Formulation and Delivery

The 21st Century Brings New Challenges and Opportunities

by Larry R. Brown

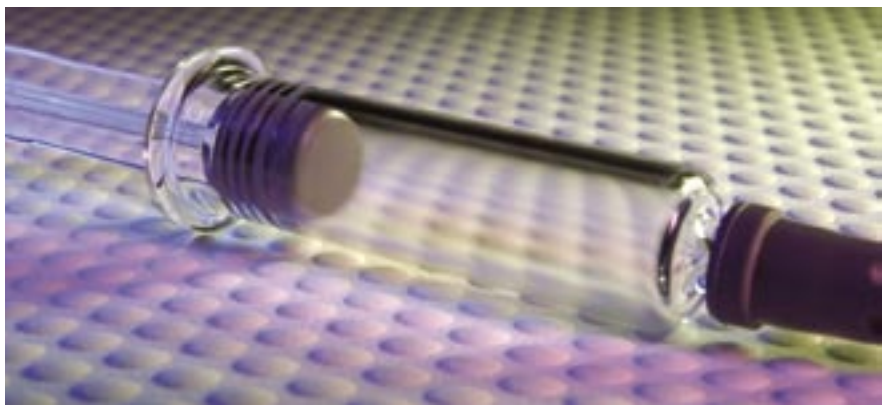
Biotechnology has created many therapeutic proteins to treat diseases that were incurable 10 years ago (Table 1). Formulating protein delivery systems so that the drugs maintain stability and remain within safe and efficacious target doses remains a challenge. Two general approaches have been used in addressing the delivery of protein therapeutics:

• One has focused on noninvasive means of transporting therapeutic proteins into patient's bodies. Two oral peptide products and three nasal products are approved in the United States. Several pulmonary insulin products are in clinical trials.

• The second technology set aimed at increasing the in vivo half-life of proteins. Several extended-release poly(lactide-co-glycolide) (PLGA) low–molecular-weight peptide products are marketed today. There are also several chemically altered protein products that have reached the market using technologies such as amino acid substitution and PEGylation.

The limited successes in protein drug delivery so far reflect the difficulty of these research and development efforts. Thus, the hypodermic needle and syringe still remains the most practical route for protein delivery despite many attempts to develop noninvasive systems or reduced-frequency administration.

Early on it was apparent that protein biopharmaceutical development was significantly more difficult than that for traditional pharmaceuticals. Molecular sizes of proteins are orders of magnitude larger than classical drugs, and their secondary and tertiary structures make them very susceptible to physical and chemical degradation. Proteins are easily denatured by heat or by agitation and often go through structural changes when exposed to water and organic solvents. So they often must be maintained at refrigerated temperatures, with stabilizing additives for long-term storage. Proteins also need sterile packaging.



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Noninvasive Administration

Oral, nasal, and pulmonary methods have been the primary noninvasive routes of protein delivery investigated so far. This field of research remains active despite evidence that the bioavailability of peptides and proteins has proven to be very low in most cases. The high cost of many such complex molecules may limit the number of protein drugs that could be economically delivered this way.

Oral Delivery: There have been numerous efforts to deliver protein molecules by the oral route. Enteric coatings and capsules can be used to protect drugs from the acidic environment of the stomach. However, avoidance of proteolytic enzymes and absorption of these relatively large molecules through a membrane designed to actively uptake only single amino acids and/or tripeptides presents many more challenges. Nobex Corporation and Emisphere Technologies have conducted interesting preclinical and clinical trials administering oral insulin using proprietary enhancer molecules. One outstanding question remains as to whether the healthcare system or the pharmaceutical industry will be able to justify the significantly added cost of oral insulin at continued low bioavailabilities.

There are already several marketed oral peptide products, but these molecules are significantly smaller than insulin, which has a molecular weight of 5,808 Da. And insulin is considered a relatively small protein. For example, desmopressin acetate (DDAVP) is a synthetic cyclic analogue of the natural pituitary hormone 8-arginine vasopressin, an antidiuretic hormone affecting renal water conservation. It is marketed by Aventis Pharmaceuticals for the treatment of diabetes insipidus,

in which patients need antidiuretic replacement therapy and primary nocturnal enuresis. DDAVP's oral bioavailability varies between 0.08% and 0.16%, but the dose ranges from 50 µg to 800 µg. That low dosage combined with the relative ease of manufacturing this relatively small peptide helps to justify the marketing of the oral dosage form, even with an oral bioavailability significantly under 1%.

