COVER STORY
June 10, 2002
Volume 80, Number 23
CENEAR 80 23 pp. 51-57
ISSN 0009-2347

TAKING A MEASURE OF CHIRAL RICHES

Researchers respond to high demand for ways to measure enantioenrichment quickly

A. MAUREEN ROUHI, C&EN WASHINGTON

"The chiral technology revolution was founded on the ability to measure enantiomeric purity," says Christopher J. Welch, a process research fellow at Merck Research Laboratories, Rahway, N.J. Research in chiral technology exploded only after chiral columns for liquid chromatography became commercially available in 1981, he notes. The columns offered a way to measure enantiomeric excess (ee) that is much better than polarimetry. "Without the reliable measurement techniques, no one could have done very much," he notes.

TEAMWORK Merck's Mirlinda Biba (left), Welch, Mohamed Shaimi, and Jennifer Chilenski explore fast analysis and separation methods for chiral mixtures.
COURTESY OF MERCK RESEARCH LABS/VISCOM

Making chiral compounds through asymmetric catalytic reactions is a key chiral technology. The pace of catalyst discovery has been ratcheted up on the synthesis side by combinatorial and other techniques. Identifying which potential catalysts in a library work, however, requires measurement of ee. How to do this rapidly has become a nagging question for researchers in the field. "Everyone is trying to get

an answer in a matter of a few seconds," Welch says.

Usually, ee is determined by separating enantiomers by high-performance liquid chromatography (HPLC) or gas chromatography with chiral stationary phases and measuring how much of each enantiomer was originally in the mixture. "Chiral HPLC is still the most important analytical technique for stereochemical analysis in the pharmaceutical industry," Welch says.

With modifications, throughput higher than a few dozen measurements per day can be achieved with these workhorse methods. For example, high-throughput HPLC with chiral detection can cut analysis time to 20 seconds per sample with 10% accuracy, which, Welch says, "is good enough when we just want to categorize reactions into lousy, middle-of-the-road, and really, really good."

Using shorter columns and smaller solvent volumes could further increase HPLC throughput. The ability to scale down is limited by detector sensitivity at small volumes, however.

On this front, work by Darryl J. Bornhop, an associate professor of chemistry at Texas Tech University, Lubbock, may provide an answer. Bornhop has developed a micropolarimeter that measures optical rotation in nanoliter volumes. The invention achieves "a quantum leap in sensitivity," according to M. G. Finn, a chemistry professor at Scripps Research Institute, in a review that will appear in *Chirality* [**14,** 534 (2002)].

Bornhop's micropolarimeter is based on the interaction between a plane-polarized laser and a silica capillary. Backscattered interference fringes are produced when the laser illuminates the capillary. Depending on how the fringes are analyzed, different measurements can be made. For example, monitoring the relative changes in intensity of a pair of adjacent high-frequency fringes can sense microdegrees of rotation. And with minor changes in the optics and detector, the polarization state of the fringes can be analyzed, allowing ee measurements by calibrating the system against a standard.

BECAUSE THE micropolarimeter is compatible with flowing streams, it can be used as a detector for capillary-scale separations and high-throughput methods requiring real-time analysis of small volumes. For high-throughput applications, Bornhop is configuring the micropolarimeter in a capillary array format, as well as investigating the use of chip-scale embodiments.

Measurement of ee could be even faster if chiral separation is bypassed. But determining ee would then require use of chiral differentiators. Various groups are working on methods along these lines.

At Scripps, Finn's group has focused on mass spectrometry because it avoids the need to use a sophisticated tag--such as a chromophore or a radiolabel--to give a signal. It could be applicable to many situations.

In the Scripps method, the products of a catalytic asymmetric reaction are made to react with a chiral reagent consisting of two enantiomeric forms of different masses. For example, if the catalytic reaction forms alcohol products, mass-tagged chiral acids might be used as the chiral reagent. The masses of the two different esters produced from the alcohols reacting with two different mass-tagged acids give the ee.

Meanwhile, in Germany, Manfred T. Reetz, a chemistry professor at the Max Planck Institute for Coal Research, has developed a mass spectrometric method based on isotopically labeled substrates. Reetz discovers and optimizes enantioselective catalysts through directed evolution. In his method, one enantiomer of a racemic substrate is labeled with deuterium. After the substrate reacts, the products of that enantiomer are differentiated from those of the other by their masses. The ee can be calculated from the parent peaks.

The system is completely automated. In a recent review, Reetz says a second-generation system using an eight-channel multiplexed sprayer for ionization can do up to 10,000 determinations per day [*Angew. Chem. Int. Ed.*, **41,** 1335 (2002)]. "Several companies are in the process of licensing this technology from us," he says.

