 minutes, and then the supernatant was directly injected into the HPLC. The reverse phase, ion pair separation was identical to that used above. UV/Vis detection showed that a large number of compounds were present in the milk extract. However, most of these are not optically active and thus do not produce a response with the ALP, the exception being a distribution of material eluting just after 2 min. With the ALP detector (see figure 16), the gentamicin contamination was easily observed and the supplier of the gentamicin contaminated milk sample was easily identified. The entire analysis took less than 15 minutes, which is a significant improvement over the extraction/cleanup/derivitization protocol used previously with UV/Vis detection. The UV/Vis protocol typically requires several hours precluding the possibility of its use in the widespread screening of milk samples.

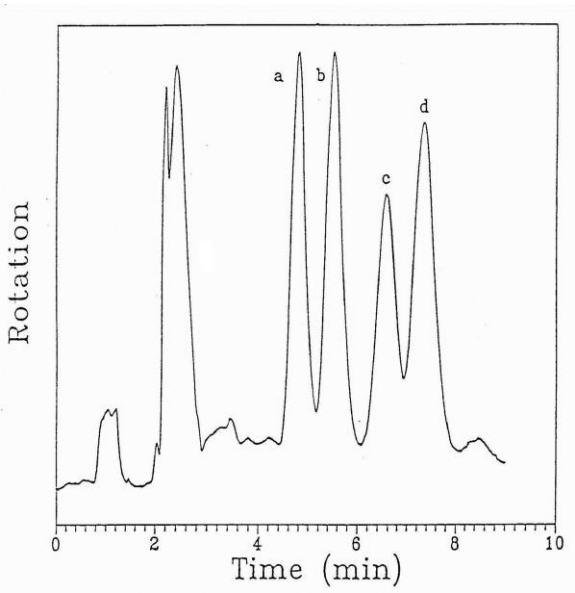


Figure 16 – Milk spiked with Gentamicin

HPLC/SFC Method Development

ALPs are used is chiral method development because they unmistakably identify enantiomers regardless of elution order and show enantiomeric separation even during overlap from non optical impurities. Compounds with multiple chiral centers exhibit noticeable differences in specific rotation between enantiomer pairs. This characteristic makes it easier to properly identify and quantitate peaks in diastereoisomer (and more complex) separations

HPLC/SFC Prep Purification - Fraction Collection

ALPs are the detector of choice in chiral prep applications. They exhibit consistent measurements of optical activity and are not affected by achiral compounds or solvents. Linear dynamic range in significantly better than any absorbance based detection technology. ALPs are robust with long lifetimes and they operate without adjustment or attention. Flow cell technology is available to accommodate almost any application. Most prep labs using ALPs rely almost entirely on the ALP for collection decisions while a UV tracks achiral impurities.

Process Monitoring[2]

The following section demonstrates the use of a ALP to monitor the conversion of a chiral reactant, of one specific rotation, into a chiral product with a different specific rotation. Figure 17 shows how the optical rotation changes during the glucose oxidase catalysis of the following reaction:

$$\beta\text{-D-glucose} + O_2 \rightarrow \text{D-gluconic acid} + H_2O_2$$

The specific rotation of the reactant, β-D-glucose, is 53 (deg. $(g/ml)^{-1}$ dm^{-1}) whereas the product, D-gluconic acid, has a specific rotation of 12.7 (deg. $(g/ml)^{-1}$ dm^{-1}). Thus, the reaction results in a net change in [α] by approximately 30, which is easily measured by ALP.

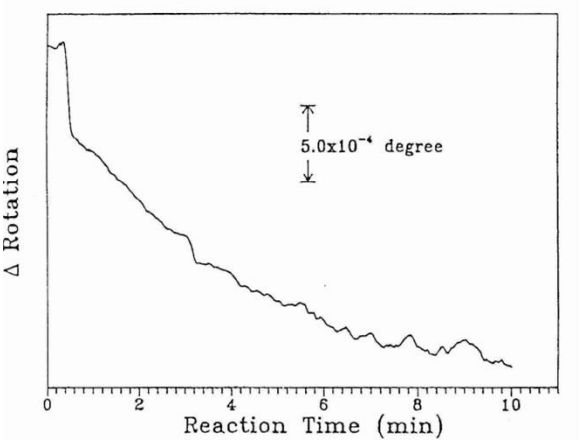


Figure 17 – Change in Rotation during Glucose Oxidase Catalyzed Conversion

In this experiment, the reaction was actually carried out in the polarimetric detector flow cell. Next, we will see how samples can be drawn from a reaction vessel at regular intervals and then analyzed by the ALP. Of course, another application would be to use the detector as an online monitor for real-time control of a continuously operating reaction system.

