12 Insulin

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INTRODUCTION

Insulin was discovered by Banting and Best in 1921 (Bliss 1982). Soon afterwards manufacturing processes were developed to extract the insulin from porcine and bovine pancreas. From 1921 to 1980, efforts were directed at increasing the purity of the insulin and providing different formulations for altering time action for improved glucose control (Brange 1987a, b; Galloway 1988). Purification was improved by optimizing extraction and processing conditions and by implementing chromatographic processes (size exclusion, ion exchange, and reversed-phase (Kroeff et al. 1989)) to reduce the levels of both general protein impurities and insulin-related proteins such as proinsulin and insulin polymers. Formulation development focused on improving chemical stability by moving from acidic to neutral formulations and by modifying the time-action profile through the use of various levels of zinc and protamine. The evolution of recombinant DNA technology led to the widespread availability of human insulin, which has eliminated issues with sourcing constraints while providing the patient with a natural exogenous source of insulin. Combining the improved purification methodologies and recombinant DNA (rDNA) technology, manufacturers of insulin are now able to provide the purest human insulin ever made available, >98 %. Further advances in rDNA technology, coupled with a detailed understanding of the molecular properties of insulin and knowledge of its endogenous secretion profile, enabled the development of insulin analogs with improved pharmacology relative to existing human insulin products.

CHEMICAL DESCRIPTION

Insulin, a 51-amino acid protein, is a hormone that is synthesized as a proinsulin precursor in the β-cells of the pancreas and is converted to insulin by enzymatic cleavage. The resulting insulin molecule is composed of two polypeptide chains that are connected by two interchain disulfide bonds (Fig. 12.1) (Baker et al. 1988). The A-chain is composed of 21 amino acids, and the B-chain is composed of 30 amino acids. The interchain disulfide linkages occur between A^{7}–B^{7} and A^{20}–B^{19}, respectively. A third intra-chain disulfide bond is located in the A-chain, between residues A^{6} and A^{11}.

In addition to human insulin and insulin analog products, which are predominately used today as first-line therapies for the treatment of diabetes, bovine and porcine insulin preparations have also been made commercially available (Table 12.1). However, all major manufacturers of insulin have discontinued production of these products, marking an end to future supply of animal-sourced insulin products. Difficulties obtaining sufficient supplies of bovine or porcine pancreata and recent concerns over transmissible spongiform encephalopathies associated with the use of animal-derived materials are major reasons for the product deletions.

The net charge on the insulin molecule is produced from the ionization potential of four glutamic acid residues, four tyrosine residues, two histidine residues, a lysine residue, and an arginine residue, in conjunction with two α-carboxyl and two α-amino groups. Insulin has an isoelectric point (pI) of 5.3 in the

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denatured state; thus, the insulin molecule is negatively charged at neutral pH (Kaarsholm et al. 1990). This net negative charge state of insulin has been used in formulation development, as will be discussed later.

In addition to the net charge on insulin, another important intrinsic property of the molecule is its ability to readily associate into dimers and higher-order associated states (Figs. 12.2 and 12.3) (Pekar and Frank 1972). The driving force for dimerization appears to be the formation of favorable hydrophobic interactions at the C-terminus of the B-chain (Ciszak et al. 1995). Insulin can associate into discrete hexameric complexes in the presence of various divalent metal ions, such as zinc at 0.33 g-atom/monomer (Goldman and Carpenter 1974), where each zinc ion (a total of two) is coordinated by a His^{B10} residue from three adjacent monomers. Physiologically, insulin is stored as a zinc-containing hexamer in the β-cells of the pancreas. As will be discussed later, the ability to form discrete hexamers in the presence of zinc has been used to develop therapeutically useful formulations of insulin.

Commercial insulin preparations also contain phenolic excipients (e.g., phenol, m-cresol, or methylparaben) as antimicrobial agents. As represented in Figs. 12.2 and 12.3d, these phenolic species also bind to specific sites on insulin hexamers, causing a conformational change that increases the chemical stability of insulin in commercial preparations (Brange