Novartis and Roche Pharmaceuticals market an oral peptide drug that has some unusual properties. Cyclosporin is a small lipophilic cyclic polypeptide of 11 amino acids indicated for the prophylaxis of organ rejection in kidney, liver, and heart transplants. It is also approved for treating psoriasis and rheumatoid arthritis. Unique chemical properties give it an oral bioavailability of 30% compared with intravenous injection. The cyclic structure helps protect cyclosporin from proteolytic endopeptidases, and its lipophilic properties favor uptake from the intestinal mucosa. The oral formulation immediately forms a microemulsion in an aqueous environment.

Opportunities to succeed with the oral delivery of protein drugs are clearly limited. Only certain small peptides — with certain unique properties — can so far be delivered this way. Another kind of "oral" delivery of insulin has been the focus of Generex Biotechnology, which in early studies claims to have obtained significant insulin transport through the buccal membranes in the mouth.

Nasal Delivery: Three peptide drugs are currently marketed for systemic distribution by the nasal route. Miacalcin nasal spray from Novartis is a calcitonin analogue with a molecular weight of 3,432 Da. It is used to treat osteoporosis and has been shown to reduce the incidence of vertebral fractures by over 50% in elderly women. The bioavailability of the nasal spray is 3% compared with an injectable form, but the administered nasal dose is only 0.2 µg. Clearly the small dosage is a key reason allowing it to be marketed despite that low nasal bioavailability.

Synarel spray is the nasal form of the lipophilic luteinizing hormone releasing hormone (LHRH) agonist, nafarelin, which is marketed by Hoffmann-La Roche, Inc. for treating endometreosis. As above, it has a relatively low molecular weight (1,322 Da). Systemic bioavailability has been in the range of 1.15–5.62%, averaging 2.82 ± 1.23%. The drug is readily absorbed by nasal mucosa, and therapeutic blood levels have been rapidly achieved and maintained over time. Hydrophobic amino acids may enhance its nasal absorption.

DDAVP has also been developed in a nasal dosage form with a bioavailability about 20 times higher than by the oral route. The molecular structure and relatively low dosage permit commercialization of this nasal dosage form. There have also been experimental studies with nasal delivery of human growth hormone and insulin. All successfully delivered peptides through the nasal route are relatively low–molecular-weight moieties. Relatively low administered dosages range 0.2–400 µg. Also, chronic use of penetration enhancers to assist drug transport across the nasal mucosa does represent a potential safety concern.

Pulmonary Delivery: Research has shown that the key to obtaining effective systemic delivery through the lungs is to have a drug reach the alveoli or deep lung. To achieve this goal, it has been determined that the aerodynamic particle size must be 0.5–3 µm. Several companies — including Nektar, Alkermes, and Aradigm — have shown that aerosolised insulin can effectively be delivered to the alveolar region of the lung using a variety of devices and formulations in diabetic patients. [Editor's Note: Exubera inhalable insulin is the first such product to be approved by the US FDA. It is made by Pfizer in collaboration with Nektar Therapeutics.] The bioavailability has been estimated to be ~10% compared with subcutaneous injection.

Table 1: Top protein-based prescription drug brands by 2003 worldwide sales

Drug Name	2003 Sales ($millions)	Indication
Erythropoeitin (and analogs)	9,451	Anemia
Insulin	5,264	Diabetes
Interferons	4,709	Hepatitis C
LHRH analogs	2,533	Prostate cancer
GCSF	2,522	Neutropenia
Rituximab	2,063	Non-Hodgkins lymphoma
Infliximab	1,729	Crohn's disease, rheumatoid arthritis
Glatiramer acetate	1,418	Multiple sclerosis

GCSF = granulocyte colony stimulating factor LHRH = luteinizing hormone releasing hormone

(Sources: Leichter SB. The Business of Insulin: A Relationship Between Innovation and Economics. *Clin. Diabetes* 21, 2003: 40–42; and *Med. Ad. News*, May 2004, 23(5): 70–72.)

Fill and Finish

BioProcess International has devoted deserved attention to fill, finish, and packaging concerns. We've been especially concerned with parenteral products — for reasons made obvious in this article. Below is a sampling of some of our best articles on the topic so far.

Cicerone MT, et al. Substantially Improved Stability of Biological Agents in Dried Form: The Role of Glassy Dynamics in Preservation of BioPharmaceuticals. *BioProcess International* 1(1) 2003: 36–47.

Eakins MN. New Plastics for Old Vials. *BioProcess International* 3(6) 2005: 52–57.

Arthur JC. A Case Study in Parenteral Filling: Modular Construction Meets the Need for Speed. *BioProcess International* 3(2) 2005: S48–S51.

DeGrazio FL. Parenteral Packaging Concerns for Biotech Drugs: Compatibility Is Key. *BioProcess International* 4(2) 2006: 12–16.