Figure 18 presents data that shows how ALPs can utilize a flow injection analysis (FIA) system for sample introduction. Duplicate injections of a mixture of glucose and gluconic acid, were made approximately 40 seconds apart to verify the elution order and detector response for the two optically active compounds.

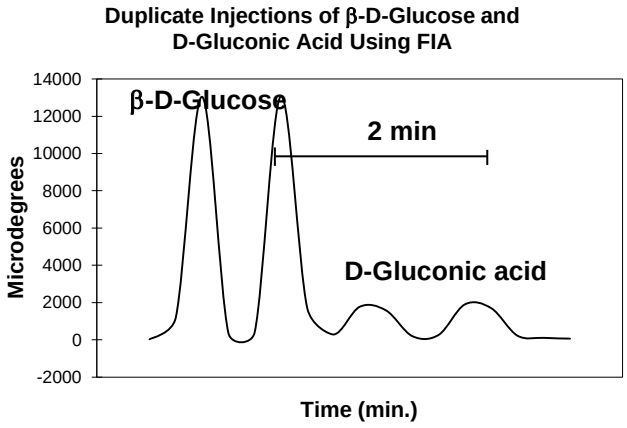


Figure 18 – FIA of Glucose and Gluconic Acid

The β-D-Glucose is the first to elute, showing a much greater response than the D-Gluconic acid which elutes about 2 minutes later. This is in agreement with the [α] values reported in the literature. This now gives us a valuable tool for fast, repetitive analysis of a reaction system.

In figure 19, we show the ALP's response as a function of the mole-fraction of β-D-glucose. The data presented here shows how a reaction could easily be followed using a polarimetric detector when a compound exhibiting one-chirality is converted into a compound with a different, or no, chirality.

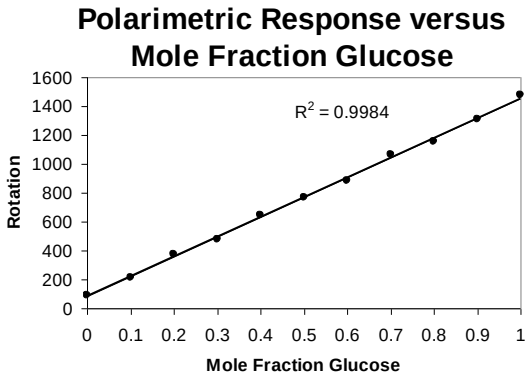


Figure 19 – Polarimetric response to Glucose concentration

A similar curve would be obtained when the mole-fraction of gluconic acid is plotted, only the slope would be less due to the smaller specific rotation of gluconic acid.

Figure 20 shows a plot of collected data illustrating the potential for using in-process analysis and control to optimize a reaction involving chiral compounds. The conversion of glucose to gluconic acid was followed via polarimetric detection. Samples from the reaction vessel were introduced into the ALP using FIA. In this experiment, the reactions were followed for 50 minutes, however, other studies extended out as much as 12 hours. The enzyme itself is optically active, resulting in the difference in starting rotation of the reaction broth.