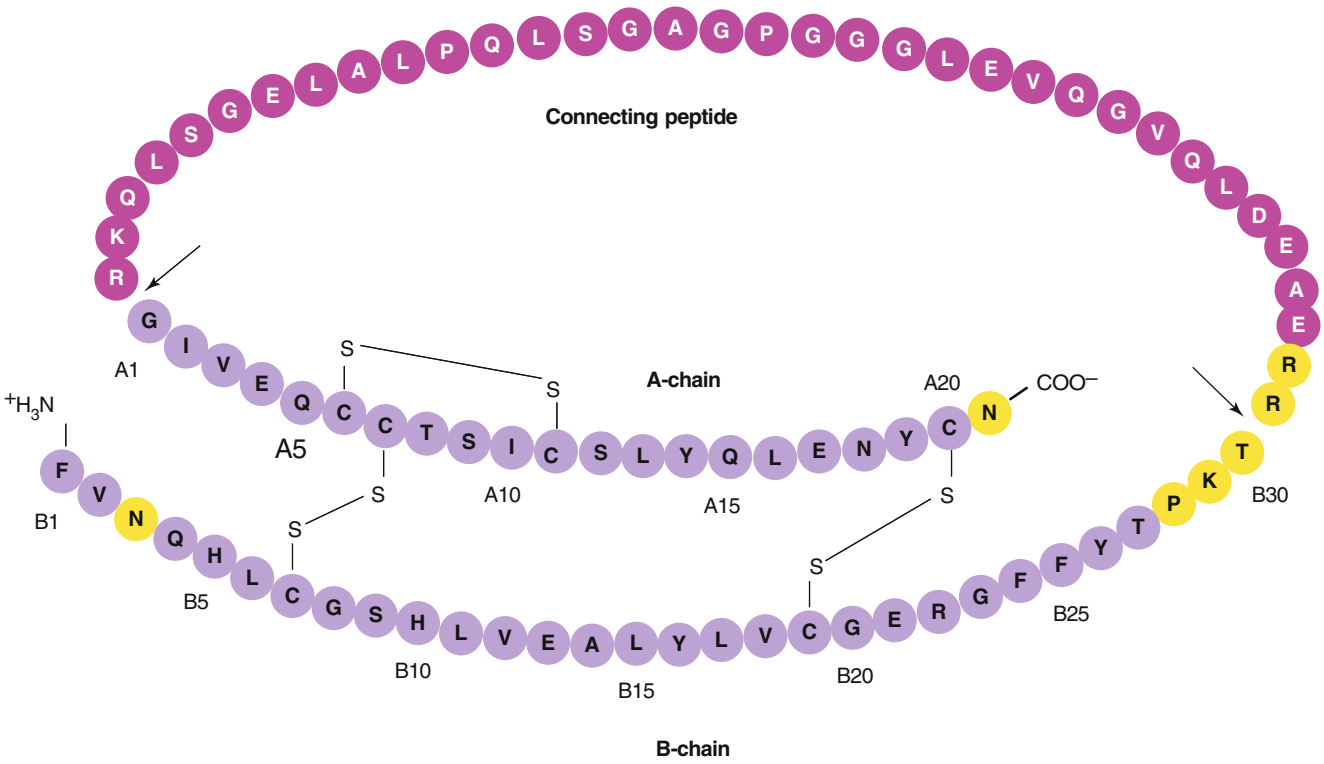

Figure 12.1 ■ Primary sequence of insulin. The shaded amino acids represent sites of sequence alterations denoted in Table 12.1.

Species	A^{21}	B^{3}	B^{28}	B^{29}	B^{30}	B^{31}	B^{32}
Human (Humulin®, Novolin®)	Asn	Asn	Pro	Lys	Thr	–	–
Insulin lispro (Humalog®)	Asn	Asn	Lys	Pro	Thr	–	–
Insulin aspart (NovoRapid®, NovoLog®)	Asn	Asn	Asp	Lys	Thr	–	–
Insulin glulisine (Apidra®)	Asn	Lys	Pro	Glu	Thr		
Insulin glargine (Lantus®)	Gly	Asn	Pro	Lys	Thr	Arg	Arg
Insulin detemir (Levemir®)	Asn	Asn	Pro	Lys-(*N*-tetradecanoyl)			

Table 12.1 ■ Amino acid substitutions in insulin analogs compared to human insulin.

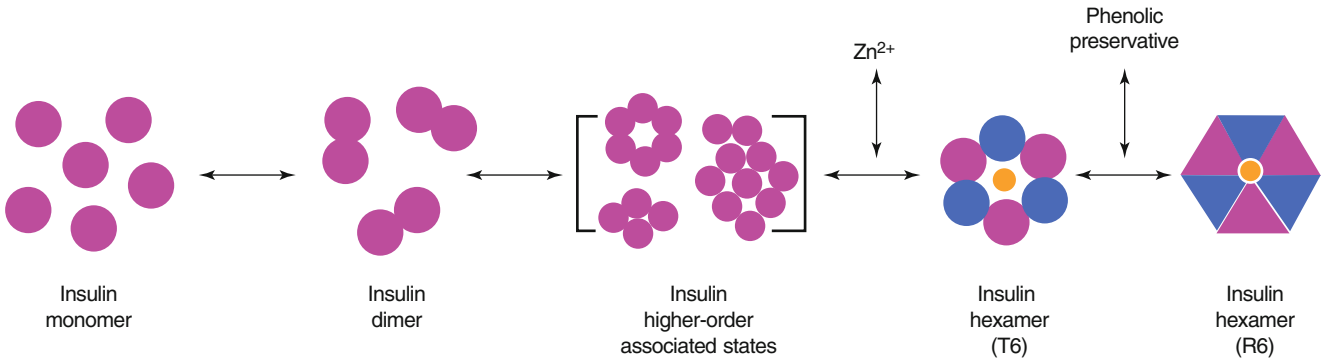

Figure 12.2 ■ Schematic representation of insulin association in the presence and absence of zinc and phenolic, antimicrobial preservatives.

