



# In the Drug Delivery Zone

## Patients Are the Priority

by Cheryl Scott

A rule of thumb in drug development states that the larger a therapeutic molecule is, the more trouble it will be to make, ship/store, and administer to patients. Biotherapeutics include proteins (such as antibodies), vaccines, some smaller peptides (such as hormones), DNA for gene-transfer therapies, cells and tissues, and to a lesser extent blood-fractionation products, allergenics, and RNA/oligonucleotides. Biomolecules are big and unwieldy, they're produced in complex mixtures by biological processes, and they face numerous challenges in storage and within the environment of a human body. Cells and tissues present an entirely different set of problems, from culture of autologous cells to immune-system problems with allografts.

The emergence of regenerative medicine and a move toward a global marketplace are creating a demand for new technologies that allow worldwide shipment of biological products while maintaining their viability or function. Effective biological packaging requires new understanding of state-of-the-art hypothermic storage and cryopreservation, the standard approaches currently used for preserving cells and tissues.

Biotech drug formulators have many concerns to juggle in their work, beginning with the physicochemical characteristics of an active molecule and



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including the reliability, cost, and availability of analytical methods used in formulation work; the array of excipients on the market; evolving delivery methods and devices; patient preferences and behavior, as well as the biology of diseases being treated; even the concerns of legal, sales, and marketing staff. So formulation is more than science, but science is its foundation.

The most basic concerns of biotherapeutic formulation are stability and structural integrity of the active molecule during transit and storage, successful delivery of the drug to its site of action, and (inevitably) speed and cost-effectiveness of development and the final product. Numerous details come into play including analytical methods and testing protocols (see the "Finer Points" box), containers and closures, delivery devices and dosage forms, excipients and stabilizers, and

compatibility of ingredients. Complicated formulation decision trees necessitate the following types of choices: method of delivery (e.g., parenteral, pulmonary), final product form (e.g., in solution, lyophilized), multidose or single-dose (with or without preservatives), ingredients (e.g., excipients, stabilizers), dosage details (e.g., frequency, concentration), logistics (e.g., transportation, storage conditions, shelf life), packaging (e.g., vials/ampules, prefilled syringes, and even labeling), and manufacturing process (e.g., scale, equipment, procedures).

### STABILITY IS KEY

Proteins and other large biomolecules are delicate and sensitive to changes in conditions such as pH, osmolality, tonicity, pressure, and temperature. Vaccines may be genes, proteins, pathogen fragments, or whole

organisms that have been killed or inactivated. Blood-fractionation products and allergens are usually proteins themselves, so they can be treated by formulators the same ways as recombinant antibodies. And peptides are merely amino-acid chains (like proteins, only smaller), which

present the least amount of trouble in biotech formulations.

Nucleic acids usually are formulated with gene-transfer vectors such as transformed viruses, lipids, or polymers. Viral vectors can be treated more or less as large proteins in formulation because they are typically

protein shells surrounding genes of interest. Synthetic vectors present fewer safety issues than viruses, but they are less efficient transfectors of DNA/RNA. They also present more challenges in formulation because aqueous suspensions of nonviral vectors tend to agglomerate over time. Simply

## THE FINER POINTS OF FORMULATION TESTING

In January 2008, BPI's associate editor Leah Rosin spoke with biotechnology consultant Nadine Ritter about one of Ritter's specialties: analytical testing of biologics. In particular, they discussed forced-degradation studies and stability as well as immunogenicity issues related to residual host-cell proteins. Here is part of their conversation.

**BPI:** What analytical methods are used in forced degradation studies?

**Ritter:** The tools used for stability testing vary by product type. Purified proteins will have a slightly different set of analytical tools than you'd apply to a complex mixture of proteins (e.g., vaccines) or a mixture of proteins and nucleic acids (e.g., gene therapy products).