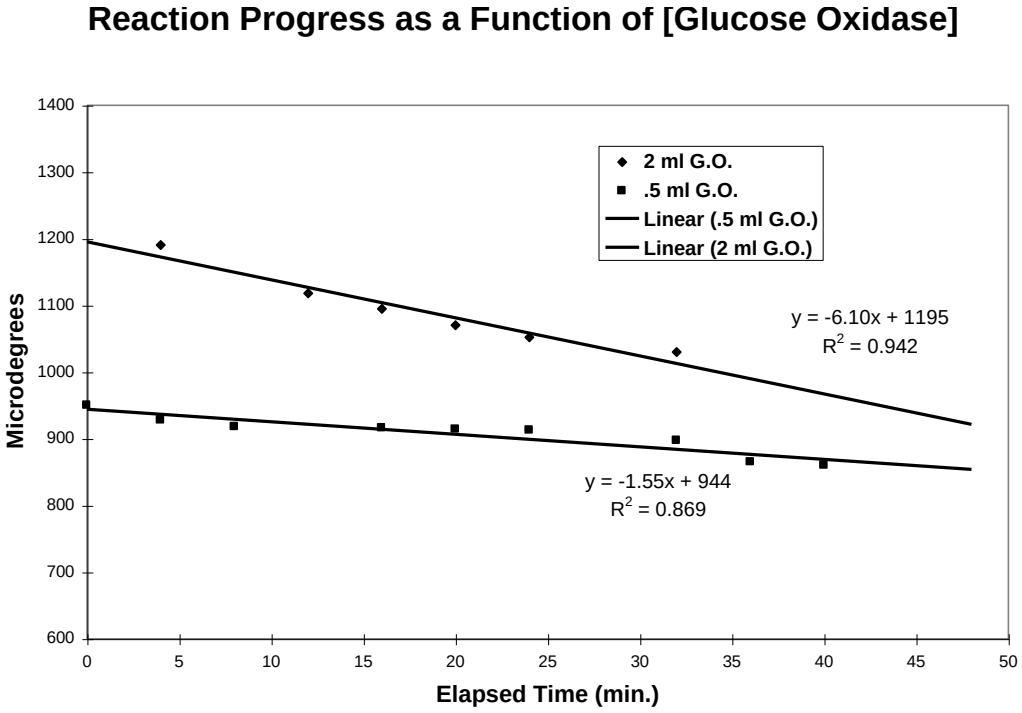


Figure 20 – Polarimetric response during Glucose Conversion

Clearly, we can see how increasing the amount of glucose oxidase catalyst (G.O.) significantly increases the reaction rate. It is also important to note that no separation was employed here. By using FIA, each sample took less than 3 minutes to analyze increasing the potential number of data points collected as compared with other techniques such as HPLC.

In table 2, on the following page, we have listed a number of medicinal and industrial enzyme systems that are suitable for monitoring with an ALP detector. Any of these systems would naturally benefit from process optimization using an ALP.

Enzyme	Substrate(s)	$[\alpha]D^{25}$, deg	Products(s)	$[\alpha]D^{25}$, deg
Serum glutamate pyruvate transmutase	L-aspartate α-ketogluterate	-25.5 0	L-glutamate oxaloacetate	+32.5 0
Serum glutamate pyruvate transmutate	L-alanine α-ketoglutarate	-13.6 0	L-glutamate pyruvate	+32.5 0
Lactate dehydrogenate	pyruvate	0	L-lactate	-2.3
α-Hydroxybutyrate dehydrogenase	α-ketobutyrate	0	α-Hydroxybutyrate	-24.5
Aspartase	fumerate	0	L-aspartase	-25.5
Fructose diphosphate alodase	D-fructose-1,6-diphosphate	+1.2	D-glyceraldehyde-3-phosphate Dihydroxyacetone phosphate	+14.5 0
Ornithine	carbamyl phosphate	0	citrullene	+3.7
Carbamyl transferase	ornithine	+11.5	phosphate ion	0
β-Fructofurano-sidase	β-sucrose	+66.4	D-fructose D-glucose	-13.3 +57.2
α-Glucosidase	α-maltose	+140.7	D-glucose	+57.2
β-Galactosidase	β-lactose	+34.2	D-glucose D-galactose	+57.2 +83.3

Table 2 – Medicinal and Industrial Enzyme Systems suitable to Polarimetric Detection

ALPs are used in large scale separation applications including liquid (LC) and super-critical fluid (SFC) chromatography as well as simulated moving bed (SMB) type systems.

ALPs can be used to monitor the real time changes in optical activity of the contents of a reactor or crystallizer, at any scale. The change in optical activity can be the result of an enantiomeric enrichment process or the conversion of a chiral reactant into a chiral product with a different specific rotation value. Usually these measurements can be made in real time and without separation by using a closed loop flow from the reactor. ALP measurements are not affected by absorbance changes and interferences from non optically active compounds in a measured stream.

1.6 Summary and Conclusion

ALPs have improved significantly over the last 10 years in both performance and acceptance. They now routinely support applications in chiral chromatography that are not possible without the use of modern laser ALPs and that result in significant improvements in throughput, robustness, and accuracy. With proper flow cell design ALPs are applicable to HPLC, SFC, SMB and Process applications covering sample sizes ranging from micrograms to tons. They are generally easy to automate and do not require periodic adjustment or attention.

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