and Langkjaer 1992). X-ray crystallographic studies have identified the location of six phenolic ligand binding sites on the insulin hexamer and the nature of the conformational change induced by the binding of these ligands (Derewenda et al. 1989). The phenolic ligands are stabilized in a binding pocket between monomers of adjacent dimers by hydrogen bonds with the carbonyl oxygen of Cys^{A6} and the amide proton of Cys^{A11} as well as numerous van der Waals contacts. The binding of these ligands stabilizes a conformational change that occurs at the N-terminus of the B-chain in each insulin monomer, shifting the conformational equilibrium of residues B1 to B8 from an extended structure (T state) to an α-helical structure (R state). This conformational change is referred to as the T<–>R transition (Brader and Dunn 1991) and is illustrated in Fig. 12.3c, d.

In addition to the presence of zinc and phenolic preservatives, modern insulin formulations may contain an isotonicity agent (glycerol or NaCl) and/or a buffer (e.g., sodium phosphate). The former is used to minimize subcutaneous tissue damage and pain on injection. The latter is present to minimize pH drift in some pH-sensitive formulations.

PHARMACOLOGY AND FORMULATIONS

Normal insulin secretion in the nondiabetic person falls into two categories: (1) insulin that is secreted in response to a meal and (2) the background or *basal* insulin that is continually secreted between meals and during the nighttime hours (Fig. 12.4). The pancreatic response to a meal typically results in peak serum insulin levels of 60–80 μU/mL, whereas basal serum insulin levels fall within the 5–15 μU/mL range (Galloway and Chance 1994). Because of these vastly different insulin demands, considerable effort has been expended to develop insulin formulations that meet the pharmacokinetic (PK) and pharmacodynamic (PD) requirements of each condition. More recently, insulin analogs and insulin analog formulations have been developed to improve PK and PD properties.

■ Regular and Rapid-Acting Soluble Preparations

Initial soluble insulin formulations were prepared under acidic conditions and were chemically unstable. In these early formulations, considerable deamidation was identified at Asn^{A21}, and significant potency loss was observed during prolonged storage under acidic conditions. Efforts to improve the chemical stability of these soluble formulations led to the development of neutral, zinc-stabilized solutions.

The insulin in these neutral, regular formulations is chemically stabilized by the addition of zinc (~0.4 % relative to the insulin concentration) and phenolic preservatives. As mentioned above, the addition of zinc leads to the formation of discrete hexameric structures (containing 2Zn atoms per hexamer) that can bind six molecules of phenolic preservatives, e.g., m-cresol (Figs. 12.2 and 12.3c). The binding of these excipients increases the stability of insulin by inducing the formation of a specific hexameric conformation (R_6), in which the B1 to B8 region of each monomer is in an α-helical conformation (Fig. 12.3d). This in turn decreases the availability of residues involved in deamidation and high molecular weight polymer formation (Brange et al. 1992a, b).

The pharmacodynamic profile of this soluble formulation (Type R) is listed in Table 12.2. The neutral, regular formulations show peak insulin activity between 2 and 3 h with a maximum duration of 5–8 h. As with other formulations, the variations in time action can be attributed to factors such as dose, site of injection, temperature, and the patient's physical activity. Despite the soluble state of insulin in these formulations, a delay in activity is still observed. This delay has been attributed to the time required for the hexamer to dissociate into the dimeric and/or monomeric substituents prior to absorption from the

