# In the Field

Pharmaceutical Science & Technology News

# **Biocatalysis for Efficient** Manufacturing

SM Pharma Chemicals (DPC, Sittard, the Netherlands) and Diversa Corporation (San Diego, CA) have signed an agreement for the discovery and development of biocatalysts, with the aim of simplifying and lowering the cost of chemical transformations. Under the agreement, DPC, a division of DSM Pharmaceutical Products, will identify the targeted chemical conversions and Diversa will develop the appropriate biocatalysts. DSM will then scale up the process to manufacture pharmaceutical intermediates and active ingredients.

Biocatalysis, in which enzymes are used to drive chemical reactions, can be used in pharmaceutical manufacturing as an alternative to standard organic chemistry. The key advantage of biocatalysts is that their use can often simplify the synthesis of complex molecules, reducing the number of steps required and the related costs, particularly when chiral molecules are involved. The selectivity of enzymes in particular can make certain chemical conversions more commercially attractive.

"The selectivity of enzymes is phenomenal," says Marcel Wubbolts, PhD, competence manager for biocatalysis

and biotransformations at DPC. As an example, Wubbolts explains how enzymes can be used to selectively modify a macrolide, a complex antibiotic that may contain various similar functional groups. "If you want to modify one specific alcohol functionality among five others to get a new product, you can do that with an appropriate enzyme, because of its selectivity. With organic chemistry, you would have to re-synthesize the whole molecule."

Enzymes can have three types of selectivity—chemoselectivity, regioselectivity, and stereoselectivity. Chemoselectivity means that enzymes, unlike some chemical catalysts, are capable of distinguishing between chemical structures such as an amino group and hydroxyl group. Regioselectivity, seen in the macrolide example, means that enzymes can distinguish between identical groups in different locations. For example, if a ring structure contains two hydroxyl groups, an enzyme can selectively modify one of them, based on its position, whereas a chemical catalyst would modify both, because they are chemically equivalent. The third type of selectivity is stereoselectivity, which

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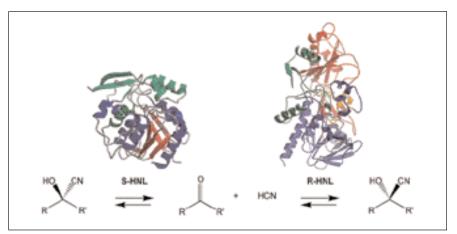


Figure 1: DPC uses an R-hydroxynitrile lyase (R-HNL) to produce an R-mandelic acid derivative for a pharmaceutical intermediate.

# **Ultrasonic Rheometer Possible PAT Candidate**

An innovative viscosity measurement technology developed at Pacific Northwest National Laboratory (PNNL, Richland, WA) could potentially help advance the pharmaceutical industry's PAT-driven push toward real-time process monitoring and control systems.



PNNL's prototype ultrasonic rheometer.

The "ultrasonic rheometer" combines ultrasonic and pressure-sensor data using sophisticated signal processing algorithms to determine the viscosity of material flowing in a pipeline in real time. Since viscosity is often a qualitycontrol parameter in blending operations, the ability to collect measurement data without interrupting production processes for off-line batch testing could provide pharmaceutical producers significant cost and time savings.

As noted by David Pfund, PNNL senior research engineer, the need for off-line viscosity measurements to either confirm specifications or ensure quality control inevitably increases production schedules as well as the amount of storage capacity required. "And, if it turns out that the product is off-specification, it will have to either be re-blended or scrapped, increasing waste."

PNNL first began work on the technology in the late 90s with funding from the Department of Energy, which was

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# NIR Spectroscopy Brings PAT into Focus

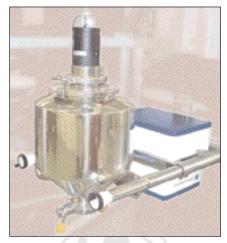
When FDA set forth its process analytical technologies (PAT) initiative, there was little doubt that it would foster new collaborations among the agency, the pharmaceutical industry, and equipment manufacturers. And, the industry's first innovations specifically targeted at meeting PAT objectives are indeed now making their debut on the marketplace. Especially notable are those incorporating near infrared (NIR) spectroscopy.

