5 Formulation of Biologics Including Biopharmaceutical Considerations

Daan J. A. Crommelin, Andrea Hawe, and Wim Jiskoot

INTRODUCTION

This chapter provides an introduction to the process of formulating biologics. In this formulation process, a drug *substance* (DS), also called active pharmaceutical ingredient (API), is turned into a drug *product* that can be administered to the patient. It addresses questions regarding the tests to be run, e.g., to characterize the DS and to ensure its stability, and the chotice of excipients and delivery system. It also discusses biopharmaceutical issues such as the route and rate of administration.

The text concentrates on formulating proteins used in therapy, but the same principles also apply to other biologics, such as vaccines and oligonucleotide based products as discussed in Chaps. 14 and 15 of this book.

Formulating a protein is not a one-step, routine process with fixed strategies. Several different, sometimes overlapping, phases can be recognized during the product development process, as depicted in Fig. 5.1. In the formulation development process one starts with preformulation activities and ends up –after months/years of running tests– with late-stage fine tuning of the selected, optimized product composition-dosage form. Therefore, the formulation used in the preclinical and clinical development phases may change according to the insights gained up to that moment: from 'initial formulation' to 'commercial drug product'.

POINTS TO CONSIDER IN THE PROCESS OF FORMULATING A THERAPEUTIC PROTEIN

■ Protein Structure and Protein Stability

Table 5.1 lists 'points to consider' when formulating a protein. An early and deep understanding of the structural properties of the protein at hand such as primary structure, higher-order structures, molecular weight, isoelectric point, post-translational modifications, hydrophobicity, and its physical (unfolding and aggregation) and chemical stability (cf. Table 5.2) as function of its direct environment (e.g., pH, ionic strength) will speed up the formulation process. This basic information helps to design a product that is stable not only on the shelf, but also under real-life conditions, e.g., during transportation, compounding (e.g., dilution in an intravenous infusion bag) and administration (Jiskoot et al., 2017; Nejadnik et al., 2018). Table 5.3 (adapted from Hawe et al., 2012) shows various stress factors a product can encounter.

D. J. A. Crommelin (✉)
Department of Pharmaceutical, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands
e-mail: d.j.a.crommelin@uu.nl

A. Hawe
Coriolis Pharma, Martinsried, Germany
e-mail: Andrea.Hawe@coriolis-pharma.com

W. Jiskoot
Division of BioTherapeutics, Leiden Academic Centre for Drug Research (LACDR), Leiden University, Leiden, The Netherlands
e-mail: w.jiskoot@lacdr.leidenuniv.nl

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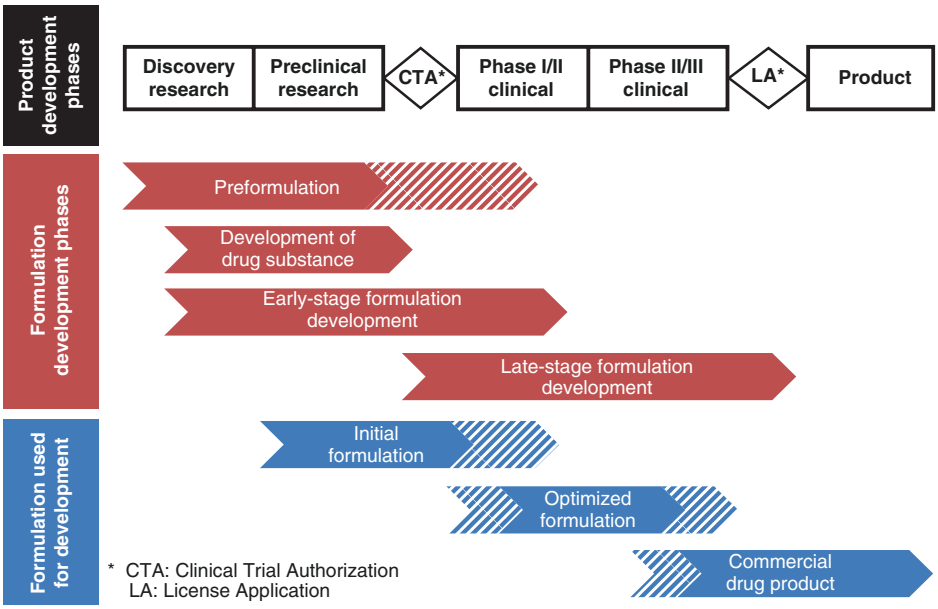

Figure 5.1 ■ Diagram of a formulation development process. Modified from Chang and Hershenson (2002)

Factor	Description/attributes/examples
API or drug substance	Type of protein, physico-chemical properties, e.g., molecular weight, pI, hydrophobicity, solubility, post-translational modifications, pegylation, physical and chemical stability and concentration, available amount, purity
Clinical factors	Patient population (e.g., age and concomitant medication), self-administration versus administration by professional, compatibility with infusion solution, indication (e.g., one-time application or chronical application)
Route of administration	Subcutaneous, intravenous injection or infusion, intramuscular, intravitreal, intra-articular, intradermal, pulmonal
Dosage form	Single- or multi-dose, prefilled syringe, dual chamber cartridge, pen cartridge; liquid, lyophilizate, frozen liquid, API concentration, injection volume, injection rate, controlled delivery/release
Primary packaging material	Glass, polymers, rubber, silicone oil, metals, leachables (anti-oxidants, plasticizers, etc.)
Excipients	Pharmaceutical quality, safety record (for intended administration route and dose), manufacturer, tested for critical impurities, stability
Analytical methods	Characterization of API, stability-indicating assays, quality control assays

Adapted from Weinbuch et al. (2018)
API active pharmaceutical ingredient

Table 5.1 ■ Points for consideration in the formulation process of pharmaceutical proteins

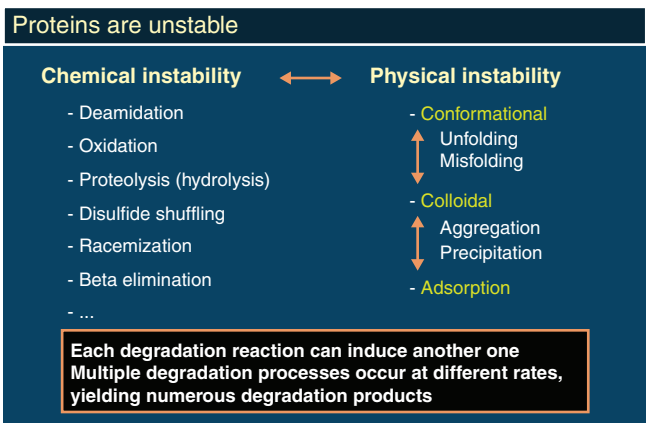

Table 5.2 ■ Chemical and physical reactions jeopardizing protein stability (cf. Table 3.1)

Stress factor	When encountered/examples
Elevated temperature, temperature excursions	Production (upstream and downstream processing); improper shipment; storage or handling deviations
Freezing, freeze-thawing	Storage of frozen (bulk) material; accidental freezing during storage or shipment; lyophilization
Mechanical stress	Production (e.g., pumping, stirring, filtration)
Light	Production; shipment; storage; handling
Oxidative stress	Production (exposure to oxygen); exposure to peroxide or metal ion impurities in excipients; shipment (cavitation)
pH changes	Production (downstream processing); freezing; formulation; dilution in infusion liquids; administration
Interfaces	Air-water interface; filters; primary packaging material; infusion bags and administration lines; particulate impurities
X-ray	Air freight transportation

Adapted from Hawe et al. (2012)

Table 5.3 ■ Stress factors a therapeutic protein may encounter

Box 1: Orthogonal vs Complementary Analytical Techniques

Orthogonal analytical techniques are combinations of techniques that monitor the same (similar) properties of a protein (in its formulation) with a different measurement principle (cf. Table 5.4).

For example,

- Size-exclusion chromatography (SEC), asymmetrical flow field flow fractionation (AF4) and analytical ultracentrifugation (AUC)
- Near-UV circular dichroism (CD) and intrinsic fluorescence spectroscopy
- Far-UV circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopy
- Light obscuration (LO), flow-Imaging microscopy and electric zone sensing

Complementary analytical techniques are techniques that measure different properties of a protein (in its formulation) with a different measurement principle (cf. Table 5.4).

For example when monitoring aggregation of the protein,

- Size-exclusion chromatography (SEC) for oligomers and dynamic light scattering for nanometer size aggregates
- Analytical ultracentrifugation (AUC) for oligomers and nanoparticle tracking analysis (NTA) for nanometer size aggregates,
- Light obscuration or flow imaging microscopy for micrometer size aggregates, visual inspection for larger, visible particles
- Techniques for sizing (see above) and methods for conformational analysis (CD, FTIR, fluorescence spectroscopy)
- Techniques for size (e.g., SEC), charge (e.g., ion-exchange chromatography) and hydrophobicity (RP-HPLC)

■ Analytical Toolbox

The need for an 'analytical toolbox' with stability-indicating orthogonal and complementary analytical techniques (see Box 1) to characterize a protein in various stages of formulation development in as much detail as possible is evident. Table 5.4 (adapted from Hawe et al., 2012) lists selected analytical methodologies that are being used to monitor protein stability.

These analytical techniques will provide the necessary data and guide the formulator through the subsequent stages of the development process ending up with the marketed drug product. More information about protein (in)stability and analytical methodology can be found in Chap. 3 of this book and in articles/books by Manning et al. (1989, 2010), Jiskoot and Crommelin (2005); Kamerzell et al. (2011); Zölls et al. (2012); Hawe et al. (2012); Weinbuch et al. (2018).