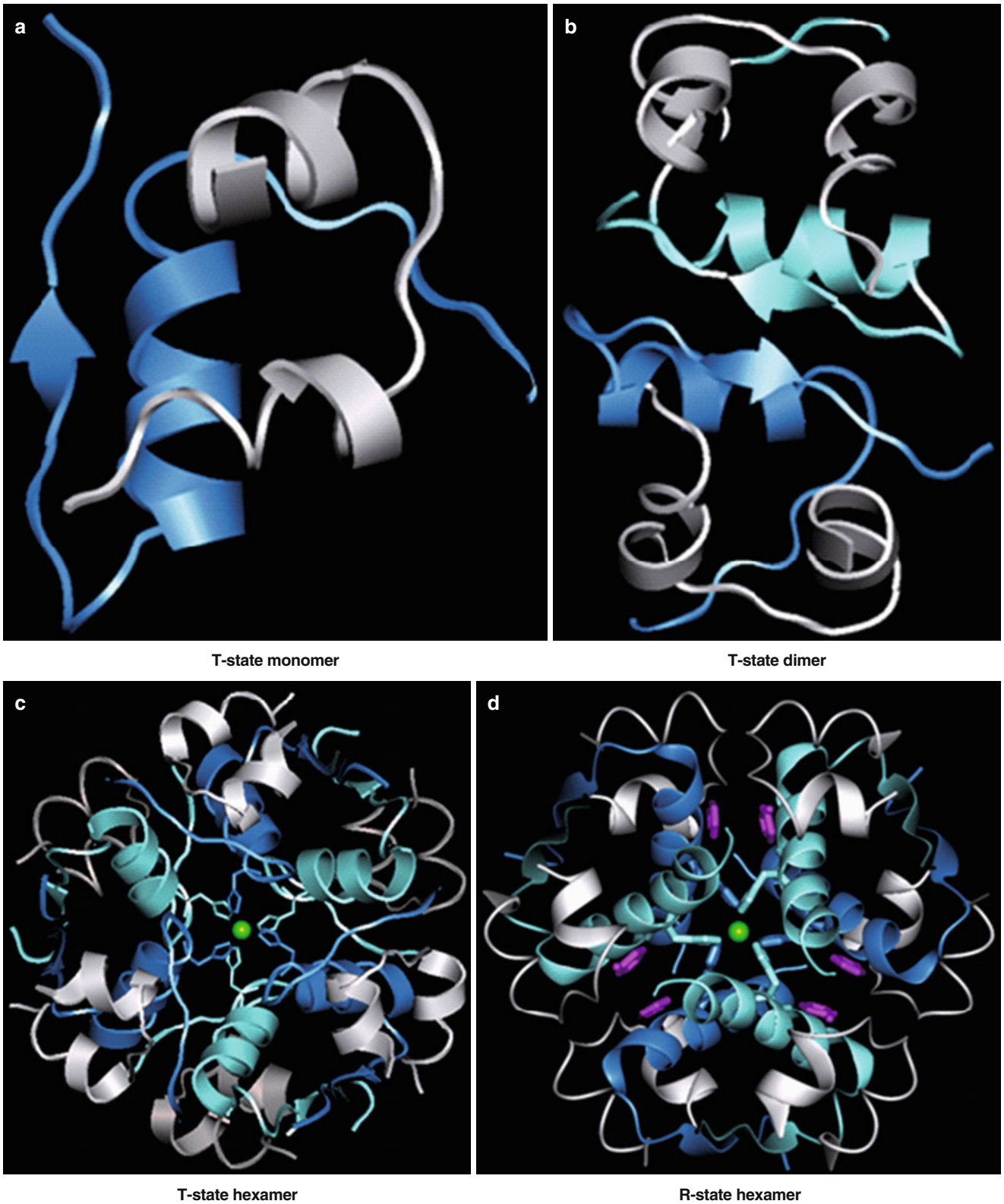

Figure 12.3 ■ (**a**) A cartoon representation of the secondary and tertiary structures of a T-state monomer of insulin, with the B1–B8 region in an extended conformation. The A-chain is colored white and the B-chain is colored blue. (**b**) A cartoon representation of the secondary and tertiary structures of a T-state dimer of insulin. The A-chains are colored white and the B-chains are colored blue and cyan. (**c**) A cartoon representation of the secondary and tertiary structures of a T-state hexamer of insulin. The A-chains are colored white, the B-chains are colored blue and cyan, and zinc is colored green. (**d**) A cartoon representation of the secondary and tertiary structures of R-state hexamer of insulin in the presence of preservative. The A-chains are colored white, the B-chains are colored blue and cyan, zinc is colored green, and preservative is colored magenta.