THE METHOD is ideal for kinetic resolution; that is, when the substrate is a racemate. "In the ideal case, the asymmetric catalyst will pick out only one enantiomer and transform it to product, which can be easily separated from the unreacted enantiomer," Reetz explains. It is also useful for desymmetrizing prochiral compounds into chiral products.

But the method does not cover all possibilities. For example, it cannot be used in the reduction of acetophenone to (*R*)- or (*S*)-phenylethanol because the precursor cannot be labeled in such a way that the products can be differentiated.

Finn's method, however, can be used for kinetic resolution and desymmetrization, as well as the ketone reduction that Reetz describes. "We take the ketone, subject it to our catalyst, and then take the reaction product and derivatize it with a mass-tagged chiral reagent," Finn explains.

Accuracies are ±3% for Reetz's method and ±10% for Finn's method. As Welch says, accuracy of ±10% is good enough to separate the good catalysts from the bad ones. Reetz, however, needs greater accuracy. "In the last phases of directed evolution, when the ee has reached 90% and you want to go even higher, an assay that's ±10% is not good enough," he says. In fact, Reetz says his group has developed a method based on nuclear magnetic resonance spectroscopy (NMR) that can perform 1,400 assays per day with an accuracy of ±2%.

Last year, Harvard University chemistry professor Matthew D. Shair excited the chiral chemistry community by showing that DNA microarray technology could be

adapted to ee measurement.

With the new technology, called reaction microarrays, picoliter amounts of a mixture are spotted onto a glass surface and then made to react with fluorescent chiral probes. When a spot is excited by laser, the fluorophores of the chiral probe emit with intensities proportional to the ee at that spot. Spots can be applied at densities of up to 100,000 per 3-sq-in glass slide, making for very high-throughput ee measurements.

Shair and graduate student Gregory A. Korbel have been optimizing the reaction microarray system. Currently, they are developing a general way to attach molecules to a glass surface through the carbon-hydrogen insertion chemistry of nitrenes.

"Every molecule we care about would have a C–H bond," Shair tells C&EN. "If you could come up with a chemistry that attached any compound irrespective of the structure, you could use reaction microarrays for almost any reaction product, as long as that molecule also had some handle to which a chiral probe can be attached."

Nitrenes are species in which a nitrogen atom bears two unpaired electrons. To generate nitrenes, Korbel coats the glass slide with an azide and then irradiates the slide with a handheld ultraviolet lamp. "What we believe happens is that N_2 is removed photochemically from the azide, leaving a nitrene."

In principle, the nitrene would insert into enantiomers of a mixture whose ee is to be determined. Preliminary results are promising, but Shair says it's difficult to know for sure what's going on. "It's hard to find out what you've done on a glass surface," he explains. "That has slowed our progress." Still, Shair is optimistic that his lab can soon use reaction microarrays to screen a new catalytic aldol reaction that he and his coworkers have been developing.

Other new ideas for determining ee include use of enzymes, immunoassays, color indicators, molecularly imprinted polymers, and metal-organic supramolecules. Some of these methods are mentioned in the reviews by Finn and Reetz and in another by Henri B. Kagan and Masaki Tsukamoto of the University of Paris-South in Orsay, France [*Adv. Synth. Catal.*, **344,** 453 (2002)].

At Brown University, associate chemistry professor Christopher T. Seto has been developing a high-throughput method that uses an enzyme to selectively transform one enantiomer of a catalytic reaction product. As an example, he and graduate student Paul Abato use the addition of diethylzinc to benzaldehyde, producing (*R*)- or (*S*)-1-phenyl-1-propanol [*J. Am. Chem. Soc.*, **123,** 9206 (2001)].

An alcohol dehydrogenase from a Thermoanaerobium species oxidizes the S enantiomer to phenyl ethyl ketone, and the rate of this oxidation is a direct measure

of ee. One hundred samples can be assayed in 30 minutes, with an accuracy of ±10%. A second assay using an alcohol dehydrogenase from *Lactobacillus kefir*, which oxidizes the R enantiomer, enables calculation of the extent of conversion.

According to Abato and Seto, alcohol dehydrogenases could be used to analyze alcohol products from a wide variety of reactions, including hydrogenation of ketones, aldol reactions, nucleophilic ring opening of epoxides, and kinetic resolution of alcohols. Other enzymes could be harnessed for other compound types: lipases and esterases for ester products of allylic oxidations with *tert*-butyl peroxybenzoate, alkene cyclopropanation with alkyl diazoacetate, and the glyoxylate ene reaction; and acylases and proteases for amide products of catalytic hydrogenation of *N*-acetyleneamines.