Already a familiar laboratory technique for raw material identification and quantitative assessment, NIR technology is expected to move on to the next logical step of being implemented on the manufacturing process line.

For example, late last year, L.B. Bohle (Bristol, PA) unveiled its integrated NIR system for monitoring blend homogeneity in real time (Figure 1). Designed for laboratory- and full-scale production, the instrument combines the company's blending technology with NIR spectroscopy and 21 *CFR* Part 11–compliant software. Data are obtained by shooting NIR light through a window mounted on the bin blender and results are sent to an on-line or remote-access computer.

Because sampling doesn't involve physically opening the bin, the material is always in containment, which is especially beneficial to manufacturers of potent materials. Also, bringing the analysis on-line saves time because test results are immediate. As Reinhard Sievert, general manager at L.B. Bohle observes, "It's much faster than the traditional method of taking a sample out of the blender, going to the lab, and analyzing the result to find out, only then, if you have blend homogeneity."

Integrating an analytical system with a complex manufacturing process was not an easy task. "You can't just take NIR technology, install it onto a blending system, and expect it to work," says Sievert. "It's a combination of the hardware, the optics, the electronics, and the software with the blending system that makes it perform well." Sievert notes at least three major attributes that had to be incorporated into the design: pre-



**Figure 1**: L.B. Bohle's integrated NIR system for real-time monitoring of blend homogeneity.

venting "pockets" where raw material may be trapped; a flush interior that won't restrict powder flow; and, most important, the ability to maintain conditions inside the blender that will allow stable readings with the NIR probe.

Although operating and maintaining the "correct" conditions is essential for project management, understanding the process is equally, if not more, important. Says Sievert, "We have customers ask us How do you scale up? How do you scale down? With what speed should we blend? If we transfer from a V-blender to a bin blender, how fast do we have to rotate the bin? How about filling levels? But what's really important here is not establishing these individual parameters, but rather achieving blend homogeneity and then understanding how these individual factors impact that homogeneity."

Establishing such correlations among manufacturing processes and how they affect product quality is a goal of the PAT initiative. As observed by Robbe Lyon, deputy director of FDA's Division of Product Quality Research, PAT "requires a level of scientific understanding of how formulation and manufacturing process factors affect product quality and performance."

Although PAT can essentially incorporate several technologies, NIR is proving to be a preferred method among analysts. In part, this is because during the past 10–15 years, the implementation of infrared cameras, array detectors, digitally tunable infrared optical

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### Bioerodable Eye Implant May Help Treat Macular Edema

A bioerodable sustained-release eye implant shows promise as an effective method for treating eye diseases such as macular edema (swelling of the retina that causes decreased vision). Data from a six-month Phase II clinical trials study revealed that patients with macular edema experienced a significant increase in vision after being surgically implanted with the investigational technology.



The Posurdex eye implant is bioerodable and provides sustained drug release.

The "Posurdex" implant from Allergan, Inc. (Irvine, CA), delivers dexamethasone (a corticosteroid) in small amounts over an extended period of time, which allows the drug to reduce the swelling of the retina while curtailing possible side effects. Recent data from other studies has provided evidence that corticosteroids can be useful for treating several retinal diseases, specifically macular edema. However, the main side effects of steroid use in the eye are glaucoma (increased pressure in the eye) or cataracts.

According to Scott Whitcup, MD, vice-president and head of the ophthal-mology therapeutic area at Allergan, the sustained-release aspect of Posurdex was one of the main factors of its success in the Phase II clinical trials. "The thought is that if a patient receives smaller amounts of steroid over time, as opposed to big boluses of steroids, then maybe you can minimize the complications but maximize the treatment effects," he says.

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**BIOCATALYSIS** continued from page 17 means that enzymes can be used to produce different stereoisomers of chiral molecules.