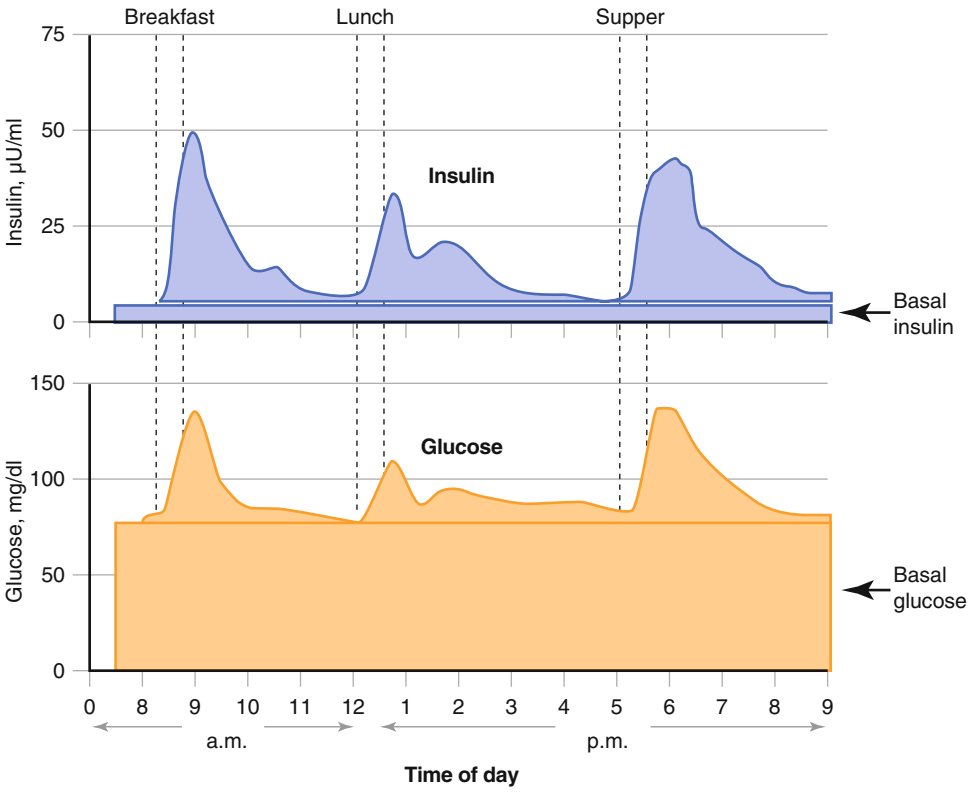

Figure 12.4 ■ A schematic representation of glucose and insulin profiles during the day in nondiabetic individuals (Adapted and reprinted from Schade et al. 1983).

interstitium. This dissociation requires the diffusion of the preservative and insulin from the site of injection, effectively diluting the protein and shifting the equilibrium from hexamers to dimers and monomers (Fig. 12.5) (Brange et al. 1990). Recent studies exploring the relationship of molecular weight and cumulative dose recovery of various compounds in the popliteal lymph following subcutaneous injection suggest that lymphatic transport may account for approximately 20 % of the absorption of insulin from the interstitium (Supersaxo et al. 1990; Porter and Charman 2000; Charman et al. 2001). The remaining balance of insulin is predominately absorbed through capillary diffusion.

Monomeric insulin analogs were designed to achieve a more natural response to prandial glucose level increases while providing dosing convenience to the patient. The pharmacological properties of these soluble formulations are listed in Table 12.3. The development of monomeric analogs of insulin for the treatment of insulin-dependent diabetes mellitus has focused on shifting the self-association properties of insulin to favor the monomeric species and consequently minimizing the delay in time action (Brange et al. 1988, 1990; Brems et al. 1992). One such monomeric analog, $Lys^{B28}ProB^{29}$-human insulin (insulin lispro; CAS Number 133107-64-9; Humalog® or Liprolog®; Eli Lilly & Co.) has been developed and does have a more rapid time-action profile, with a peak activity of approximately 1 h (Howey et al. 1994). The sequence inversion at positions B28 and B29 yields an analog with reduced self-association behavior compared to human insulin (Fig. 12.1; Table 12.1); however, insulin lispro can be stabilized in a preservative-dependent hexameric complex that provides the necessary chemical and physical stability required by insulin preparations. Despite the hexameric complexation of this analog, insulin lispro retains its rapid time action. Based on the crystal structure of the insulin lispro hexameric complex, Ciszak et al. (1995) have hypothesized that the reduced dimerization properties of the analog, coupled with the preservative dependence, yield a hexameric complex that readily dissociates into monomers after rapid diffusion of the phenolic preservative into the subcutaneous tissue at the site of injection (Fig. 12.6). Consequently, the substantial dilution (10^5) of the human insulin zinc hexamers is not necessary for the analog to dissociate from hexamers to monomers/dimers, which is required for absorption.

It is important to highlight that the properties engineered into insulin lispro (Humalog®) not only provide the patient with a more convenient therapy but also improve control of postprandial hyperglycemia and reduce the frequency of severe hypoglycemic events (Holleman et al. 1997; Anderson et al. 1997).

Since the introduction of insulin lispro, two additional rapid-acting insulin analogs have been introduced to the market. The amino acid modifications made to the human insulin sequence to produce these analogs are depicted in Table 12.1. Like insulin lispro,