Early this year, a team led by Charles Mioskowski and Alain Wagner at the University Louis Pasteur, in France, reported a method based on competitive enzyme immunoassays for high-throughput screening of catalysts for enantioselective ketone reduction [*Angew. Chem. Int. Ed.,* **41,** 124 (2002)]. The team demonstrated the principle with reduction of benzoyl formic acid to mandelic acid. Binding of the products to an indiscriminate antibody gives the total yield of the reaction. Binding of one enantiomer with an enantioselective antibody gives ee with an accuracy of ±9%.

Testing a catalyst library prepared by combining four metals and 22 chiral diamine-based ligands, the researchers were able to complete 176 assays in one day. With the routine use of competitive enzyme immunoassays in biology and diagnostics, the researchers believe that antibody-based methods have great potential in high-throughput screening for catalyst discovery. Others have proposed using immunosensor techniques in space missions to detect extraterrestrial enantioenrichment [*Enantiomer,* **6,** 153 (2001)].

MEANWHILE, in the Netherlands, a color test for ee has been developed by chemists Richard A. van Delden and Ben L. Feringa at the University of Groningen. The test is based on the unique optical properties of cholesteric liquid crystal (LC) phases. Dopants with high chiral induction--or helical twisting power--induce these LC phases to reflect in the visible range. The wavelength of reflection is inversely proportional to the dopant ee. Enantiomeric excesses of 50% and higher can be visualized [*Angew. Chem. Int. Ed.,* **40,** 3198 (2001)]. In a recent study with methyl phenyl glycine, the researchers demonstrated visual detection of the full range of ee by monitoring the color of the doped LC [*Chem. Commun.,* **2002,** 174].

However, the helical twisting power of most simple organic molecules is too weak for color induction. To overcome this problem, van Delden and Feringa modify the analyte by attaching a "mesogenic unit"--a structure that resembles the LC host molecules. The change dramatically increases the helical twisting power, and even at low dopant concentrations colored LC phases are obtained.

"The method requires no chiral auxiliaries and only microgram quantities of analyte," van Delden says. "And it should be possible to custom design the method for a large variety of analytes."

In addition, "automation should be readily feasible," van Delden says. "Microarrays of colored dots would allow high-throughput screening since color inspection is instantaneous. It should even be possible to calibrate the method to allow ee determination in the presence of other compounds; for example, starting materials, side products, or even a chiral catalyst."

A similar approach has been proposed by Gloria Proni, of New York University, New York City, and Gian Piero Spada, of the University of Bologna, in Italy, to detect enantioenrichment in an extraterrestrial environment [*Enantiomer,* **6,** 171 (2001)].

Closer to home, at the American Chemical Society national meeting held in April in Orlando, Fla., Ken D. Shimizu, an assistant professor of chemistry at the University of South Carolina, Columbia, described a rapid assay of ee based on molecularly imprinted polymers (MIPs).

"It's difficult to measure ee, because it is hard to differentiate enantiomers based on chirality," Shimizu says. "It's a lot easier to measure concentrations. So we've been developing ways to differentiate enantiomers such that the outcome is a difference in concentrations."

MIPs are highly cross-linked polymers that are prepared in the presence of a template molecule, for example, one enantiomer. The template molecules form cavities within the polymer, and when the molecules are removed, the cavities remain. These cavities enable the polymer to bind the imprinted enantiomer to a greater extent than its opposite enantiomer.

MIPs are easy to make and fairly inexpensive, Shimizu says. Measurement of ee involves simply adding ground polymer to a reaction mixture and then determining the total concentration of enantiomers remaining in solution.

MIPs deplete the mixture of the enantiomer with which they were originally imprinted to a far greater extent than they deplete the other enantiomer. Through calibrations, the concentration of the remaining mixture can be correlated to the initial enantiomeric composition. Potentially, 1,000 measurements can be done in one day instead of the two weeks they might take with conventional chiral chromatography. The method also can be general, in that MIPs could be easily tailored to just about any molecule, Shimizu says.

Also at the ACS meeting, Wenbin Lin, an assistant professor of chemistry at the University of North Carolina, Chapel Hill, described a family of metal-organic supramolecules that can be applied to high-throughput sensing of ee. The

molecules are squares with rhenium carbonyl complexes at the corners and 4,4'-bis(pyridyl)-1,1'-binaphthyl units at the sides [*J. Am. Chem. Soc.*, **124**, 4554 (2002)]. Molecular squares of this type fluoresce strongly, and the fluorescence is quenched in the presence of chiral amino alcohols. The extent of quenching can be related to the alcohol's ee.

"If you have an optical means to detect optical purity, all you have to do is add this sensory material, shine light, and see which ones light up and which ones don't," Lin tells C&EN. He adds that optical techniques based on fluorescence are some of the best for sensing because they are very sensitive, able to detect concentrations as low as 10^{-7} M.

"As measurement techniques get better, the jobs of people making chiral materials will get easier and easier," Welch says. "Any revolution from nothing to something always requires a way to keep score."

Top