■ Physical and Chemical Stability

Already in an early stage, data on physical stability (colloidal and conformational stability) and on chemical stability of the API and formulations are collected. Monitoring and controlling aggregate formation is particularly important, because protein aggregates are

Type of degradation product	Examples of analytical techniques
Soluble aggregates (dimers, trimers, oligomers) and fragments	Size-exclusion HPLC/UPLC, AF4, analytical ultracentrifugation, SDS-PAGE, CE-SDS
Nanometer-sized aggregates	Dynamic light scattering; nanoparticle tracking analysis; AF4; Taylor dispersion analysis; turbidimetry/nephelometry; static light scattering
Micrometer-sized aggregates	Light obscuration; light microscopy; flow imaging microscopy; coulter counter; fluorescence microscopy; turbidimetry/nephelometry; Raman microscopy
Visible particles	Visual inspection; (semi-)automated visual inspection
Conformational changes	Circular dichroism, infrared, intrinsic fluorescence, extrinsic fluorescence spectroscopy and secondary-derivative UV spectroscopy
Chemical changes	Reversed-phase HPLC/UPLC; (HPLC-)mass spectrometry; ion-exchange chromatography; (capillary) isoelectric focusing

AF4 asymmetrical flow field-flow fractionation, *CE-SDS* capillary electrophoresis sodium dodecyl sulfate, *HPLC* high performance liquid chromatography, *UPLC* ultra performance liquid chromatography
Adapted from Hawe et al. (2012)

Table 5.4 ■ Examples of protein degradation products and techniques to analyze them

Type of stress	Examples of stress conditions	Anticipated instability types
Temperature	Real time (2–8 °C; up to several years) Accelerated (e.g., 25 °C, 40 °C, up to several months)	Aggregation, conformational changes, chemical changes
Mechanical	Shaking (50–500 rpm, hours-days) Stirring, 50–500 rpm, hours-days) Freeze-thawing, (1–5 cycles, from 25 °C to –20 °C or –80 °C)	Aggregation, adsorption, conformational changes
Oxidation	H_2O_2 (1–5%, 1–2 days)	Chemical changes, aggregation, conformational changes
Humidity*	0–100% relative humidity	Aggregation, conformational changes, chemical changes

* Specifically for lyophilized products. Adapted from Weinbuch et al. (2018)

Table 5.5 ■ Examples of accelerated stability and forced degradation studies

readily formed under a variety of conditions and have been associated with enhanced risk of immunogenicity of therapeutic proteins (cf. Chap. 7). It should be emphasized that aggregation of proteins can happen at concentrations much below their solubility and at temperatures far below their denaturation temperature. Proteins in solution have an increased tendency to aggregate upon mechanical stress and interaction with interfaces. Therefore, protein stability should not only be studied under quiescent conditions, but tests should be performed on protein stability in tubes, pipes, columns and pumps, during handling and the fill-finish process to ensure stability during manufacturing.

For shelf life assessment of the drug product typically 'real time'data at 2–8 °C is collected in combination with data from forced degradation studies. These include collecting stability information under stress conditions such as exposure to elevated temperatures e.g., 25 °C and 40 °C, to mechanical stress (cavitation, shear, interfacial effects) or to light. This information is used to assess the overall robustness of a formulation during manufacturing. The ICH (International Conference on Harmonization) guideline Q5C provides global information about accelerated stability testing of biological products but does not outline exact conditions for forced degradation studies, except light stress. Table 5.5 lists a typical set of stress test conditions that are used in practice. With respect to temperature stress, one should realize that protein degradation typically does not follow Arrhenius kinetics; this is due to the complexity of the different degradation reactions that may run parallel to each other (Manning et al., 2010). Therefore, accelerated degradation studies at elevated temperatures can never replace real-time experiments (Hawe et al., 2012). Typically, a shelf life of at least 18–24 months for the drug product in its final primary packaging container (e.g., vial, syringe, cartridge pen, autoinjector) is desired. Various strategies are available to increase a protein's shelf life to or beyond the preferred 24 months range. These follow later in this chapter.

Primary Packaging

The vast majority of therapeutic proteins is parenterally administered by injection. Various primary packaging materials are available to the formulator, such as vials, cartridges and syringes (see Fig. 5.2 and Sacha et al. 2010; Sacha et al. 2015). The choice of the primary packaging material depends on a number of factors. For chronic therapy, the subcutaneous route of administration is often preferred as the patient can self-administer the drug. Convenience of use then excludes vial containers. One can choose among pen injectors and prefilled syringes (cf. Fig. 5.2). Pen injectors are cartridge-based syringes for multidose administration. They are typically used when frequent subcutaneous injections of variable doses of the drug are required, such as with insulin. The patient has to insert the needle her/himself. Prefilled syringes for subcutaneous administration are gaining increased popularity, especially in combination with an autoinjector to facilitate controlled and reproducible self-administration.

Typically, vials and the barrel of pre-filled syringes consist of glass. Glass has the advantage of transparency, which allows visual inspection of the injected solution. Pre-filled glass syringes are coated with silicone oil that acts as a lubricant to help moving the plunger. Fully polymer-based syringes start to offer an alternative option. A disadvantage of pre-filled glass syringes is that a small fraction of the silicone oil coating can be released in the solution in the form of (subvisible) oil droplets. Proteins can adsorb to these silicone oil droplets, which potentially leads to aggregation. Adding non-ionic surfactants to the formulation can prevent protein adsorption and aggregation. Furthermore, in the manufacturing process of glass syringes tungsten, which has been associated with protein aggregate formation as well, may end up in the product.

On storage of glass vials and syringes, the glass surface can release (heavy) metal ions; organic compounds may leach out of the polymer-based materials as used in vial stoppers, syringe plungers and barrels of polymer-based syringes. The formulator should collect information on these leachables and take proper action when necessary, such as changing packaging material or its vendor, add coatings or adjust formulation characteristics such as the pH.

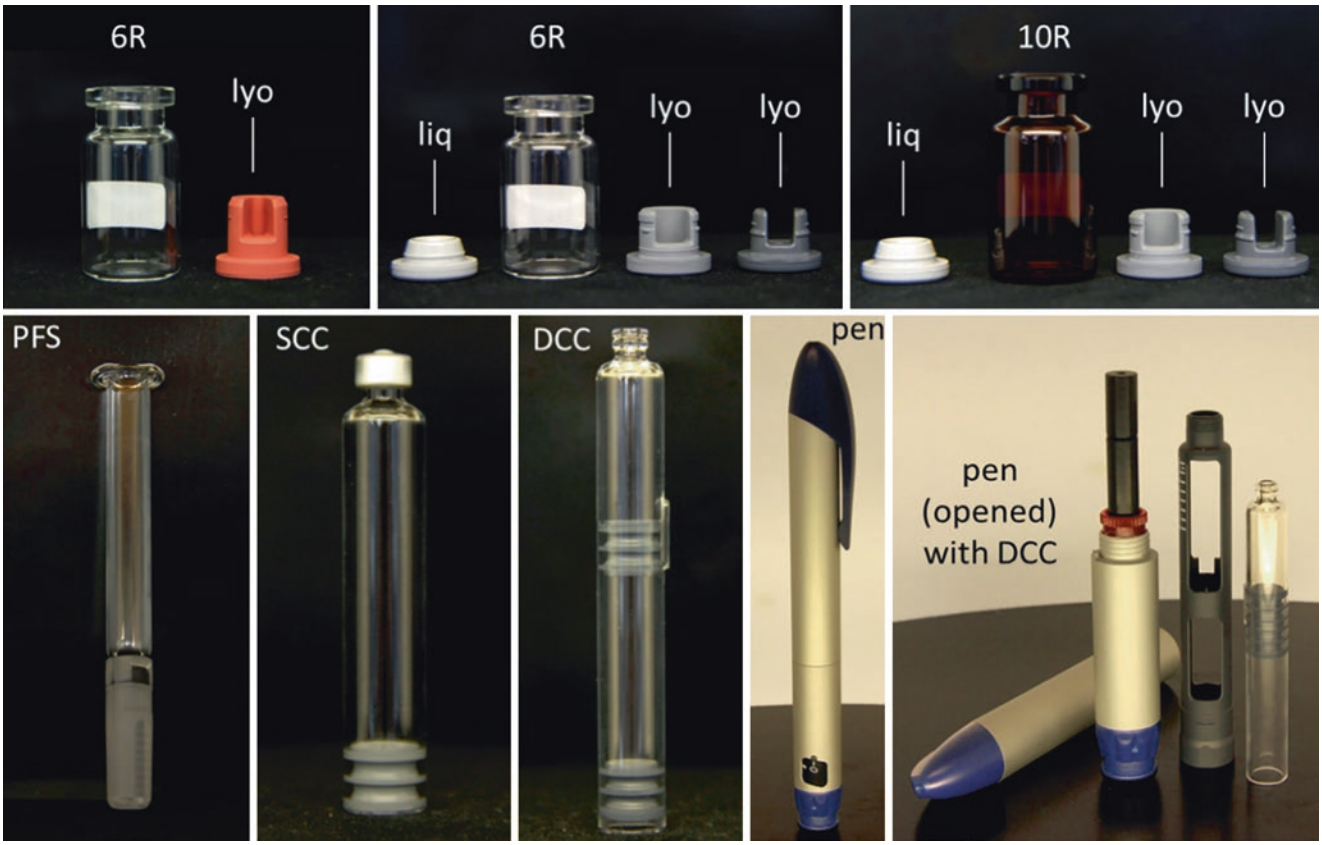

Figure 5.2 ■ Examples of primary packaging materials and a pen device (pen): 6-ml (6R) vials and 10-ml (10R) vial with corresponding stoppers for liquid (liq) and lyophilized (lyo) formulations; (empty) pre-filled syringe (PFS), single-chamber cartridge (SCC) and dual-chamber cartridge (DCC). Scaling of the pictures is not identical. Photos taken by Matthias Wurm

Higher gauge (G) –i.e., thinner– needles are preferred by patients as the pain sensation during injection decreases with a decreasing needle diameter. However, these narrow needles will negatively affect the 'syringeability': the force required to inject a certain volume at a certain rate through a needle with a certain length. The narrower the needle, the more force is needed. The same occurs when increasing the viscosity of the protein solution as is seen with highly (>50 mg/ml) concentrated monoclonal antibody solutions for subcutaneous injection.

■ Formulation Development of Marketed Products

A marketed drug product may undergo changes in its formulation and the way it is used. For instance, the company may introduce a new type of primary packaging material, a change in route of administration, a change in master cell bank, or new column material in the purification process. Such changes may affect protein structure and its stability profile and thereby the safety and efficacy profile of the product. Chapter 7 (immunogenicity) mentions an example of a formulation change of an epoetin product that caused a dramatic increase in the incidence of anti-drug antibody induced pure red cell aplasia, a serious adverse effect. Dependent on the proposed change, regulatory bodies may request new data to exclude changes in structure and shelf life of the new product and ensure that protein efficacy and safety have not changed. These comparability studies may include clinical studies, if analytical, preclinical and pharmacokinetic studies are considered insufficient to prove the claim of 'comparability' (cf. Chap. 12, biosimilars).

■ Excipients

In a protein formulation one finds, apart from the API, a number of excipients that are selected to serve different purposes. This selection process should be carried out with great care to ensure therapeutically effective and safe products. The nature of the protein (e.g., stability) and its therapeutic use (e.g., multiple injection systems for chronic use) can make these formulations complex in terms of excipient profile and manufacturing (freeze-drying, aseptic preparation). Table 5.1 mentions clinical factors, route of administration and dosage form as points to consider when designing the formulation. For example, the choice of an intravenously administered product (hospital setting) versus a subcutaneously administered product (self-administration) impacts the selection of excipients.

Both the choice of the excipient and its concentration are important. For instance, low concentrations of polysorbates may stabilize the protein (see below), while higher concentrations may cause denaturation. On the other hand, too low concentrations of polysorbates may result in particle formation during storage caused by polysorbate degradation when the cleaved fatty acids (from the polysorbate) are no longer solubilized by the remaining polysorbate; moreover, polysorbate degradation may lead to a surfactant concentration that is insufficient to stabilize the protein.