Type[b]	Description	Appearance	Components	Action (h)[a]		
				Onset	Peak	Duration
R[c]	Regular soluble insulin injection	Clear solution	Metal ion: zinc (10–40 mcg/mL)	0.5–1	2–4	5–8
			Buffer: none			
			Preservative: m-cresol (2.5 mg/mL)			
			Isotonicity agent: glycerin (16 mg/mL)			
			pH: 7.25–7.6			
N	NPH insulin isophane suspension	Turbid or cloudy suspension	Metal ion: zinc (21–40 mcg/mL)	1–2	2–8	14–24
			Buffer: dibasic sodium phosphate (3.78 mg/mL)			
			Preservatives: m-cresol (1.6 mg/mL), phenol (0.73 mg/mL)			
			Isotonicity agent: glycerin (16 mg/mL)			
			Modifying protein: protamine (~0.35 mg/mL)			
			pH: 7.0–7.5			
70/30	70 % insulin isophane suspension, 30 % regular insulin injection	Turbid or cloudy suspension	Metal ion: zinc (21–35 mcg/mL)	0.5	2–4	14–24
			Buffer: dibasic sodium phosphate (3.78 mg/mL)			
			Preservatives: m-cresol (1.6 mg/mL), phenol (0.73 mg/mL)			
			Isotonicity agent: glycerin (16 mg/mL)			
			Modifying protein: protamine (~0.241 mg/mL)			
			pH: 7.0–7.8			
50/50	50 % insulin isophane suspension, 50 % regular insulin injection	Turbid or cloudy suspension	Metal ion: zinc (21–35 mcg/mL)	0.5	2–4	14–24
			Buffer: dibasic sodium phosphate (3.78 mg/mL)			
			Preservatives: m-cresol (1.6 mg/mL), phenol (0.73 mg/mL)			
			Isotonicity agent: glycerin (16 mg/mL)			
			Modifying protein: protamine (~0.172 mg/mL)			
			pH: 7.0–7.8			
L[d]	Lente insulin zinc suspension	Turbid or cloudy suspension	Metal ion: zinc (120–250 mcg/mL)	1–2	3–10	20–24
			Buffer: sodium acetate (1.6 mg/mL)			
			Preservative: methylparaben (1.0 mg/mL)			
			Isotonicity agent: sodium chloride (7.0 mg/mL)			
			Modifying protein: none			
			pH: 7.0–7.8			
U[d]	Ultralente extended insulin zinc suspension	Turbid or cloudy suspension	Metal ion: zinc (120–250 mcg/mL)	0.5–3	4–20	20–36
			Buffer: sodium acetate anhydrous (1.6 mg/mL)			
			Preservative: methylparaben (1.0 mg/mL)			
			Isotonicity agent: sodium chloride (7.0 mg/mL)			
			Modifying protein: none			
			pH: 7.0–7.8			

[a]The time-action profiles of Lilly insulins are the average onset, peak action, and duration of action that are taken from a composite of studies. The onset, peak, and duration of insulin action depend on numerous factors, such as dose, injection site, presence of insulin antibodies, and physical activity. The action times listed represent the generally accepted values in the medical community

[b]US designation

[c]Another notable designation is S (Britain). Other soluble formulations have been designed for pump use and include Velosulin® and HOE 21PH®

[d]Discontinued

Table 12.2 ■ A list of neutral U-100 insulin formulations.

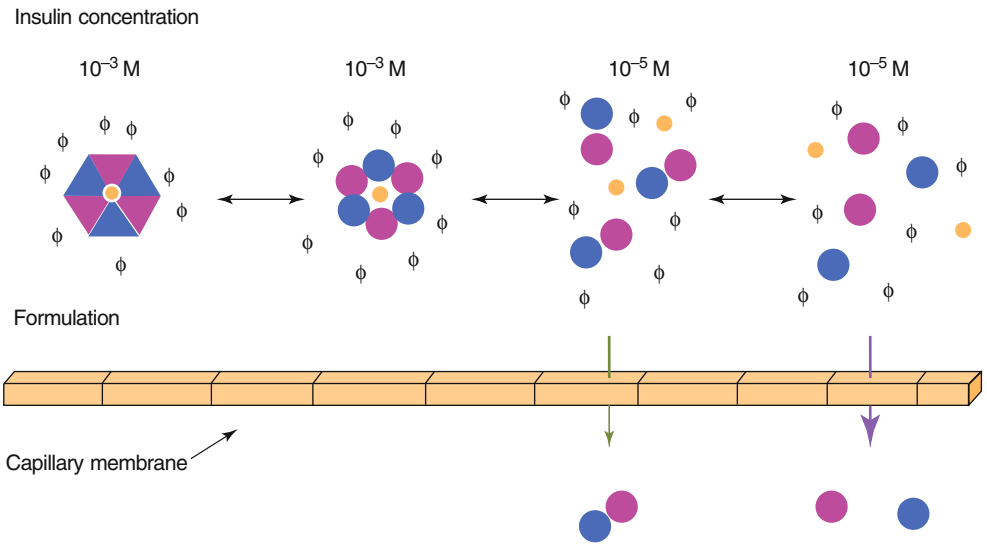

Figure 12.5 ■ A schematic representation of insulin dissociation after subcutaneous administration.