A major goal of forced degradation studies — and people miss this a lot — is that you're using samples of the force-degraded material to demonstrate experimentally whether or not the methods that you've selected for stability testing are actually capable of measuring changes in the product. It's easy to say things like, "My product is not degrading because we don't see anything changing." Well, how do you know you could see it degrading? It's always hard to prove a negative. Protein chemists have a good idea that some methods can show some types of degradants. For example, we know that SEC-HPLC should pick up high-molecular-weight species (e.g., aggregates) and that SDS-PAGE should pick up lower molecular-weight protein fragments. But until we actually degrade a product under a systemic set of physical and chemical conditions to generate defined sets of degradants, we can't

actually prove those methods are sensitive and specific enough to indicate stability for a particular product. It isn't enough to assume they'll be suitable any more than to assume they'll be linear or accurate or precise. We have to validate their suitability for the intended use. It's simple to say, "The methods have been validated as stability-indicating," but like any other type of validation exercise, certain data is required to support that claim.

Another objective of forced-degradation studies is to characterize product degradation pathways and identify specific degradants. For this, we go beyond the release/stability analytical "tool kit" to more definitive, orthogonal methods. For example, you might use analytical ultracentrifugation or light scattering to identify and characterize aggregates resolved by SEC-HPLC. Or you might use in-line mass spectrometry to perform fragment analysis of peaks separated by RP-HPLC of peptide digests.

**BPI:** What are some good sources of information on the current state of analytics for biotechnology products?

**Ritter:** Two separate publications came out in the past couple years from the CMC Strategy Forum that address analytics for different types of biotech products. One is on monoclonal antibodies and the other on live attenuated vaccines and gene therapy products. Both papers were drawn from forum discussions among expert scientists from industry and regulatory agencies. It is important to recognize that the tool kit continually changes with advances in technology and breakthroughs in new

applications. That is the C in CGMP: *current* practices. However, we've been using a basic set of protein analytical methods pretty much since the beginning of biotech: electrophoretic, chromatographic, and spectrophotometric methods. Then of course there are functional assays that vary by product type.

But it's challenging to design our particular analytical strategies because even as guidance documents are published by regulatory agencies, they're nearly obsolete because product and test-method technologies advance so quickly. So regulators aren't too prescriptive in their official technical recommendations, referring often to "where appropriate" or "as applicable" in the use of analytical methods. I think compilations like the CMC Forum papers can provide a more accurate snapshot of what's going on right now in the field. They're not official guidance documents, but they do represent input from a variety of perspectives, including regulatory scientists who see a broad array of product types and analytical technologies. From the feedback we've received, these papers seem to be very useful in that regard.

**BPI:** OK, so what about host-cell protein analysis?

**Ritter:** Another misunderstood parameter in analytical testing, with safety concerns similar to product degradants, is possible immunogenicity. We know that parenterally delivered proteins (or aggregates or fragments) can become antigenic under certain circumstances, potentially

triggering an immunogenic response in patients. When our production "factory" is a living organism (e.g., *E. coli* or CHO cells), it generates hundreds of proteins from which we try to isolate just the ones we want. Some host-cell proteins can copurify with our target protein. We know the immune system can recognize a very, very small amount of highly antigenic proteins. Our mass-based methods of analysis are typically neither sensitive nor specific enough to pick up low levels of such residuals. So for an analytical tool, we turn to the same biological "detection" mechanism the body uses — namely the immune system. We make a mixture of total host-cell proteins from our expression system and use that mixture to generate immune sera, containing a set of polyclonal antibodies that recognize epitopes present in that mixture. Then we use that PAb preparation as the driving reagent in immunoassays to measure residual host-cell proteins in process intermediates and bulk drug substance. It is critically important to confirm that the PAb preparation adequately recognizes the mixture of proteins from our expression system. Whether an anti-host-cell protein PAb reagent is custom-generated or commercially obtained, it must be verified immunoreactive against the majority of individual proteins present in a sponsor's own host-cell mixture. Only then can it be used in an ELISA format. Without visual confirmation of antibody-host-cell specificity, (e.g., using Western blotting), we might not recognize whether our ELISA is missing a subset of residual proteins from our own expression system.

put, stabilization of gene products is also stabilization of their transfer vectors. Formulation and delivery have been the primary challenge to companies developing gene-transfer products, whether as therapies or vaccines. Because chemical degradation of DNA can cause mutation as well as negate therapeutic activity, any kind of destabilization is of great concern. For those choosing synthetic over viral vectors, freezing or freeze-drying (lyophilization) appear to be only options. Naked DNA fares much better at colder temperatures, even better in a freeze-dried state. However, much work is yet to be done in nucleic acid formulations before we see confirmed shelf lives approaching the drug-product norm of two years.