"Enzymes are really one of the best tools to address chirality in manufacturing," says Wubbolts. Chiral molecules exist in two or more different forms, or stereoisomers, which have the same

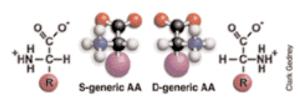


Figure 2: Examples of left-handed (S) and right-handed (R) stereoisomers of a generic amino acid.

chemical composition but different configurations that are not superimposable on each other. For example, most amino acids exist in two forms, depending on how the various component groups are arranged around the central carbon atom. In the right-handed (R) stereoisomer, the amino group is on the right, whereas in the left-handed (S) configuration, the amino group is on the left (see Figure 2). Pairs of stereoisomers are referred to as enantiomers.

The pharmaceutical importance of chirality was first discovered in the 1960s with the tragic case of thalidomide, which was given to pregnant women for morning sickness. However, although one of the stereoisomers of the drug had the desired sedative effect, the other form was a teratogen, causing birth defects. As a consequence, FDA now requires that chiral molecules be tested to determine the behavior of the enantiomers in the human body. In most cases, the functionality is not the same, which means that the final pharmaceutical product must be enantiopure, containing only the correct isomer. If a racemic mixture is produced (i.e., one that contains both enantiomers), the stereoisomers must be separated, which can be done with selective co-crystallization, chromatography, or biocatalysis. In this case the maximal production yield will be 50%. Alternately, other chiral production methods, which again include biocatalysis, can be used, which will produce an enantiopure compound with 100% maximal yield.

In many cases, the use of biocatalysts makes the process more cost effective. In one application, DPC uses an R-hydroxynitrile lyase (R-HNL) to produce an Rmandelic acid derivative for a pharmaceutical intermediate (see Figure 1), a 100% maximal yield route. "One of the traditional prejudices against biocatalysis is that even when you can make

> something, it's frequently the wrong stereoisomer," notes Wubbolts. "Unlike chemical catalysis you typically cannot simply use the stereochemical opposite catalyst. However, in this case we used the R-enzyme, but we also have an S-enzyme avail-

able, so we can address both the S-enantiomer and the R-enantiomer."

Biocatalysts, in addition to being used in manufacturing chiral compounds, can also simplify other complex synthesis processes. "We have examples where we have been able to replace an eightstep organic synthesis route with a twostep process," says Ronald Gebhard, R&D director of DPC. Gebhard also notes that biocatalysis generally uses very mild reaction conditions, because many reactions can be performed in water instead of organic solvents. This means that sensitive functionalities on organic molecules can be left unprotected during the process, which can also save steps. "Nature is efficient, and sometimes it allows us to shortcut classical organic chemistry," Gebhard says.

Because biocatalysts can simplify chemical conversions and reduce costs, they can make the production of certain molecules, not easily produced using chemical catalyts, commercially viable. "There are products in pharmaceutical pipelines that never made it beyond the discovery phase because nobody could synthesize them to scale in an economically attractive way," says Wubbolts. "Using biocatalysts gives you new routes to new products."

DSM currently has more than 25 biocatalysis processes for life sciences applications, about 15 of which are used in pharmaceutical manufacturing. Through the partnership with Diversa, the company expects to be able to increase its

biocatalyst toolbox more quickly.

"What DPC is looking for from us are unique activities where the known enzymes just don't perform well," says Mark Burk, PhD, vice-president of research and development at Diversa. "Our technologies, which stem from a genomics approach to enzyme discovery, allow us to do that."

Diversa starts its enzyme-identification process by sampling the entire global environment, particularly extreme conditions of temperature and pH, to find organisms that produce unique enzymes. They then extract and process the DNA from the organisms contained in those samples, and clone the DNA into a host such as Escherichia coli. "In any given environmental sample there are probably 5-10,000 different organisms, generally bacteria and fungi, most of which have never been observed or characterized before," notes Burk. Each E. coli clone, which contains unique DNA, then becomes part of a genomic library that the company can screen for a specific kind of activity. Through this process, Diversa has created more than 2500 genomic libraries, each containing 10<sup>6</sup>–10<sup>8</sup> unique clones that can be screened.