Table 5.6 lists components commonly found in presently marketed formulations. Clearly, an excipient can have different functions. For instance, sugars may be added for achieving isotonicity, as conformation stabilizer in liquid products, and as bulking agent and lyoprotectant in freeze-dried products. Kamerzell et al. (2011) discuss in detail the role of different classes of excipients in protein formulations and their mechanism of action.

In the following sections the reasons for including excipients from this list to protein products are discussed in more detail.

Solubility Enhancement

Approaches to enhance protein solubility include the selection of the proper pH (see below) and ionic strength conditions. Addition of amino acids, such as

Excipient class	Function	Examples
Buffers	pH control, tonicity	Histidine, phosphate, acetate, citrate, succinate
Salts	Tonicity, stabilization, viscosity reduction	Sodium chloride
Sugars[a], polyols	Tonicity, stabilization, cryoprotection, lyoprotection[b], bulking agent[b], reconstitution improvement[b]	Sucrose, trehalose, mannitol, sorbitol
Surfactants	Adsorption prevention, solubilization, stabilization, reconstitution improvement[b]	Polysorbate 20, polysorbate 80, poloxamer 188
Amino acids	Stabilization, viscosity reduction, tonicity, pH control, bulking agent[b]	Arginine, glycine, histidine, lysine, proline
Anti-oxidants	Oxidation prevention	Methionine, sodium edetate
Preservatives[c]	Bacterial growth prevention	m-cresol, benzyl alcohol, phenol

Adapted from Weinbuch et al. (2018)
[a]Only non-reducing sugars
[b]For freeze-dried products
[c]Multi-dose containers

Table 5.6 ■ Common excipients in protein drug products

arginine, or surfactants can also help to increase the solubility. The mechanism of action of these solubility enhancers depends on the type of enhancer and the protein involved and is not always fully understood. As an example, Fig. 5.3 shows the dramatic effect of the arginine concentration on the apparent solubility of tissue plasminogen activator (alteplase).

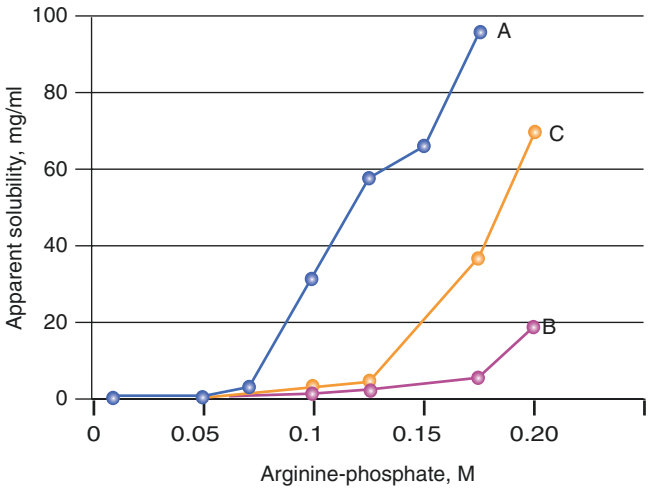

Figure 5.3 ■ Effect of arginine concentration on the apparent solubility of (A) type I and (B) type II alteplase, and (C) a 50:50 mixture thereof at pH 7.2 and 25 °C (From Nguyen and Ward 1993)

Protection Against Adsorption, Interfacial Stress and Aggregation in the Bulk Solution

Most proteins are prone to adsorb to interfaces. Upon adsorption, proteins tend to expose hydrophobic sites, normally present in the core of the native protein structure when an interface is present. Examples of interfaces are the formulation liquid–air interface, the liquid–container wall interface, or interfaces formed between the liquid and utensils used to administer the drug (e.g., infusion bags and lines, syringes, needles). Importantly, adsorbed, partially unfolded protein molecules not only present a loss of API but also may form aggregates, leave the surface, return to the aqueous phase, and form larger aggregates. Figure 5.4 (adapted from Sediq et al. 2016) shows a schematic representation of this mechanism of aggregation at a solid surface. A similar situation may occur at gas-liquid interfaces. For some proteins the reconstitution protocol for the freeze-dried cake explicitly stipulates to swirl the vial instead of shaking it to avoid protein exposure to large liquid-air interfaces.

Many protein formulations include a surfactant to reduce protein adsorption. Surfactants readily adsorb to hydrophobic interfaces with their own hydrophobic groups and render this interface hydrophilic by exposing their hydrophilic groups to the aqueous phase. Protein accumulation at the interface is suppressed and thereby aggregate formation. The most commonly used surfactants for parenteral use are poly-

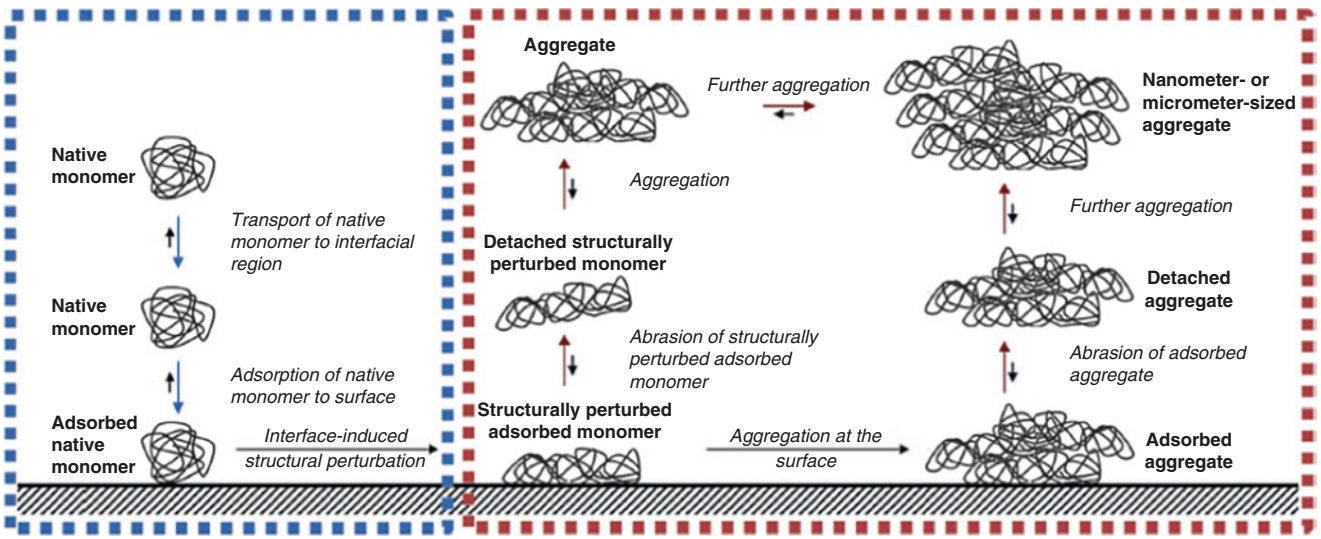

Figure 5.4 ■ Schematic representation of the suggested mechanism of stirring-induced protein aggregation. The left part (framed in blue) depicts the process of protein adsorption onto solid surfaces with potential perturbation of the native structure of the protein on adsorption. This process is followed by aggregation at the surface and in the bulk (framed in red). Contact sliding results in abrasion of the adsorbed protein layer, leading to renewal of the surface for adsorption of a fresh protein layer. Addition of surfactants, such as polysorbate 20, and avoidance of contact stirring will inhibit the steps shown as blue and red arrows, respectively. Adapted from Sediq et al. (2016)

sorbate 20 and 80. Poloxamer 188 is also used and is gaining importance because of issues with polysorbate degradation (Martos et al., 2017). Furthermore, 2-hydroxypropyl-beta-cyclodextrin can prevent adsorption and is accepted as an excipient for parenteral use as well. Human serum albumin prevents adsorption, but is nowadays rarely used because of potential infectious content and interference with analytical characterization of the API.

Apart from interface-induced aggregation, aggregates may be formed in the bulk of a solution because of colloidal and/or conformational instability (Chi et al. 2003). Sugars, selection of a proper pH value and buffer components may mitigate the tendency to this bulk aggregation.

Glucose may perfectly act as a conformational stabilizer, but will induce chemical instability through the Maillard reaction. Primary amino groups of the proteins react with the reducing sugar, resulting in brownish/yellow solutions. Sucrose should not be used below pH 6 because of hydrolysis, leading to formation of fructose and glucose, both being reducing sugars. Polyols such as mannitol and sorbitol may be used as well, also at low pH.

These excipients (sugars and polyhydric alcohols) may not be inert; they may influence protein stability. For example, sugars and polyhydric alcohols can stabilize the protein structure through the principle of "preferential exclusion" (Arakawa et al. 1991). They enhance the interaction of the solvent with the protein and are themselves excluded from the protein surface layer; the protein is preferentially hydrated. This results in an increased conformational stability of the protein.

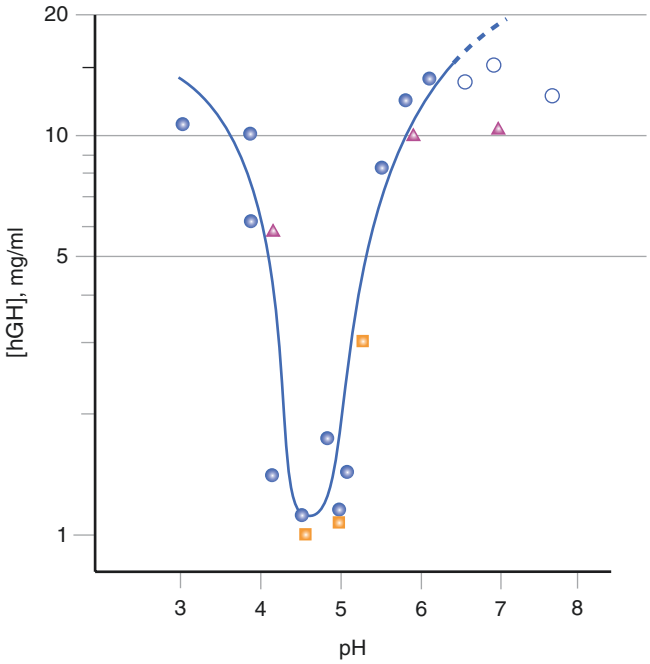

Figure 5.5 ■ A plot of the solubility of various forms of hGH as a function of pH. Samples of hGH were either recombinant hGH (circles), Met-hGH (triangles), or pituitary hGH (squares). Solubility was determined by dialyzing an approximately 11 mg/ml solution of each protein into an appropriate buffer for each pH. Buffers were citrate, pH 3–7, and borate, pH 8–9, all at 10 mM buffer concentrations. Concentrations of hGH were measured by UV absorbance as well as by RP-HPLC, relative to an external standard. The closed symbols indicate that precipitate was present in the dialysis tube after equilibration, whereas open symbols mean that no solid material was present, and thus the solubility is at least this amount (From Pearlman and Bewley 1993)

Buffer Selection

Buffer selection is an important part of the formulation process, because of the pH dependence of protein solubility, as illustrated in Fig. 5.5. Moreover, both the pH and the buffer species itself can have profound effects on the physical (aggregation) and chemical stability of proteins (Zbacnik et al. 2017) (cf. Fig. 5.6). Buffer systems regularly encountered in protein formulations are phosphate, citrate, histidine, succinate, glutamate and acetate. Highly concentrated protein solutions (protein concentration >50 mg/ml) may not need a buffer as they have sufficient intrinsic buffer capacity (Bahrenburg et al., 2015). Even short, temporary pH changes can cause protein aggregation. These conditions can occur, for example, during elution of a monoclonal antibody from a protein A column at low pH (see Chap. 4) or during the freezing step in a freeze-drying process, when one of the buffer components is crystallizing and the other is not. For instance, in a sodium phosphate buffer, Na_2HPO_4 crystallizes faster than NaH_2PO_4. This causes a pronounced drop in pH during the freezing step (see below). In the presence of high concentrations of a sugar, which is typically added as lyo- and cryoprotectant during lyophilization, the effect of pH changes is less pronounced. Other buffer components do not crystallize but form amorphous systems, and then pH changes are negligible.