Type[b]	Description	Appearance	Components	Action (h)[a]		
				Onset	Peak	Duration
Humalog®	Rapid-acting soluble insulin analog for injection	Aqueous, clear, and colorless solution	Metal ion: zinc (19.7 mcg/mL) Buffer: dibasic sodium phosphate (1.88 mg/mL) Preservatives: m-cresol (3.15 mg/mL), phenol (trace) Isotonicity agent: glycerin (16 mg/mL) pH: 7.0–7.8	0.25–0.5	0.5–2.5	≤5
Humalog® Mix75/25™	75 % insulin lispro protamine suspension and 25 % insulin lispro for injection	Turbid or cloudy suspension	Metal ion: zinc (25 mcg/mL) Buffer: dibasic sodium phosphate (3.78 mg/mL) Preservative: m-cresol (1.76 mg/mL), phenol (0.715 mg/mL) Isotonicity agent: glycerin (16 mg/mL) Modifying protein: protamine (0.28 mg/mL) pH: 7.0–7.8	0.25–0.5	0.2–2.5	14–24
NovoLog®	Rapid-acting soluble insulin analog for injection	Aqueous, clear, and colorless solution	Metal ion: zinc (19.6 mcg/mL) Buffer: disodium hydrogen phosphate dihydrate (1.25 mg/mL) Preservative: m-cresol (1.72 mg/mL), phenol (1.50 mg/mL) Isotonicity agents: glycerin (16 mg/mL), sodium chloride (0.58 mg/mL) pH: 7.2–7.6	0.25[c]	0.75–1.5[c]	3–5[c]

Table 12.3 ■ A list of human-based U-100 insulin analog formulations.

Type[b]	Description	Appearance	Components	Action (h)[a]		
				Onset	Peak	Duration
Novolog® Mix 70/30	70 % insulin aspart protamine suspension and 30 % insulin aspart for injection	Turbid or cloudy suspension	Metal ion: zinc (19.6 mcg/mL) Buffer: dibasic sodium phosphate (1.25 mg/mL) Preservatives: m-cresol (1.72 mg/mL), phenol (1.50 mg/mL) Isotonicity agents: sodium chloride (0.58 mg/mL), mannitol (36.4 mg/mL) Modifying protein: protamine (0.33 mg/mL) pH: 7.2–7.44	<0.5[d]	1–4[d]	≤24[d]
Apidra®	Rapid-acting soluble insulin analog for injection	Aqueous, clear, and colorless solution	Metal ion: none Buffer: tromethamine (6 mg/mL) Preservative: m-cresol (3.15 mg/mL) Isotonicity agent: sodium chloride (5 mg/mL) Stabilizing agent: polysorbate 20, (0.01 mg/mL) pH: ~7.3	~0.3[c]	0.5–1.5[c]	~5.3[c]
Humalog® Basal (EU) or Humalog® NPL (Japan)	Intermediate-acting insulin lispro protamine suspension for injection	Turbid or cloudy suspension	Metal ion: zinc (0.0225 mcg/mL) Buffer: dibasic sodium phosphate (3.78 mg/mL) Preservatives: m-cresol (1.76 mg/mL), phenol (0.8 mg/mL) Isotonicity agent: glycerin (16 mg/mL) Modifying protein: protamine (0.376 mg/mL) pH: 7.0–7.5	1–2	2–8	14–24
Lantus®	Long-acting soluble insulin analog for injection	Aqueous, clear, and colorless solution	Metal ion: zinc (30 mcg/mL) Buffer: none Preservative: m-cresol (2.7 mg/mL) Isotonicity agent: glycerin (20 mg 85 %/mL) Modifying protein: none pH: ~ 4		Constant release with no pronounced peak[c]	10.8 to >24.0[d]
Levemir®	Long-acting soluble insulin analog for injection	Aqueous, clear, and colorless solution	Metal ion: zinc (65.4 mcg/mL) Buffer: dibasic sodium phosphate (0.89 mg/mL) Preservatives: m-cresol (2.06 mg/mL), phenol (1.8 mg/mL) Isotonicity agents: mannitol (30 mg/mL), sodium chloride (1.17 mg/mL) Modifying protein: none pH: 7.4		3–14[c]	5.7–23.2[c]

[a]The time-action profiles of Lilly insulins are the average onset, peak action, and duration of action taken from a composite of studies. The onset, peak, and duration of insulin action depend on numerous factors, such as dose, injection site, presence of insulin antibodies, and physical activity. The action times listed represent the generally accepted values in the medical community

[b]US designation

[c]DRUGDEX® System [Internet database]. Greenwood Village, Colo: Thomson Micromedex. Updated periodically

[d]PDR® Electronic Library™ [Internet database]. Greenwood Village, Colo: Thomson Micromedex. Updated periodically

Table 12.3 ■ (continued)