If the screening process identifies an enzyme that isn't exactly what is needed, Diversa uses directed evolution technologies to mutate the genetic information to be able to produce an enzyme with the desired characteristics. Through this process, they create new libraries of evolved enzyme variants, which again are screened using their high-throughput screening techniques. "This combination of enzyme discovery and evolution usually takes us where we want to be, with an enzyme suitable for commercialization" says Burk.

Once the new biocatalysts are identified, DPC will scale them up for commercial production of pharmaceutical actives and intermediates. "Scale up is a challenge," comments DPC's Gebhard. "Not only do you have to use the enzymatic reaction on a large scale, but you also must be able to scale up the production of the enzyme."

Wubbolts estimates that across the industry, approximately 10% of chiral BIOCATALYSIS continued on page 224

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looking for a way to continuously monitor transfer lines at a radioactive waste tank farm. Subsequently, the laboratory has been looking at applying the technology to process-control/blending applications in the cosmetic and food industries. So far, the group hasn't had a pharmaceutical industry sponsor, but is anxious to explore that possibility. Says Pfund, "What we currently have is a technique, a (3rd-generation) prototype instrument, and some proof-of-concept measurements."

#### **Principle**

The basic principle behind the ultrasonic rheometer is that by measuring the velocity of a fluid passing through a pipe, and correlating these measurements with pressure-drop data, the viscosity of the material can be determined as a function of shear rate.

As explained by Pfund, the velocity field in a pipeline flow is indicative of the rheological behavior of the material. Viscosity is expressed as the ratio of shear stress (force/unit area) to the shear rate (rate change of shear strain).

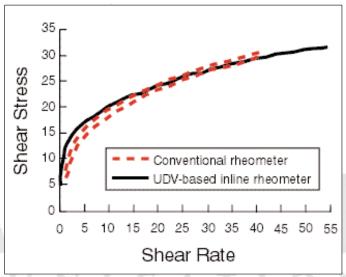
For any type of pipeflow, the flow velocity will be zero at the walls of the pipe and maximum at the center line. Many materials (such as water) exhibit a constant (shear-independent) viscosity profile and are referred to as "Newtonian" fluids. In these cases, fluid velocity will vary quadratically with relative distance from the centerline of the pipe, defining a parabola-shaped profile. Other materials, however, are known as "shear-thinning" fluids. These include one common example (ketchup) but also a host of important polymers and colloidal substances.

With shear thinning fluids, the higher the rate of shear (velocity/height), the lower will be the viscosity. The maximum velocity in these cases will persist for some distance away from the centerline of the pipe, and fluids will tend to flow through the pipe with a central "plug." The shape of the velocity profile will therefore reflect the shear-thinning behavior of the fluid.

#### Instrument

The ultrasonic rheometer makes use of

multiple data sources. Velocity profile information is obtained using an ultrasonic doppler velocimeter. A series of ultrasonic pulses are transmitted into the pipe and the backscatter echo signal returned (corresponding to the structural properties of the material) is collected. Since the pulses and return echoes are time-gated, the relative range of the returns can be calculated. Then, since based on whether the return signal was travelling away from or toward the trans-



Completed rheogram based on ultrasonic doppler velocimeter (UDV) results plotted against results from a conventional rheometer.

ducer (transmitter/receiver), it will have a distinctive doppler-shift characteristic proportional to the velocity of the fluid.

To complete the viscosity calculations, pressure-drop measurements are taken over long sections of pipe to obtain pressure-drop/unit length data. The pressure drop measurements indicate the shear-stress at the pipe wall and, since shear-stress (amount of force/unit area on a layer of moving fluid in a pipe) varies linearly with distance from the pipe wall, and the shear stress at the center is zero, it's possible to determine shear stress throughout the pipe. The ratio of shear stress to shear rate is the viscosity, and by plotting this, viscosity versus shear rate can be obtained.