Protection Against Oxidation

Methionine, cysteine, tryptophan, tyrosine, and histidine are amino acid residues that are readily oxidized (see Chap. 3). As these amino acid residues occur in almost all proteins, oxidative degradation is a regular threat to the stability of proteins. The sensitivity of an amino acid residue towards oxidation depends on its position within the protein, as this determines its accessibility for oxidative reagents. Replacement of oxygen by inert gases (e.g., argon) in the vials or minimizing the headspace, such as in pre-filled syringes, helps reducing oxidative stress. Moreover, one may consider the addition of antioxidants, such as methionine, which competes with methionine residues for oxidation. Interestingly, some antioxidants can accelerate protein

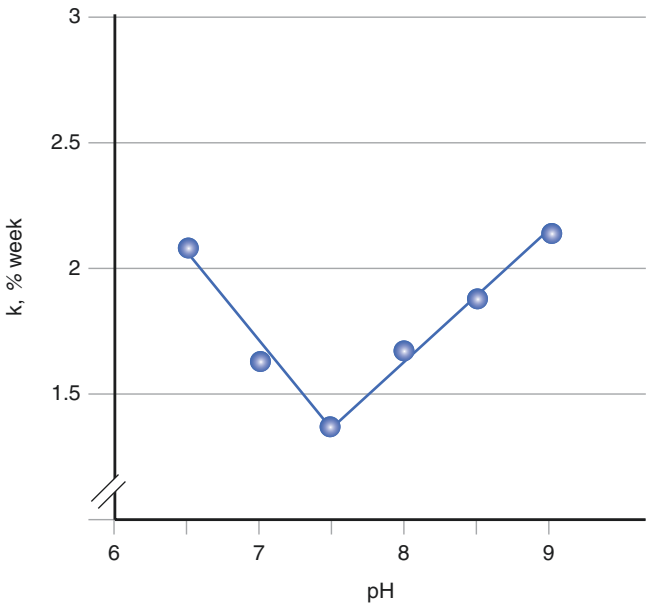

Figure 5.6 ■ pH stability profile (at 25 °C) of monomeric recombinant α1-antitrypsin (rAAT) by size exclusion-HPLC assay, k degradation rate constant. Monomeric rAAT decreased rapidly in concentration both under acidic and basic conditions. Optimal stability occurred at pH 7.5 (Adjusted from Vemuri et al. 1993)

oxidation (Vemuri et al. 1993). Ascorbic acid, for example, can act as an oxidant in the presence of trace amounts of heavy metals which may be present as impurities. To reduce the catalytic activity of heavy metals, one may consider introducing chelators such as EDTA (ethylenediaminetetraacetic acid) (Kamerzell et al. 2011).

Preservation

Proteins may be marketed in containers designed for multiple injections. After administering the first dose, contamination with microorganisms may occur. Therefore, formulations in multi-dose containers must contain a preservative. Common antimicrobial agents include phenol, meta-cresol, benzyl alcohol, and chlorobutanol (Kamerzell et al., 2011). These preservative molecules can interact with the protein, which may compromise both the activity of the protein and the effectivity of the preservative. An example is the well-established interaction between insulin and phenols (Chap. 18). A caveat is the incompatibility of polysorbates and m-cresol, which may lead to precipitation.

Tonicity Adjustment

For proteins the regular rules apply for adjusting the tonicity of parenteral products. Formulation excipients, such as buffers and amino acids, contribute to the tonicity. Disaccharides, polyols and sodium chloride are commonly added to reach isotonicity.

Protection Against Freezing and Drying

Cryoprotectants are excipients that protect a protein during freezing or in the frozen state (mainly sugars: sucrose, trehalose and sugar alcohols: mannitol, sorbitol). This is relevant for the development of frozen liquid formulations. The key stabilizing mechanisms is 'preferential exclusion'. As explained above, these additives ('water structure promoters') enhance the interaction of the solvent (water) with the protein and are themselves excluded from the protein surface layer; the protein is preferentially hydrated.

Lyoprotectants protect the protein in the lyophilized state (e.g., sugars). The key mechanisms of protection described for lyoprotectants are (cf. Mensink et al. 2017): (1) the 'water replacement theory': replacement of water as stabilizing agent by forming hydrogen bonds with the protein and (2) the 'vitrification theory': formation of a glassy amorphous matrix keeping protein molecules separated from each other.

When stored in the presence of reducing sugars, such as glucose and lactose, the Maillard reaction (see above) may occur also in the dried state and the cake color turns yellow brown. Therefore, reducing sugars should not be used as lyoprotectant and non-reducing sugars such as sucrose (above pH 6) or trehalose are preferred.

■ Freeze-Drying of Proteins

The abundant presence of water in liquid protein formulations promotes chemical and physical degradation processes. This explains why proteins in solution often do not meet the preferred stability requirements for industrially produced pharmaceutical products (shelf life >2 years), even when kept permanently under refrigerator conditions (cold chain).

Freeze-drying may provide the required stability (Constantino and Pikal 2004). During freeze-drying water is removed through sublimation. The freeze-drying process consists of three steps: (1) freezing (if required, this includes an annealing step), (2) primary drying, and (3) secondary drying. Figure 5.7 shows what happens with chamber pressure and temperature over time during these stages.

Although aimed to improve protein stability, freeze-drying may cause irreversible damage to the protein. This is particularly true when applying improper lyophilization process conditions (see below) and/or selecting improper excipients (see above). Table 5.6 lists excipients typically encountered in successfully freeze-dried protein products.

Freezing

In the freezing step (see Fig. 5.7) the temperature of the solution (typically in vials) is lowered. Ice crystal formation does not start right at the thermodynamic

or equilibrium freezing point, but supercooling occurs. That means that ice crystallization often only occurs when reaching temperatures of −15 °C or lower. During ice crystallization the temperature temporarily rises in the vial, because of the generation of crystallization heat. During the cooling stage, concentration of the protein and excipients occurs because of the growing ice crystal mass at the expense of the liquid aqueous phase. This can cause precipitation of one or more of the excipients, which may result in pH shifts (see above and Fig. 5.8) and ionic strength changes. It may also induce protein denaturation. Cooling of the vials is done through lowering the shelf temperature. Selecting the proper cooling scheme for the shelf –and consequently the vials– is important, as it dictates the degree of supercooling and ice crystal size. Small crystals form during fast cooling; large crystals form at lower cooling rates. Small ice crystals are required for porous cakes and fast sublimation rates (Pikal 1990).

Ice nucleation is a random and stochastic approach, which may lead to inhomogeneity in pore size and cake structure. Controlled nucleation can be applied to assure freezing at a defined temperature. This results in reduced primary drying times and shorter reconstitution times of highly concentrated lyophilized protein formulations (Geidobler and Winter 2013).

When choosing the freezing temperature it is important to assure that the product is fully frozen. Crystallizing compounds (e.g. NaCl, mannitol) need to be cooled below the eutectic temperature (Te) and compounds forming amorphous structures (e.g. sugars) below the glass transition temperature of the maximally freeze-concentrated solution (Tg'). In the amorphous phase the viscosity changes dramatically in the