Zandbergen J-E, Monge M. Disposable Technologies for Aseptic Filling: A Case Study. *BioProcess International* 4(6) 2006: S48–S51.

Smith KA. Considerations for Aseptic Filling of Parenterals: A CMO Perspective. *BioProcess International* 4(10) 2006: 12–17.

Reynolds G. The Market Need for Reconstitution Systems. *BioProcess International* 4(10) 2006: 18–21.

However, concerns have been raised about the safety of inhaled preparations and whether inhaled insulin will compromise lung capacity or damage lung tissue over long-term use. In August 2004, the European regulatory authorities determined that the Exubera product was not licensable at that time. [Editor's Note: British medical authorities would not recommend it "because it could not be proven to be more clinically or cost effective than existing treatments."] The extended path to regulatory approval of inhaled insulin clearly illustrates the hurdles that innovative companies must endure in order to bring such novel products to market.

Even so, several second-generation technologies are under development. Epic Therapeutics has developed a remarkably monodispersed, 1–3 μm formulation of insulin microspheres suitable for deep lung delivery. These microspheres are almost completely made up of insulin, and they show excellent chemical stability over time. The same technology is also serving as a testing tool for the delivery of other systemic proteins (e.g., human growth hormone and α1-antitrypsin for the treatment of chronic emphysema) through the lungs. Also, Syntonix has linked the FcRn region of an antibody to large protein molecules, apparently enabling receptor-dependent uptake. Further development of this approach may improve transport of protein drugs across epithelial cell barriers over the diffusion-based technologies.

Chemical Alterations for Better Half-Life

Certain chemical alterations of protein molecules can extend their activity or perhaps hasten their onset of action. PEGylation, glycosylation, and amino acid alterations of proteins have led to several successful marketed products.

PEGylation: Companies such as Nektar and Enzon are covalently attaching flexible strands of polyethylene glycol (PEG) to protein molecules. PEGylation generally masks a protein's surface, effectively increasing the protein's molecular size, reducing renal ultrafiltration, inhibiting antibodies or antigen processing cells, and reducing degradation by proteolytic enzymes. Thus, the protein's distribution is significantly altered. Numerous studies have showed that extended serum half-lives can be obtained by chemically adding the PEG molecule to therapeutic proteins. Table 2 shows the effect of increasing the molecular weight of PEG on the superoxide dismutase (SOD) enzyme, clearly showing that the half-life of SOD is increased 450-fold when 72,000 molecular weight PEG is compared with the native protein.

Table 2: Polyethylene glycol (PEG) molecular mass and half-life data for three different PEG chains bound to the superoxide dismutase (SOD) enzyme

Protein	Half-Life (hours)
SOD	0.08 in rats
SOD w/PEG 1,900 Da	1.50 in rats
SOD w/PEG 5,000 Da	11.00 in rats
SOD w/PEG 72,000 Da	36.00 in mice

Several approved PEGylated proteins are currently in clinical use. The first FDA-approved such product was Enzon's Adagen enzyme, used to treat severe combined immunodeficiency disease. Also from Enzon, the Oncaspar PEG-modified L-asparaginase enzyme is used as a chemotherapeutic agent for acute lymphoblastic leukaemia. PEGylated interferons are in development from Hoffmann-La Roche, Inc. and Schering-Plough. Pfizer's Somavert product is a PEGylated hGH receptor antagonist used in the treatment of acromegaly. Amgen's Neulasta product is used to decrease the incidence of infection, manifested by febrile neutropenia.

PEGylating a protein is not a simple chemical reaction. Problems encountered when conjugating a protein of interest to PEG can significantly decrease its activity. Variations in the number of PEG chains bound to a protein lead to polydispersity of the newly formed molecules. There can also be difficulties in determining the exact sites of conjugation in polypeptides.

Glycosylation: Darbepoetin-α is an erythropoiesis-stimulating protein similar to recombinant human erythropoietin, marketed by Amgen under the Aranesp trade name. It differs from human erythropoietin by the addition of two N-linked oligosaccharide chains and an increase in the number of sialic acid residues. Those result from amino acid substitutions in the peptide backbone, which do not interfere with receptor binding. But the carbohydrate chains increase the molecular weight of the glycoprotein from ~30,000 Da for human erythropoietin to 37,000 Da for darbepoetin-α. The latter has a threefold longer terminal half-life in humans than erythropoietin, leading to a decrease in frequency of administration and potentially greater patient compliance.

Amino Acid Substitutions: Recently, several novel insulin formulations have been developed that dramatically affect insulin's pharmacokinetic properties. This has been accomplished by substituting some amino acids in the primary structure of the protein without changing the molecule's biological activity. Eli Lilly has developed and marketed a fast-onset product called LysPro insulin, in which two amino acids have been reversed from native human insulin for a more soluble monomeric structure. NovoNordisk developed its insulin-aspart analogue for similarly rapid onset of action effect.