According to Pfund, the rate at which measurements can be taken varies according to a number of factors, but the principle consideration is the fluid flow rate—lower flow rates require relatively longer measurement times since the

lower frequency doppler returns will need to be averaged over a longer period of time to provide adequate resolution. "Individual pulse sequence data sets may be collected every 100th of a second, but with correlation of multiple scans and processing time etc., you may be looking at time increments in the range of 10 seconds," says Pfund.

Clearly, the data correlation, automation and reporting software supporting the instrument is an important compo-

nent of its capability. The PNNL development effort included input and expertise from a number of different groups within the laboratory including fluiddynamics, electronic systems and non-destructive testing. Written in C, the software is fully-integrated with the instrument.

As is the case with many potential in-line process

measurement technologies being considered for pharmaceutical production applications, additional analytical groundwork will likely need to be done before an actual implementation can be accomplished. As pointed out by Pfund, "It's not always clear (to users) what kind of data will be required to do this operation. Many existing quality control, or product-approval specifications don't directly relate to viscosity, but are only indicative of it." As an example, he notes that a customary test in the food industry to determine if a batch of tomato paste is on spec is to put the product behind a small "dam" and measure how fast it takes to "slump" when the dam is removed. "The point is that there's not always a documented quantitative relationship between observables and viscosity, and detailed measurements will often need to be taken up-front to establish specific acceptable viscosity parameters," says Pfund.

John Haystead

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The study, which monitored the effects of the steroids on macular edema and how the implants were tolerated, followed 306 patients who had been diagnosed with macular edema associated with diabetes, retinal vein occlusions, uveitis, and postcataract surgery. The patients were given one of three treatments: a single implant with either a 350-µg or 700-µg dose of dexamethasone or observation without drug therapy. The results showed that patients who had been implanted with the 700µg dose had the greatest improvement in vision—significantly more of these patients exhibited a three-line increase (relating to an eye chart) in visual acuity compared to the control group.

The bioerodable Posurdex is constructed of a mixture of poly lactic and poly glycolic acid. These polymers are also mixed with the drug. Therefore, when the body begins to break the implant down, it results in a controlled drug release (which could be over a period of approximately six weeks, according to data from animal studies). After the drug has been absorbed by the body, all that is left of the implant is essentially carbon dioxide and water, which pose no risk to the patient.

Data from the study also showed a low incidence of complications, no increase in the presence of cataracts or eye infection, and a glaucoma rate potentially less than what has been reported in other investigational methods of delivering steroids to the eye.

Although improved visual acuity is the type of dose response and efficacy that Allergan hopes to present to FDA and other regulatory bodies to receive approval to market the technology commercially, the technology must still also perform favorably in follow-on Phase III clinical trials (which are planned to begin in the second quarter of 2004). Currently, Posurdex is positioned to be the first approved treatment for macular edema. If Phase III trials are successful, Allergan anticipates marketing Posurdex in 2006 or 2007.

With approximately 750,000 people in the United States and 2.5 million people worldwide suffering from some form of macular edema, Whitcup esti-

mates that the market potential for a technology such as Posurdex is probably \$500 million to \$1 billion in peak sales.

Allergan hopes to use the Posurdex implant with additional drugs to treat other retinal diseases such as age-related macular degeneration, a condition that affects approximately 12 million people in the United States. The company currently has an antiangiogenic program of drugs that inhibits new blood vessel growth in the retina, countering the overproduction of growth factors,



Allergan has developed an applicator with a 22-gauge needle for inserting Posurdex in an office-based surgery setting.

which can destroy the retina and cause blindness. "Retinal disease is one of the biggest unmet medical needs in the ophthalmology field, and we felt that it was important to not only have novel compounds but also have a novel way to deliver them to the back of the eye," Whitcup says.

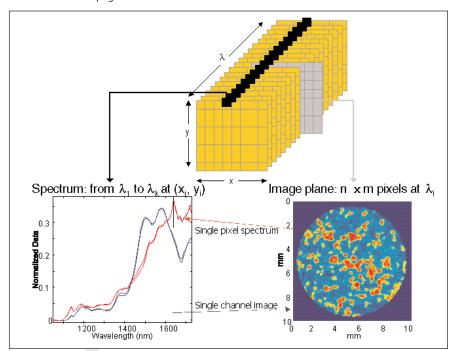
Patients in the Phase II study were required to go to a medical operating room to be surgically implanted with Posurdex, however, Allergan has now developed an applicator with a 22-gauge needle for inserting Posurdex into the eye that can be used in an office-based surgery setting such as the ophthalmologist's office, thereby making the process more comfortable and convenient for the patient.