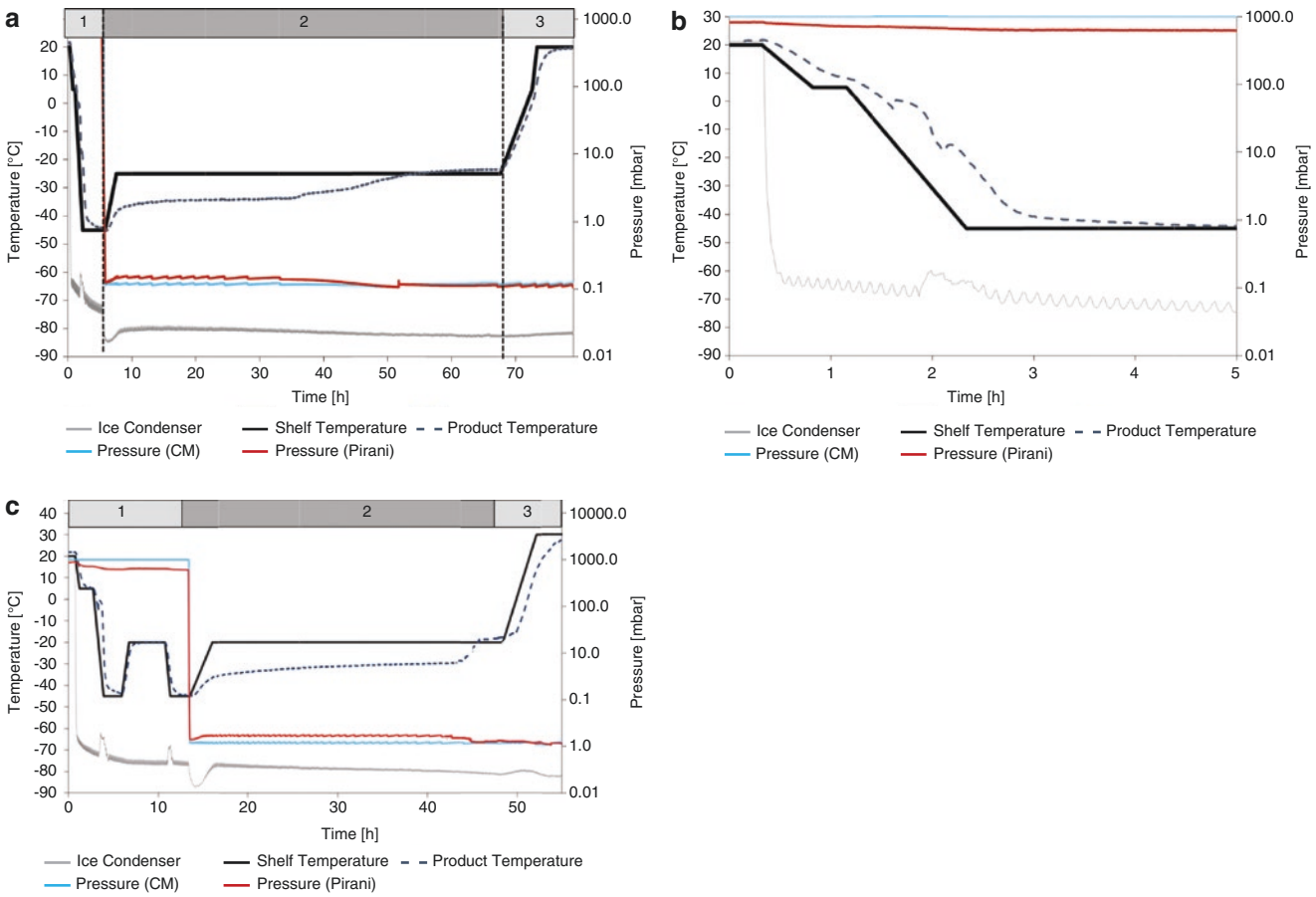

Figure 5.7 ■ **(a)** Example of freeze-drying showing shelf temperature, product temperature, pressure (by Pirani and CM and ice condenser temperature. 1 = freezing stage, 2 = primary drying stage, 3 = secondary drying stage. See the text for explanation of CM and Pirani measurement. **(b)** Zoom in on the freezing stage. **(c)** Similar to **(a)**, but now with annealing step in the freezing stage (temperature rise form −45 °C tot −20 °C and drop again to −45 °C)

temperature range around Tg′: A "rubbery" state exists above and a glass state below the Tg′.

Lyophilized formulations can be either amorphous (e.g. sugar-based) or (partially) crystalline (e.g. mannitol-based). To assure that bulking agents such as mannitol crystallize, an annealing step can be included to promote crystallization during freezing: The frozen mass is then kept at a temperature above the Tg′ for some time and then refrozen, before moving towards primary drying. At the start of the primary drying stage, no "free and fluid" water should be present in the vials. Minus forty to minus fifty degrees Celsius is a typical freezing temperature range where sublimation is initiated through chamber pressure reduction.

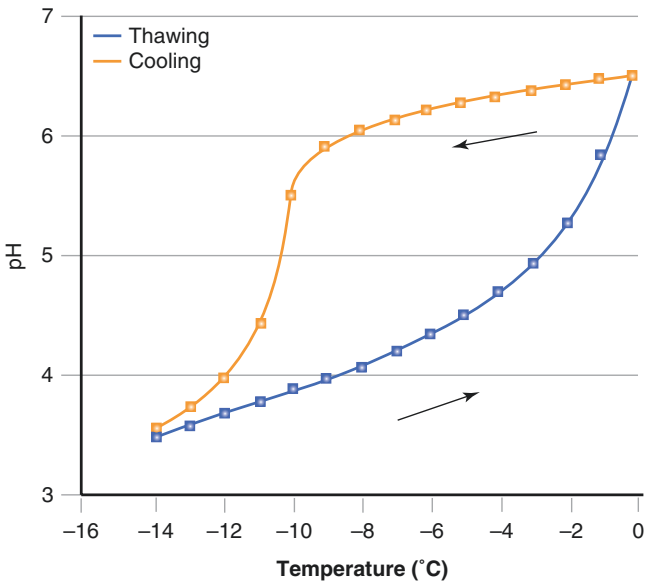

Figure 5.8 ■ The effect of freezing-thawing on the pH of a citric acid–disodium phosphate buffer system (Cited in Pikal 1990)

Primary Drying

In the primary drying stage (see Fig. 5.7), sublimation of the water mass in the vial starts by lowering the pressure. The water vapor condenses on a condenser, with a (substantially) lower temperature than the shelf with the vials (typically −80 °C). Sublimation costs energy (about 2500 kJ/g ice). The supply of heat from the shelf to the vial prevents the vial temperature to drop. Thus, the shelf is heated during this stage.

The pressure (vacuum) and the heat supply rate control the resulting product temperature. It is important to keep the product temperature below the Tg′ (e.g., determined by differential scanning calorimetry (DSC)) or the collapse temperature (Tc) (e.g., determined by freeze-drying microscopy). At and above the collapse temperature the material softens and cannot support its own structure anymore (see below). Collapse causes a strong reduction in sublimation rate and poor cake formation, resulting in non-elegant cake appearance and long reconstitution times. Although cake appearance is negatively affected, collapse may result in products with acceptable protein stability (Schersch et al. 2010). An example of a DSC scan providing information on the Tg′ is presented in Fig. 5.9.

Heat reaches the vial through (1) direct shelf–vial contact (conductance), (2) radiation, and (3) gas conduction (Fig. 5.10). Gas conduction depends on the pressure: if one selects relatively high gas pressures, heat transport increases because of a high conductivity. However, it reduces mass transfer, because of a low driving force: the pressure between equilibrium vapor

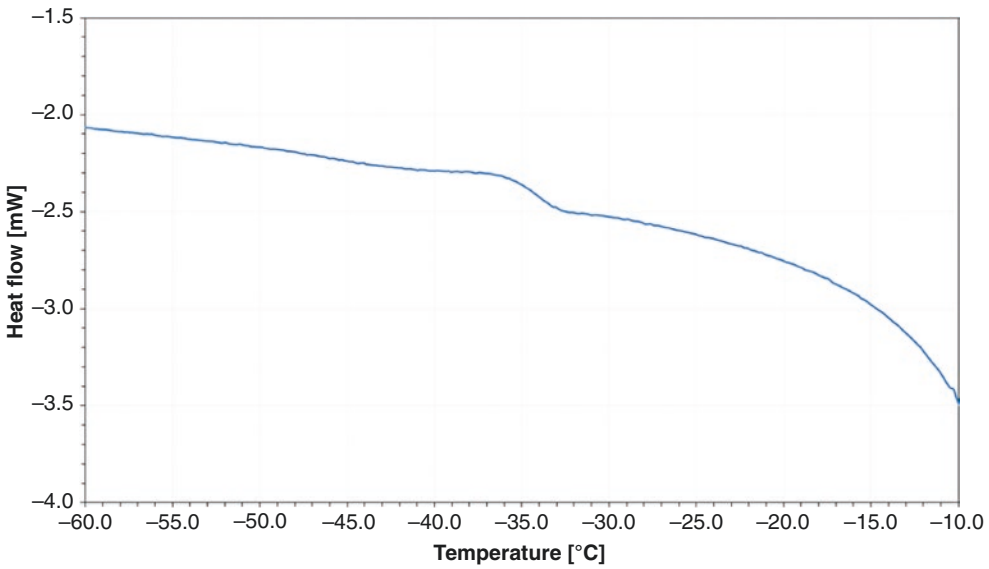

Figure 5.9 ■ Differential Scanning Calorimetry trace of a 5% sucrose solution with a Tg about −35 °C

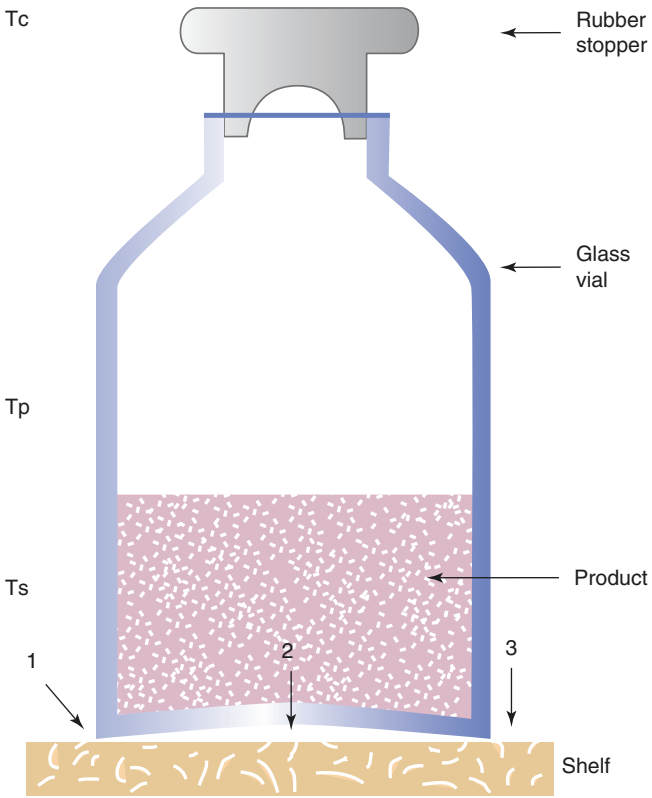

Figure 5.10 ■ Heat transfer mechanisms during the freeze-drying process: (1) Direct conduction via shelf and glass at points of actual contact. (2) Gas conduction: contribution heat transfer via conduction through gas between shelf and vial bottom. (3) Radiation heat transfer. Ts shelf temperature, Tp temperature sublimating product, Tc temperature condensor. Ts > Tp > Tc

pressures at the interface between the frozen mass/dried cake and the chamber pressure (Pikal 1990). During the primary drying stage, one transfers heat from the shelf through the vial bottom and the frozen mass to the interface frozen mass/dry powder, to keep the sublimation process going.

During this drying stage the product temperature should never reach Te or Tg′/Tc, as the cake may collapse. Typically a safety margin of 2–5 °C is used. For highly concentrated protein formulations Tc is typically higher than Tg′, and the products can be dried at product temperatures above Tg′ (below Tc) without resulting in collapse. Therefore, knowledge of Te and Tg′ is of great importance to develop a rationally designed freeze-drying protocol.

Heat transfer resistance decreases during the drying process by the reduction of the transport distance as the interface retreats. With the mass transfer resistance (transport of water vapor), however, the opposite occurs. Mass transfer resistance increases during the drying process, as the dry cake becomes thicker. Therefore, parameters such as chamber pressure and shelf temperature are not necessarily constant during the primary drying process. They should be carefully chosen and adjusted as the drying process proceeds.

When all frozen or "unbound" water (i.e., not bound to protein or excipients) has been removed, the primary drying step has finished (Fig. 5.7). The primary drying process can be monitored by following individual vials (e.g., product temperature of individual vials) or batch methods e.g., comparative pressure measurements, manometric temperature measurement/pressure rise test, mass spectrometry to monitor gas composition in the chamber or tunable diode laser absorption spectroscopy to provide information on the sublimation rate.

The end of the primary drying stage can be measured by thermocouples within the product vials: the end of primary drying is reached when product temperature and shelf temperature become equal, or when the partial water pressure drops (Pikal 1990).