**Protein Stability:** Formulators have much more experience with recombinant proteins. They have identified three principal methods of degradation in protein formulations: deamidation, oxidation, and aggregation, all of which are most likely to occur in aqueous solutions. As amphoteric molecules, proteins are most stable at their isoelectric point: the pH value at which their net charge is zero, but that is often the same point at which they are least soluble as well. This creates a powerful dilemma for protein formulation and delivery.

Proteins are attracted to interfaces, such as where air and water meet, so they tend to cluster along the walls of their container or clump around bubbles in solution. Simply shaking a vial could destroy the efficacy of the product within. Some denaturation is reversible — if it occurs during processing. In final products, there is no going back. Lyophilization is a way to prevent this type of degradation.

The results from accelerated stability tests, in which product is stored at higher temperatures than are normal for storage and monitored for signs of denaturation and degradation, are commonly used as supporting data in applications for market authorization. However, regulators expect to see real-time testing of final products at normal storage conditions that is, several months' intermittent

sampling of before- and after-shipment material. Company management may want to rush things: "If it's stable after three months, isn't that a good indication of—?" But a formulator's answer must be, "Not necessarily." After three months, the protocol and results can be reported, but those data should be amended every three months following until a firm expiration date has been established and confirmed.

### DRUG DELIVERY OPTIONS

The easiest and most economical way to formulate a biomolecule is in solution. Most approved biotherapeutics on the world markets (with the exception of three topicals, a few oral peptides, and certain tissue products) are parenteral dosage forms intended for injection into patients intravenously, intramuscularly, intracavitarily, and so on. About half are sold in solution or suspension form. If stability can be achieved this way, it is the first choice of biotech formulators.

Solution formulations are typically refrigerated at 28 °C, with a minimum target shelf life of two years. Most biologics are unstable at or above room temperature. Although normal concentrations are 110 mg/mL, several higher-concentration formulations are currently in development, and some sustained-release "depot" formulations are already on the market.

Solution formulations are not always an option. Many biomolecules simply will not remain stable in solution long enough. Removal of water from the formulation is very often the answer. So about half of the biotherapeutics currently sold are shipped and stored in freeze-dried form — conveniently at ambient temperatures. Such products are reconstituted in a "just add water-for-injection" procedure immediately before use. Of course, biotech formulations offer no easy answers. The keys to successful lyophilization processes are precise control of time, temperature, and pressure — all of which can be determined only through characterization and testing. One recent trend is toward use of nonaqueous (organic) solvents such as

ethanol for increased sublimation rates and improved product stability.

Some formulation scientists are working on ways to improve the *in vivo* half-life of proteins once they are systemically delivered to patients, usually by the old familiar parenteral route. High-concentration, extended-release, and chemically altered products are in development. And nanotechnology may offer new options in the future. Needles and syringes — especially with the new trend toward prefilled syringe packaging — remain the most practical option for protein delivery despite many attempts to develop noninvasive systems over the years.

**Other Delivery Routes:** Despite the inherent problems of biologics when it comes to stability, solubility, and bioavailability, some companies are working toward methods of delivery outside the parenteral norm. Two oral peptide drugs and three nasal products are approved in the United States, and inhaled insulin is on the market as well.

The most problematic alternative is also the most attractive: Patients would much rather take a pill than get a shot any day. But one primary function of our digestive systems is to break down incoming proteins into usable amino acids, so getting such large molecules through that route intact is a nearly insurmountable challenge.

Transdermal delivery (e.g., "the patch") is familiar to smokers trying to quit, women on hormone replacement therapy, and people who suffer from severe motion sickness. But there have been challenges in its use for delivery of peptides, proteins, and other macromolecules that cannot easily permeate the outer skin layer. One company with a promising technology is Halozyme Therapeutics of San Diego, CA ([www.halozyme.com](http://www.halozyme.com)). It uses recombinant human PH20, an enzyme that degrades hyaluronic acid, which is the space-filling, gel-like substance that makes up a major component of skin. The enzyme temporarily breaks down that gel to facilitate penetration and diffusion of drugs and fluids injected under the skin or in the muscle.