Whitcup notes that even with the large number of people diagnosed with macular degeneration, there's still only one approved therapy on the market to treat their illness. "Clearly, there are patients who still need help, so we're excited to be working on this for them," he says.

Posurdex was initially developed by Oculex Pharmaceuticals, Inc., which was acquired by Allergan in November 2003.

Ronelle Russell

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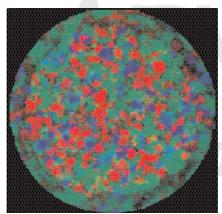


Figure 3: NIR chemical image of a tablet showing distinctive agglomerations of three active pharmaceutical ingredients: acetaminophen (red), aspirin (green), and caffeine (blue).

filters, improved computer memory, and faster data processing speeds have made NIR chemical imaging possible. Information that could once only be represented as a series of spectra is now in the form of a two-dimensional image (see Figures 2 and 3).

As FDA's Lyon observes, "The advantage of chemical imaging compared to other sensor technologies is the ability to analyze the spatial distribution of the component materials in blends, granules, or finished dosage forms. This approach has the potential to monitor processes to reveal the extent of ingre-

**Figure 2:** Each pixel on the 2-D image is represented by a spectrum. A single-channel image at a particular wavelength provides chemical and spatial information.

dient blending, particle-size distributions, agglomeration of component particles, and the presence of polymorphs, hydrates, and other trace contaminants."

Thus when implemented on or near the process line, NIR imaging can contribute significantly to the understanding of manufacturing processes. As Neil Lewis CEO of Spectral Dimensions, Inc. (Olney, MD) observes, "There's nothing new about the technology. What is new is that FDA is pushing to modernize the industry such that NIR and the PAT initiative have really begun to take off." He adds, "The PAT initiative is really about understanding the complex dosage form, and chemical imaging provides a tremendous handle on that because it allows you to get at not only the chemical species, but the spatial relationships, which has a bearing on factors such as dissolution."

High-resoution images provide spatial and chemical information about the structure of a product's matrix (the distribution and particle size of the active and the excipients). Knowledge of this matrix can be used to predict characteristics such as ease of manufacture and performance during quality testing.

With this objective in mind, FDA recently entered into a cooperative research and development agreement with Pfizer to investigate the application of NIR chemical imaging in process manufacturing. According to Lyon, the current status of that project is that "a prototype process imaging system is ready for deployment and initial testing, and a study plan has been developed and protocols are being finalized."

Spectral Dimensions is also planning to introduce an NIR system for the process line. According to Lewis, the company "recently produced an instrument designed exclusively for process applications." Meanwhile, the company continues to offer and improve upon its off-the-shelf NIR chemical imaging equipment and software that can be incorporated at or near the process line. According to Lewis, this is possible because the instruments have no moving parts. "You can effectively take the equipment, put it into a NEMA enclosure, and take it right to the factory floor." Chemical imaging then becomes not just a way to detect root-cause problems in the tablet, but also a way to monitor what's happening during manufacture. Says Lewis, "What and how much we may be able to learn about the complexities of pharmaceutical manufacturing from NIR chemical imaging is still relatively unknown, but early indications suggest that it might be a great deal."

**Maribel Rios** 

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pharmaceutical intermediate manufacturing is performed using biocatalysts. Few large pharmaceutical companies use biocatalysis in drug discovery and lead development because there is not enough time to develop suitable biocatalysts and because most chemists are more comfortable with classical organic chemistry methods.

As a result, DPC sometimes finds that they must switch technologies for scale up. "If biocatalysis were used all along, that would save in development time over the entire route," notes Wubbolts.

Laura Bush