Other amino acid substitutions can achieve the opposite effect. Insulin glargine is a 24-hour long-acting recombinant insulin analogue produced by Aventis. It differs from human insulin in that one asparagine amino acid is replaced with a glycine and two arginine amino acids are added. Insulin glargine is designed to have low aqueous solubility at physiological pH. It is injected in aqueous solution at pH 4, at which time it precipitates at physiological pH and forms a slow-dissolving depot of hexameric insulin. This mimics physiological basal insulin release.

Chemical alterations of existing proteins have led to dosage forms with significantly altered pharmacokinetics

compared with their native molecules. These methods are not panaceas: PEGylation and amino acid substitutions can alter a protein's biological activity, toxicity, or bioavailability.

Needles and Syringes

Virtually all protein drugs are administered in aqueous solutions using needles and syringes. The quality of today's disposable needles is such that the pain perception is almost negligible. Insulin needles today are available in tiny 31-gauge sizes. There is no doubt that many people have a psychological aversion to hypodermic injections. However, studies with insulin and growth hormone injections to children suggest that most proteins injected in aqueous solution are in fact painless.

An Expert's Opinion

Formulating proteins such that they maintain their stability and are delivered within their efficacious and safe target doses remains a challenge. Tables 3A and 3B summarize the various technologies investigated for protein delivery. Insulin delivery will remain the focus of many researchers and companies looking to solve the protein delivery problem. This is only natural as the number of patients afflicted with diabetes in the world continues to increase. The pulmonary route for noninvasive protein delivery has received much deserved attention during recent years. Pulmonary delivery of insulin has been shown to be effective and well tolerated by patients participating in clinical trials. Reservations about untoward effects will need to be weighed against the benefits.

The limitation of polymeric drug delivery to only small peptides with wide therapeutic indices has been disappointing, but it highlights the complexity of pharmaceutical product development compared with academic proof of principle. One important lesson from the experience is that the presumed advantages of sustained-release dosing need to be critically compared with chronic but virtually painless daily injections using 30-gauge needles.

Table 3A: Alternative protein delivery technologies (noninvasive routes of administration)

Route	Applicability	Comments
Nasal	Small peptides	Low bioavailability, need for enhancers
Oral	Small peptides	Low bioavailability, limited to low-dose drugs, hepatic route may be advantageous for insulin
Pulmonary	Peptides, proteins	10% bioavailability, effective protein deliery shown, questions about chronic delivery for some drugs
Buccal	Peptides, proteins	Uncertain reproducibility and bioavailability
FcRn (oral/ pulmonary)	Large–molecular-weight proteins and peptides	Antibody-Fc region drug link enables the active receptor-dependent uptake; significant bioavailability, but an early stage technology

Table 3B: Alternative protein delivery technologies (sustained-release technologies)

Method	Applicability	Comments
Amino-acid substitutions	Peptides, proteins	Several successful examples; bioavailability can be preserved; limited to small but significant changes in half-life; selective drug applicability; potential immunogenicity, carcinogenicity
PEGylation and glycosylation	Peptides, proteins	Potential for altering bioavailability; increased biological half-life for several days; chemistry not compatible with all proteins
Biodegradable polymers	Proteins, peptides	Drug must have wide therapeutic index; loadings are generally low; protein stability issues limit potential drug candidates; difficult manufacturing
Buccal	Peptides, proteins	Uncertain reproducibility and bioavailability

The mixed successes seen thus far in altering proteins' half-lives by polymer matrix formation or by chemical changes to native molecules are likely to be dictated by the nature of the newer protein-based molecules submitted for regulatory approval. Most are monoclonal antibodies with molecular weights in the 150,000 Da range — not likely candidates for noninvasive delivery routes. They are also particularly sensitive to changes in tertiary structure, which might affect their binding to target receptors. Furthermore, the doses of these monoclonal antibodies are very high. Such doses are likely to preclude any formulation changes that significantly increase the mass of injected material that would make a drug's administration uncomfortable for patients.

Thus, polymeric delivery systems for such macromolecules are unlikely. There will be a need for novel monoclonal antibody formulation development to allow injection of such large doses. PEGylated forms of some antibodies are likely. Similarly, peptide substitutions in other recombinant proteins will probably lead to some new and interesting pharmacokinetic properties for analogues of old drugs. The small efficiency increases in noninvasive approaches will probably never be applicable to the vast majority of protein drugs. This will preserve the role of the needle and syringe for the foreseeable future.

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