## WHAT'S BETTER FOR PATIENTS?

In January 2008, BPI's associate editor Leah Rosin spoke with Mary Cromwell, a senior scientist in formulations at Genentech, about drug delivery options. They discussed methods of creating sustained-release and high-concentration biologic formulations as a way of lessening the number of injections patients need to receive. Here is part of their conversation.

**BPI:** What do you think is the biggest general challenge in biopharmaceutical formulation?

**Cromwell:** I think creating high-concentration of liquid formulations for subcutaneous delivery is a huge challenge. Minimizing protein aggregation because the subcutaneous route is believed to be more susceptible to creating an immune response.

Some proteins at high concentration are very viscous. So you run into issues with the physical capabilities of auto-injectors.

**BPI:** (laughing) Giant needles, that's a solution!

**Cromwell:** You can go so far, but then your patients really don't like it. And just the pain on injection with a large volume. . .

**BPI:** What other delivery method is currently showing and most promise?

**Cromwell:** I believe it really depends on your indication. I think you're going to see inhaled and transdermal being used quite frequently for local delivery. For systemic delivery, some significant challenges still haven't been overcome, and people have been working on these for decades — the Holy

Grail being the oral route.

**BPI:** Does the orally delivered insulin give you any optimism?

**Cromwell:** I think if it is achieved, it will be with a limited number of proteins. Insulin is a fairly small protein compared to something like an antibody.

**BPI:** So do you think injectibles will always be the most common delivery method?

**Cromwell:** For proteins, yes.

**BPI:** What criteria designate a product for alternative delivery options? We were talking about size and if they were local . . . ?

**Cromwell:** We look at the competitive market for the product. You also have the option of sustained release as another delivery option to reduce the number of injections you have to give.

One thing we look at whenever we receive a new project is where does this drug need to go? Is it more suitable as an injectible versus local delivery? The size — is it amenable to another delivery route? The volume that needs to be given, does that make it amenable to a different injectible route? And let's see, you would have to look at the dose you're actually achieving. Usually, that is what kills the other routes: if bioavailability is very, very low compared to what you need for efficacy. On top of that, we also look at what the competitive marketplace is for a particular drug candidate. If we have to go with some injectible, does a sustained-release type formulation make sense or a long-acting formulation to at least minimize the number of injections?

Transmucosal delivery encompasses many possible routes of administration including buccal lozenges, nasal sprays, and suppositories. But most patients would prefer even a shot to a suppository. There is little interest in the buccal idea as well, though for more similar reasons to those preventing most products from taking the oral route.

Nasal-spray vaccines such as the FluMist product from MedImmune are typically liquid-solution formulations either inhaled or swabbed into the nasal cavity. This presents a lot of mucosal surface area through which drugs can interface with the circulatory system. Bioavailability levels reported so far have been low. However, this is the most common route by which we become infected with cold and flu viruses, which may suggest a possible route for gene therapies that use viral vectors.

The most promising alternative to parenteral delivery of biomolecular drugs may be through the lungs. Their alveoli offer a very large surface area with direct access to the bloodstream. Spray-drying (a form of lyophilization) is growing in popularity for creating inhalable powders. But aerosols typically begin as liquid solution formulations that are nebulized by jet, ultrasonic

technology, or mechanical means such as liquid atomizers and ultrasonic mesh devices. The metered-dose inhalers familiar to asthmatics are not an option here because of their harsh treatment of products and difficulties with their propellants. In fact, in developing Exubera insulin for inhalation, Pfizer Inc. had to create a new delivery device along with it.

Because all these are newly evolving technologies, formulators working on such products have even more hurdles to overcome than those working on parenteral biomolecules. They can find less information in scientific literature regarding excipient use with these products and these routes of administration. Many excipients that work just fine for injectible products are too strong-smelling for inhalation, for example, or may cause unacceptable burning sensations in contact with delicate mucosal membranes. Biomolecules can denature from exposure to air-water interfaces during nebulization, and oxidation may result from mixing with air, both of which can drastically reduce the amount of drug that arrives intact.