Comparative pressure measurement with a Pirani gauge and a capacitance manometer (CM) is another approach next to thermocouples to detect the end of primary drying. This is based on the gas composition dependent reading of a Pirani gauge, whereas a capacitance manometer reading is not influenced by the gas composition. Consequently, the Pirani gauge (which is typically calibrated under nitrogen or air) shows higher pressure values than the CM in the presence of water vapor in the chamber, i.e., as long as primary drying is progressing. The end of primary drying, i.e., when sublimation of "unbound" water has finished, is indicated when the pressure measured by the Pirani gauge and the CM show the same signal.

Secondary Drying

In the secondary drying stage, the temperature is slowly increased to remove "bound" water; the chamber pressure is still kept low. The temperature should stay all the time below the collapse/eutectic temperature, which continues to rise as the residual water content drops. Typically, the secondary drying step ends when the product has been kept at 20–40 °C for several hours. The residual water content of the product (e.g., determined by a Karl Fischer assay) is a critical, end point-indicating parameter. Values as low as 1% residual water in the cake have been recommended; however, this needs to be evaluated on a product specific basis and certain products may require higher or lower residual moisture contents to result in a stable product. Figure 5.11 (Pristoupil 1985; Pikal 1990) exemplifies the decreasing stability of freeze-dried hemoglobin with increasing residual water content.

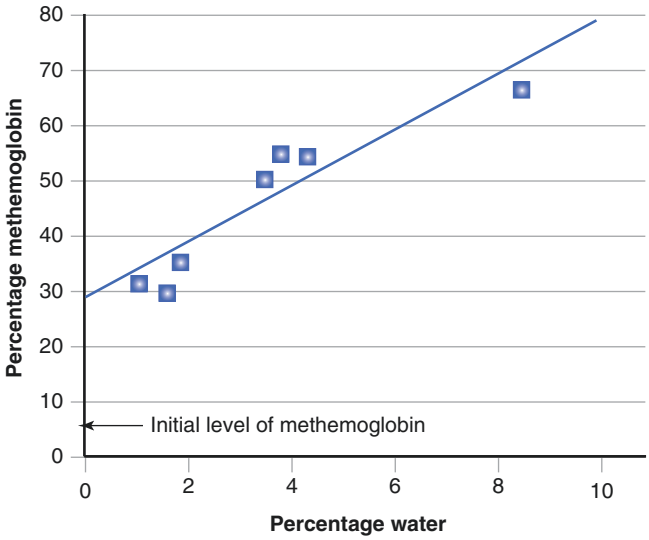

Figure 5.11 ■ The effect of residual moisture on the stability of freeze-dried hemoglobin (−6%) formulated with 0.2 M sucrose; decomposition to methemoglobin during storage at 23 °C for 4 years (From Pikal 1990). Data reported by Pritoupil et al. (1985)

HANDLING OF PHARMACEUTICAL PROTEINS POST-PRODUCTION

In the formulation process, the manufacturer will expose the protein product under development to a number of stress factors as mentioned in Table 5.3. The composition of the final formulation of the protein product will reflect the outcome of those (laboratory) stress experiments in combination with real-time stability testing: optimum formulation conditions for chemical and physical stability of the protein will be chosen. In spite of all these efforts, pharmaceutical proteins remain sensitive to 'real life' handling and may readily show degradation reactions that obviously affect both efficacy and safety. Therefore, the manufacturer—together with the regulatory authorities—creates a package insert text that points out to health care professionals and patients the conditions that should be maintained for the product, e.g., storage temperature window, avoidance of shaking/shear, exposure to light. As an example, the package insert of trastuzumab states: 'Swirl the vial gently to aid reconstitution. Trastuzumab may be sensitive to shear-induced stress, e.g., agitation or rapid expulsion from a syringe. DO NOT SHAKE' (FDA web site).

Surprisingly, little information is available on the actual storage and administration practices for pharmaceutical proteins in hospitals. Anecdotal information of exposing protein solutions to high shear conditions (shaking, use of pneumatic tube transport) and the use of incorrect administration techniques can be found in the literature. This is also true for the patient's home setting. However, recently, real data became available of a group of rheumatoid arthritis patients of whom only a small minority stored their protein product (anti-TNF-alpha therapy) in the prescribed temperature window, i.e. 2–8 °C. Both freezing and storing above 25 °C occurred (Vlieland et al., 2016). The consequences of this behavior for the stability of this protein, in particular aggregate formation, may be an increased chance of formation of anti-drug antibodies (Jiskoot et al., 2017; Vlieland et al., 2018). Considering the clinical importance of these medicines and the high prices paid, it is time to teach health care professionals and patients the importance of' 'Good Handling Practices for Biologicals' (Nejadnik et al., 2018).

DELIVERY OF PROTEINS: ROUTES OF ADMINISTRATION

■ The Parenteral Route of Administration

Parenteral administration is here defined as administration via those routes where a needle is used, including intravenous (IV), intramuscular (IM), subcutaneous (SC), intracutaneous, intraperitoneal (IP) and intravitreal injections. Chapter 6 provides more information on the pharmacokinetic behavior of recombinant proteins. It suffices here to state that the blood half-life of biotech products can vary over a wide range. For example, the blood circulation half-life of tissue plasminogen activator is a few minutes, whereas monoclonal antibodies have half-lives of a few days to weeks.

A simple way to expand the mean residence time for short half-life proteins is to switch from IV to IM or SC administration. One should realize that by doing that, changes in disposition may occur with a significant impact on the bioavailability (slower uptake in the blood compartment and lower extent of absorption) and therapeutic performance of the biologic. For instance, the extent of absorption of SC administered protein injections (compared to IV administration) may be as low as 30% (Richter et al., 2012; Kinnunen and Mrsny, 2014). This change in disposition is caused by various factors such as: (1) the prolonged residence time at the IM or SC site of injection compared to IV administration, differences in protein environment upon injection and enhanced exposure to degradation reactions (peptidases), and (2) differences in disposition.

Regarding point 1: For instance, diabetics can become "insulin resistant" through high tissue peptidase activity (Maberly et al. 1982). Other factors that can contribute to absorption variation are differences in level of activity of the muscle at the injection site and also massage and heat at the injection site. The state of the tissue, for instance, the occurrence of pathological conditions, may be important as well.

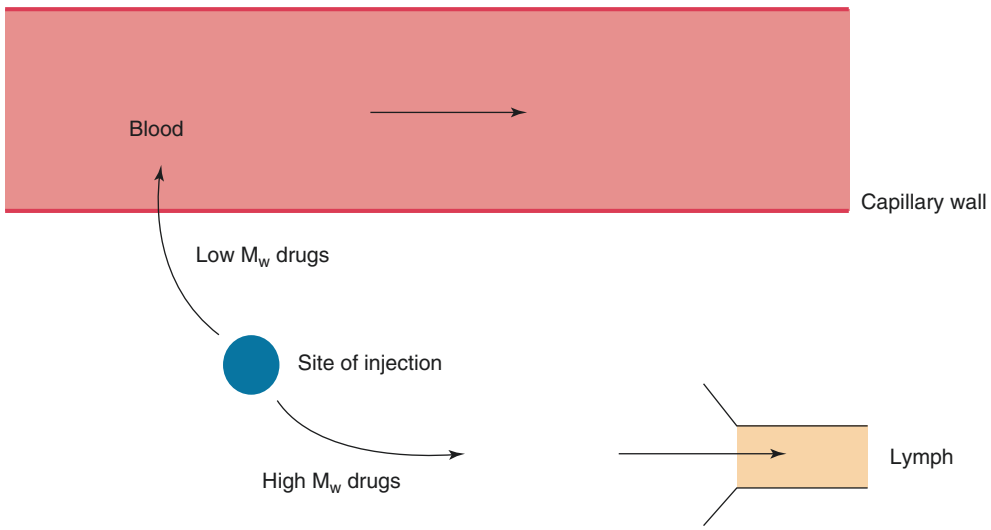

Figure 5.12 ■ Routes of uptake of SC- or IM-injected drugs

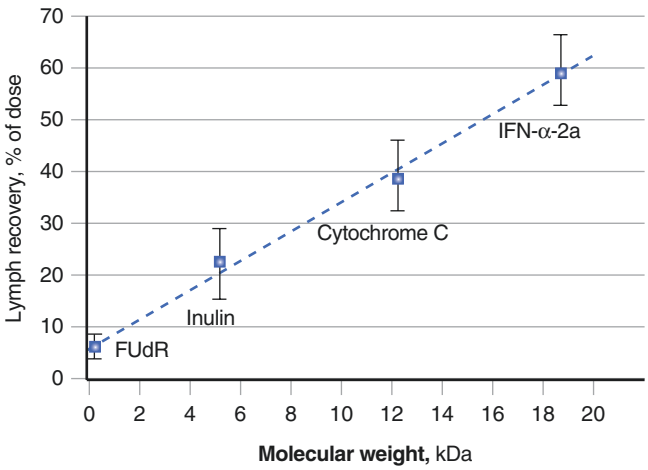

Figure 5.13 ■ Correlation between the molecular weight and the cumulative recovery of rIFN alpha-2a (Mw 19 kDa), cytochrome c (Mw 12.3 kDa), insulin (Mw 5.2 kDa), and FUdR, 5-fluorodeoxyuridine, (Mw 256.2 Da) in the efferent lymph from the right popliteal lymph node following SC administration into the lower part of the right hind leg of sheep. Each point and bar shows the mean and standard deviation of three experiments performed in separate sheep. The line drawn is the best fit by linear regression analysis calculated with the four mean values. The points have a correlation coefficient r of 0.998 ($p < 0.01$) (From Supersaxo et al. 1990)

Regarding point 2: Upon administration, the protein may reach the blood through the lymphatics or enter the blood circulation through the capillary wall at the site of injection (Figs. 5.12 and 5.13). The fraction of the administered dose taking this lymphatic route depends on the molecular weight of the protein (Supersaxo et al. 1990). Lymphatic transport takes time (hours), and uptake in the blood circulation is highly dependent on the injection site. On its way to the blood, the lymph passes through draining lymph nodes. There, contact is possible between lymph contents and cells of the immune system, such as macrophages and B and T lymphocytes residing in the lymph nodes.

■ Other Routes of Administration

For several reasons, e.g., ease of administration, patient friendliness and cost, alternative administration routes to the parenteral route would be welcome for the successful systemic delivery of recombinant proteins. This is particularly true for the oral route. Nature, unfortunately, does not allow us to use the oral route of administration for therapeutic proteins if a high (or at least constant) bioavailability is required. The two main reasons (A and B) for this failure of uptake are: (A) protein degradation in the GI (gastrointestinal track) and (B) poor permeability of the wall of the GI tract in case of a passive transport process.

Regarding point A: Protein degradation. The human body has developed a very efficient system to break down proteins in our food to amino acids or di- or tripeptides. These building stones for body proteins are actively absorbed for use in newly formed proteins. The stomach secretes pepsins, a family of aspartic proteases. They are particularly active between pH 3 and 5 and lose activity at higher pH values. Pepsins are endopeptidases capable of cleaving peptide bonds distant from the ends of the peptide chain. They preferentially cleave peptide bonds between two hydrophobic amino acids. Other endopeptidases are active in the gastrointestinal tract at neutral pH values, e.g., trypsin, chymotrypsin, and elastase. They have different, complementary peptide bond cleavage characteristics. Exopeptidases, proteases degrading peptide chains from their C- or N-terminus, are present as well. In the intestinal epithelium, brush border and cytoplasmic proteases of the enterocytes continue to cut proteins into fragments down to amino acids, di- and tripeptides.

Regarding point B: Permeability. High-molecular-weight molecules with a hydrophilic 'coat' such as therapeutic proteins do not readily penetrate the intact and mature epithelial barrier of the intestinal lumen. Active transport of intact proteins over the GI-epithelium has not been described. This leaves diffusion to and partitioning into the enterocyte membrane as the sole pathway for mass transfer. Diffusion coefficients and partition coefficients are low for intact therapeutic proteins leading to very low extent of uptake. Some oral vaccines, however, are available on the market, as discussed in Chap. 14. For those products only a (small) fraction of the antigen has to reach its target site to illicit an immune response.

Some proteins are administered locally for local therapy. For instance, ranibizumab (Fab fragment), aflibercept (a recombinant fusion protein consisting of vascular endothelial growth factor (VEGF)-binding portions from the extracellular domains of human VEGF receptors 1 and 2, which are fused to the Fc portion of human IgG1) and bevacizumab are proteins to block neovascularization in the retina. When injected in the vitreous cavity of the eye, they interact with vascular endothelial growth factor (VEGF) and slow down wet, age-related macular degeneration. Another example of administering a biologic near its site of action is dornase alfa (Pulmozyme). It is taken via inhalation to break down DNA in sputum of cystic fibrosis patients (cf. Chap. 22).

Apart from the oral route, the eye and lungs (as mentioned above when discussing local therapy), the nose, rectum, oral cavity (buccal absorption) and skin have been studied as potential sites of application. Table 5.7 lists the potential pros and cons for the different relevant routes. Moeller and Jorgensen (2009) and Jorgensen and Nielsen (2009) describe "the state of the art" in more detail. The nasal, buccal, rectal, and transdermal routes all are of little clinical relevance if systemic action is required. In general, bioavailability is too low and varies too much. The pulmonary route may be the exception to this rule.

FDA approved the first pulmonary insulin formulation (Exubera®) in January 2006. However, the supplier took it off the market in 2008 because of poor market penetration. Inhalation technology plays a critical role when considering the prospects of the pulmonary route for the systemic delivery of therapeutic proteins. Dry powder inhalers and nebulizers are the delivery systems considered and tested. The fraction of protein that is ultimately absorbed depends on (1) the

Route of administration
Oral
+ Easy to access, proven track record with "conventional" medicines, sustained/controlled release possible
– Negligible bioavailability for proteins
Nasal
+ Easily accessible, fast uptake, proven track record with a number of "conventional" medicines, probably lower proteolytic activity than in the GI tract, avoidance of first pass effect, spatial containment of absorption enhancers is possible
– Reproducibility (in particular under pathological conditions), safety (e.g., ciliary movement), negligible bioavailability for proteins
Pulmonary
+ Relatively easy to access, fast uptake, proven track record with "conventional" medicines, substantial –in the 10% range- fractions of insulin are absorbed, lower proteolytic activity than in the GI tract, avoidance of hepatic first pass effect
– Reproducibility (in particular under pathological conditions, smokers/nonsmokers), safety (e.g., immunogenicity), presence of macrophages in the lung with high affinity for particulates
Rectal
+ Easily accessible, partial avoidance of hepatic first pass, probably lower proteolytic activity than in the upper parts of the GI tract, spatial containment of absorption enhancers is possible, proven track record with a number of "conventional" drugs
– Negligible bioavailability for proteins
Buccal
+ Easily accessible, avoidance of hepatic first pass, probably lower proteolytic activity than in the lower parts of the GI tract, spatial containment of absorption enhancers is possible, option to remove formulation if necessary
– Negligible bioavailability of proteins, no proven track record yet
Transdermal
+ Easily accessible, avoidance of hepatic first pass effect, removal of formulation if necessary is possible, spatial containment of absorption enhancers, proven track record with "conventional" medicines, sustained/controlled release possible
– Negligible bioavailability of proteins
Intravitreal
+ Direct access to vitreous, delivery close to the target site
– Not suitable for systemic effects

+ Relative advantage, – Relative disadvantage

Table 5.7 ■ Alternative routes of administration to the IV, IM and SC route for biopharmaceuticals

fraction of the inhaled/nebulized dose that is actually leaving the device, (2) the fraction that is actually deposited in the lung, and (3) the fraction that is being absorbed, i.e., total relative uptake (TO%) = % uptake from device × % deposited in the lungs × % actually absorbed from the lungs. For insulin, TO% is estimated to be about 10% (Patton et al. 2004). The fraction of insulin that is absorbed from the lung is about 20%. The reproducibility of the blood glucose response to inhaled insulin was equivalent to SC-injected insulin. These figures demonstrate that insulin absorption via the lung may be a promising route, but the fraction that reaches the blood circulation is small and commercial success failed to materialize until now.

DELIVERY OF PROTEINS BY THE PARENTERAL ROUTE: APPROACHES FOR RATE-CONTROLLED DELIVERY

Presently used therapeutic proteins widely differ in their pharmacokinetic characteristics (see Chap. 6). If they are recombinant counterparts of endogenous agents such as insulin, tissue plasminogen activator, growth hormone, epoetin, interleukins, or factor VIII, it is important to realize why, when, and where –by which cells– they are secreted. Cells can communicate with each other through the endocrine, paracrine and/or autocrine pathway leading to secretion of mediator molecules (Table 5.8).

The presence of these mediators may activate a complex cascade of events that needs to be carefully controlled. Therefore, key issues for their therapeutic success are (1) access to target cells, (2) retention at the target site, and (3) proper timing of delivery.

In particular, for paracrine- and autocrine-acting proteins, such as tumor necrosis factor and interleukin-2 severe side effects were reported upon parenteral (IV or SC) administration. The occurrence of these side effects limits the therapeutic potential of these compounds. Therefore, the delivery of these proteins at the proper site, rate, and dose is crucial for their therapeutic success.

Endocrine hormones
A hormone secreted by a distant cell to regulate cell functions distributed widely through the body. The bloodstream plays an important role in the transport process
Paracrine-acting mediators
The mediator is secreted by a cell to influence surrounding cells, short-range influence
Autocrine-acting mediators
The agent is secreted by a cell and affects the cell by which it is generated, (very) short-range influence

Table 5.8 ■ Communication between cells: chemical messengers

Various technologies similar to those used for "small, low molecular weight" medicines may achieve rate control. E.g., for insulin one can choose from a spectrum of options (see Chap. 18). Moreover, continuous/"smart" infusion systems are on the market for insulin, see below.

■ Mechanical Pumps

In general, proteins are parenterally administered as an aqueous solution. Only recombinant vaccines and a number of insulin formulations are (colloidal) dispersions. For continuous and controlled administration of these solutions pump systems are used: continuous infusion. These pumps typically deliver the protein formulation via the intravenous route. However, patients may receive subcutaneously up to 25 ml over prolonged periods (up to 1 h) with a pump system (20% immune globulin solution; Hizentra, 2017). Table 5.9 lists some of the technologically feasible options. They are briefly touched upon below.

Pumps can be chosen in various sizes/prices, being portable or not, for inside/outside the body, with/without sophisticated rate control software. A pump system needs constant attention as it may fail because of power failure (batteries serve as backup power supply), problems with the syringe, accidental needle withdrawal, leakage of the catheter, and problems at the injection or implantation site. Moreover, long-term protein drug stability may become a problem. The protein should be stable at 37 °C or ambient temperature (for internal/implanted and external devices, respectively) between two refills.

Controlled administration of a drug does not necessarily imply a constant input rate. Pulsatile or variable-rate delivery is the desired mode of input for a number of proteins. For these biologics pumps should provide options for a flexible input rate. Insulin is a prime example of a therapeutic protein, where there is a need to adjust the input rate to the needs of the body. Today by far most experience has been gained with pump systems with adjustable input rates in an ambulatory setting with this protein drug. Even with high-tech pump systems,

• Continuous infusion with pumps. Input: Preset with limited variability. E.g. elastomer or spring driven pumps
• Continuous infusion with pumps. Input: Variable, controlled by health care professional or patient. E.g. mechanically/electronically driven (smart) pumps
• Rate control through implants. Input: limited control. E.g. biodegradable, polymer based microspheres
• Rate control through a closed-loop approach/feedback system. E.g. biosensor-pump combination

Table 5.9 ■ Controlled release and input systems for parenteral delivery

the patient still has to collect data to adjust the pump rate. This implies invasive sampling of body fluids on a regular basis, followed by calculations and setting of the required input rate. Progress made on developing the concept of closed-loop systems integrating these three actions: monitoring, calculating and choosing the rate of administration, i.e. a "natural" biofeedback system, is discussed under 'Biosensor-Pump Combinations'.

Biodegradable Microspheres

Polylactic acid–polyglycolic acid (PLGA)-based delivery systems are being used extensively for the delivery of therapeutic peptides, in particular for luteinizing hormone-releasing hormone (LHRH) agonists, such as leuprolide in the therapy of prostate cancer. The first LHRH agonist-controlled release formulations were implants, rods containing leuprolide with dosing intervals of 1–3 months. Later, microspheres loaded with leuprolide entered the market with dosing intervals up to 6 months. Considerations to design these controlled release systems are (1) the drug has to be highly potent (only a small dose is required over the dosing interval) and stable in the dosage form until release, (2) a sustained presence in the body is required, and (3) no adverse reactions at the injection site should occur. Only two such microsphere products for sustained delivery of therapeutic proteins instead of peptides made it to the market. Nutropin Depot® released recombinant human growth hormone over prolonged periods (monthly injection). Introduced in 1999, it was taken off the market in 2004 because of low perceived added therapeutic value, manufacturing problems and costs. A glucagon-like protein-1 (GLP-1, 39 amino acids) slow release formulation (Bydureon™) based on PLGA microspheres for once a week administration to type II diabetics was released in 2012.