Development of a nonparenteral formulation can also mean

development of a delivery device. This complicates the intellectual property picture for a product and adds regulatory steps as well. Manufacturing issues are raised when bulk product formulations must be stored before being loaded into dosage devices. If salt has been used as a tonicifying excipient, it precludes storage of the solution in stainless steel tanks. Frozen storage is a good idea if it doesn't cause product damage.

### IN THE ZONE

At the 2008 BIO International Convention, there is a Drug Delivery Product Focus Zone where you will find several companies involved in various aspects of research and development described herein. **Alkermes** of Cambridge, MA ([www.alkermes.com](http://www.alkermes.com), Booth #1245), has technology platforms for injectible extended-release and pulmonary delivery of both small- and macromolecules. The company has two commercially available products and a robust pipeline based on those technologies. **Bend Research Inc.** of Bend, OR ([www.bendres.com](http://www.bendres.com), Booth #1444), specializes in novel pharmaceutical delivery technologies, particularly

## DRUG DELIVERY BREAKOUT SESSIONS

Look for the following breakout sessions at the 2008 BIO International Convention in San Diego to learn more about the topics addressed in this chapter. Find more information online at [www.bio2008.org/subpage.aspx?pagename=cv08\\_attendee\\_sessions#tracks](http://www.bio2008.org/subpage.aspx?pagename=cv08_attendee_sessions#tracks).

Aiming for the Bull's-Eye:  
The Pursuit of Personalized,  
Targeted Therapeutics

What the Vector Is Happening Here?  
Myth vs. Reality in Gene Therapy

Combination Therapies:  
Best of Both Worlds



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oral solubilization using a licensed technology developed by Pfizer. And **Capsugel** is a division of Pfizer headquartered in Peapack, NJ ([www.capsugel.com](http://www.capsugel.com), Booth #1345) that creates innovative oral dosage forms for the pharmaceutical and dietary supplement industries.

**DelSite Biotechnologies, Inc.** of Irving, TX ([www.delsite.com](http://www.delsite.com), Booth #1446), uses technology based on a proprietary polysaccharide that changes from a liquid to a gel when used therapeutically to provide for controlled/sustained release of biomolecules and vaccines. It can be used to deliver drugs by injection, intranasally, and topically. In February 2008, this subsidiary of Carrington Laboratories, Inc., began making test batches of the first bird-flu vaccine intended for needle-free self-administration, a gel-based intranasal formulation.

**Hovione** is a multinational contract services company based in Portugal ([www.hovione.com](http://www.hovione.com), Booth #1349) that offers process development, regulatory affairs, and manufacturing of clinical

and commercial APIs and regulated intermediates for oral, topical, inhaled, and injectible products. One specialty is in dry-powder inhalation formulations based on accumulated expertise in micronization, lyophilization, and spray drying for optimizing crystal and particle characteristics.

Last but not least, **Inovio Biomedical Corporation** is based in San Diego, CA ([www.inovio.com](http://www.inovio.com), Booth #5245), not far from the location of BIO's 2008 International Convention itself. This company is most interested in DNA vaccines. It's a leader in developing delivery solutions based on electroporation, which uses brief, controlled electrical pulses to create temporary pores in cell membranes and enable dramatically increased cellular uptake of useful biopharmaceuticals. This technology is available to other companies for licensing.

In fact, there are many drug delivery technologies available for licensing, whether from Big Pharma players such as Pfizer or small academic research institutions. It's up to product developers to determine which will apply to their own projects — and Chapter Seven discusses how they approach the intellectual property issues involved in making such decisions. Some contract fill-finish organizations (e.g., Althea Technologies, Formatech, and Baxter Biopharma Solutions) have their own formulations and/or delivery technologies that are part of their service offerings. Many biopharmaceutical developers, in fact, outsource these highly specialized functions. Considering all the details only just touched upon here, it's not hard to imagine why. 🌐

**Cheryl Scott** is senior technical editor of BioProcess International, 1574 Coburg Road #242, Eugene, OR 97401; 1-646-957-8879; [cscott@bioprocessintl.com](mailto:cscott@bioprocessintl.com).