Biosensor-Pump Combinations

If input rate control is desired to stabilize a certain body function, then this function or a suitable biomarker should be monitored. An algorithm converts this data into a drug-input rate and corresponding pump settings. If there is a known relationship between plasma level of the biomarker and pharmacological effect, these systems contain (Fig. 5.14):

1. A biosensor, measuring the (plasma) level of the biomarker
2. An algorithm, to calculate the required input rate for the delivery system
3. A pump system, able to administer the drug at the required rate over prolonged periods

The concept of a fully integrated closed-loop delivery of proteins still has to overcome a number of conceptual and practical hurdles. A simple relationship between plasma level and therapeutic effect does not always exist (see Chap. 6). There are many exceptions known to this rule; for instance, "hit and run" drugs can have long-lasting pharmacological effects after only a short exposure time. Also, drug effect–blood level relationships may be time dependent, as in the case of downregulation of relevant receptors on prolonged stimulation. Finally, if circadian rhythms exist, these will be responsible for variable PK/PD (pharmacokinetic/pharmacodynamic) relationships as well.

If PK/PD relationships can be established, as with insulin in selected groups of diabetics, then integrated

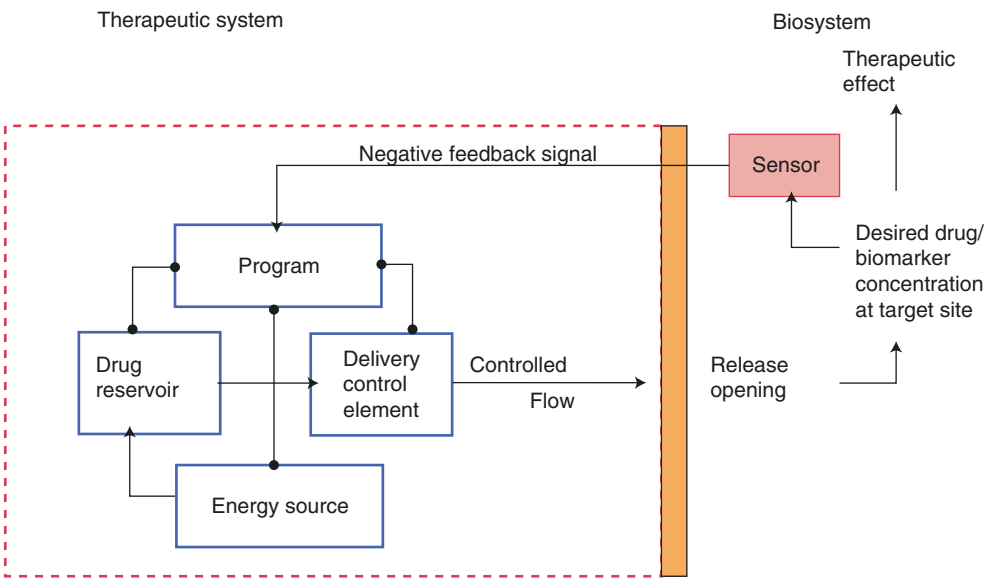

Figure 5.14 ■ Therapeutic system with closed control loop (From Heilmann 1984). (1) A biosensor, measuring the plasma level of the protein. (2) An algorithm, to calculate the required input rate for the delivery system. (3) A pump system, able to administer the drug at the required rate over prolonged periods

biosensor–pump combinations are available that almost act as biofeedback systems (Schaepelynck et al. 2011; Hovorka 2011). In 2016, FDA approved an integrated diabetes management system: insulin pump, continuous glucose monitoring biosensor, and diabetes therapy management software (Runge and Brown, 2016). The biosensor measures interstitial fluid glucose levels every 5 min and sends the outcome (wireless) to a therapy management algorithm. This software program adjusts the insulin pump settings to deliver an appropriate dose of insulin for basal glucose levels. However, the patient still has to inject a bolus before meals. Biosensor stability, robustness, absence of histological reactions and handling postprandial highs are still challenges in the design of fully integrated closed loop systems for chronic use. Trevitt et al. (2016) describe the state of the art in this fast-moving field.

DELIVERY OF PROTEINS BY THE PARENTERAL ROUTE: HALF-LIFE EXTENSION BY MODIFICATION OF THE API

Chemical modifications can change protein characteristics. For example, insulin half-life can be prolonged by exploiting the long circulation time of serum albumin and its high binding affinity for fatty acids such as myristic acid. In insulin detemir (Levemir®) lysine replaces the C-terminal threonine of insulin and myristic acid is chemically coupled through this lysine. After subcutaneous injection the myristic acid–insulin combination reaches the blood circulation and binds to albumin. Thereby the half-life of insulin is prolonged from less than 10 min to over 5 h. A similar approach is used with glucagon-1-like peptide (GLP-1 (7–37)) for the treatment of diabetes. Conjugating myristic acid to GLP-1 (amino acids 7–37) (liraglutide marketed as Victoza®) increases the plasma half-life from 2 min to over 10 h.

Another chemical modification approach that has been very successful in prolonging plasma circulation times and dosing intervals is the covalent attachment of polyoxyethylene glycol (PEG) to proteins. Figure 5.15 shows an example of this approach. Commercially highly successful examples that were developed later are PEGylated interferon alfa-2a and -2b and PEGylated hG-CSF (human granulocyte colony stimulating factor, filgrastim; see Chaps. 24 and 27). Furthermore, genetic modification of APIs has been successful in a number of cases. For example, fusion proteins with human serum albumin or Fc-parts (e.g., etanercept, aflibercept) from monoclonal antibodies are strategies to prolong the half-life. Both serum albumin and antibodies are physiological molecules with a long plasma half-life (see e.g. Chap. 8). With epoetins another approach was followed. Darbepoetin alfa (Aranesp) is a hyperglycosylated form of epoetin. It has the same mechanism of action but has a three-fold longer half-life compared to epoetin.

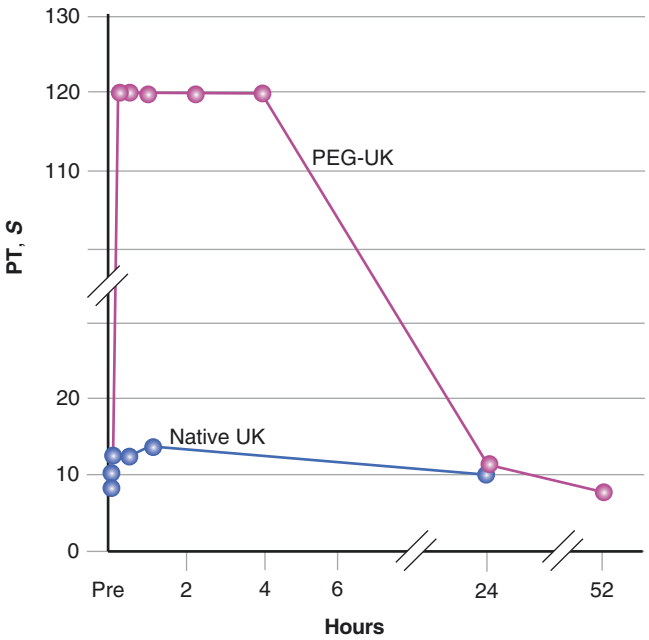

Figure 5.15 ■ Influence of chemical grafting of polyethylene glycol (PEG) on the ability of urokinase (UK) to affect the prothrombin time in seconds (PT) in vivo in beagles as function of time after a single administration (through Tomlinson 1987)

CONCLUDING REMARKS

In order to formulate a protein API successfully and turn it into a medicinal product that can be administered to a patient, the first requirement is an in depth understanding of the chemical and physical characteristics of the molecule in question, including its stability under the preferred storage conditions. A set of stability-indicating, complementary and orthogonal analytical techniques (see Box 1) should be available to help in successfully selecting the route of administration, the proper excipients, the packaging material for a stable product (freeze-dried or not).

In spite of considerable efforts made over almost 100 years, the parenteral route is the only one that allows us to administer protein-based medicines for systemic delivery to the patient. All other routes of administration failed so far.

SELF-ASSESSMENT QUESTIONS

■ Questions

1. A protein, which is poorly water soluble around its pI (pH 7.4), has to be formulated for subcutaneous administration. What conditions would one select to produce a water-soluble, injectable solution?
2. Why are many proteins to be used in the clinic formulated in freeze-dried form? Why is, as a rule, the presence of a lyoprotectant required? Why is it

important to know the glass transition temperature or eutectic temperature of the system?
3. Why should glucose be avoided as an excipient in protein formulations?
4. Why has oral delivery of therapeutic proteins failed so far?
5. What is the impact on the pharmacokinetics when changing from the IV to the SC route of administration of a therapeutic protein?
6. A company decides to explore the possibility to develop a biofeedback system for a therapeutic protein. What information should be available for estimating the chances for success?
7. What is the function of a preservative? For which type of protein formulations are they required? Example? Potential disadvantage?
8. Why do many protein formulations contain a surfactant? Example? Potential disadvantage?

Answers

1. Both solubility and stability should be considered. As both the aqueous solubility and the stability will be pH dependent, information on the solubility and stability as function of pH should be collected. The pH should be controlled by using a buffer. If needed, other excipients can be added to improve both the physical and the chemical stability of the protein, and to achieve isotonicity.
2. Chemical and physical instability of proteins in aqueous media is usually the reason to dry the protein solution.

   Freeze-drying is then the preferred technology, as other drying techniques do not give rapidly reconstitutable dry forms for the formulation and/or because elevated temperatures necessary for drying jeopardize the integrity of the protein. Lyoprotectants protect the proteins from degradation during the freeze-drying process.

   The collapse temperature (Tc) should not be exceeded (a few degrees below Te or Tg/Tg′), as otherwise collapse of the cake occurs. Collapse slows down the freeze-drying process rate, and collapsed material does not rapidly dissolve upon adding water for reconstitution.
3. Because the protein will degrade through the Maillard reaction, as glucose is a reducing sugar. This is true for both liquid and lyophilized formulations.
4. Because of the hostile environment in the GI tract regarding protein stability and the poor absorption characteristics of proteins (high molecular weight/often hydrophilic).
5. Both the extent and rate of uptake into the blood circulation are affected. When changing from IV to SC administration, the AUC (area under the curve) and the absorption rate are reduced.
6. Information that should be available
   - The desired pharmacokinetic profile (e.g., information on the PK/PD relationship/circadian rhythm)
   - Chemical and physical stability of the protein on long-term storage at body/ambient temperature
   - Availability of a biosensor system (stability in vivo, precision/accuracy)
   - Availability of a reliable pump system (see Table 5.9)
7. A preservative is included to neutralize contaminations in containers. A multi-dose formulation needs a preservative. Examples of preservatives used in protein formulations are phenol, meta-cresol, benzyl alcohol, and chlorobutanol. A disadvantage of preservatives is that they may interact with the protein, affecting their own and/or the protein's performance.
8. Surfactants reduce adsorption to interfaces and by doing so prevent aggregation. Examples of commonly used surfactants are polysorbate 80 and 20. A disadvantage of surfactants is that too high concentrations may cause denaturation (unfolding) of the protein. A disadvantage of polysorbates is that they can hydrolyze, which reduces their stabilizing potential and may lead to insoluble degradation products (a.o. fatty acids).

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