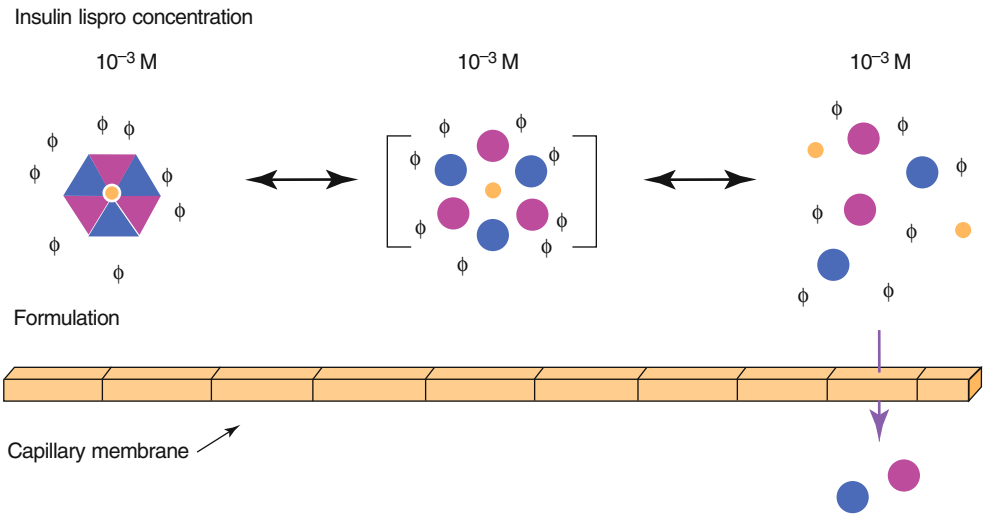

Figure 12.6 ■ A schematic representation of insulin lispro dissociation after subcutaneous administration.

both analogs are supplied as neutral pH solutions containing phenolic preservative. The design strategy for Asp^{B28}-human insulin (insulin aspart; CAS Number 116094-23-6; NovoRapid® or NovoLog®; Novo Nordisk A/S) (Brange et al. 1988, 1990) involves the replacement of Pro^{B28} with a negatively charged aspartic acid residue. Like $Lys^{B28}ProB^{29}$-human insulin, Asp^{B28}-human insulin has a more rapid time action following subcutaneous injection (Heinemann et al. 1997). This rapid action is achieved through a reduction in the self-association behavior compared to human insulin (Brange et al. 1990; Whittingham et al. 1998). The other rapid-acting analog, Lys^{B3}-Glu^{B29}-human insulin (insulin glulisine; CAS Number 160337-95-1; Apidra®, Sanofi-Aventis), involves a substitution of the lysine residue at position 29 of the B-chain with a negatively charged glutamic acid. Additionally, this analog replaces the Asn^{B3} with a positively charged lysine. Scientific reports describing the impact of these changes on the molecular properties of this analog are lacking. However, the glutamic acid substitution occurs at a position known to be involved in dimer formation (Brange et al. 1990) and may result in disruption of key interactions at the monomer-monomer interface. The Asn residue at position 3 of the B-chain plays no direct role in insulin self-association (Brange et al. 1990), but it is flanked by two amino acids involved in the assembly of the Zn^{2+} insulin hexamer. Despite the limited physicochemical information on insulin glulisine, studies conducted in persons with either type 1 (T1DM) or type 2 diabetes (T2DM) (Dreyer et al. 2005; Dailey et al. 2004) confirm that the analog displays similar pharmacological properties as insulin lispro. Interestingly, insulin glulisine is not formulated in the presence of zinc as are the other rapid-acting analogs. Instead, insulin glulisine is formulated in the presence of a stabilizing agent (polysorbate 20) (Table 12.3). The surfactant in the formulation presumably minimizes higher-order association. Since the purely monomeric formulated Apidra® demonstrates only a slightly faster PK profile and no difference in PD properties from Novolog® (insulin aspart) and Humalog® (insulin lispro), the hexameric breakdown of the two latter formulations must be rapid relative to the rate-limiting step, subcutaneous absorption (Home 2012).

In addition to the aforementioned rapid-acting formulations, manufacturers have designed soluble formulations for use in external or implanted infusion pumps. In most respects, these formulations are very similar to regular insulin (i.e., hexameric association state, preservative, and zinc); however, buffer and/or surfactants may be included in these formulations to minimize the physical aggregation of insulin that can lead to clogging of the infusion sets. In early pump systems, gas-permeable infusion tubing was used with the external pumps. Consequently, a buffer was added to the formulation in order to minimize pH changes due to dissolved carbon dioxide. Infusion tubing composed of materials having greater resistance to carbon dioxide diffusion is currently being used and the potential for pH-induced precipitation of insulin is greatly reduced. All three of the commercially available rapid-acting insulin analogs are approved for use in external infusion pumps.

■ Ultrarapid Initiatives

The efforts of developing an artificial pancreas, an external pump that can rapidly control blood glucose coupled with a continuous blood glucose monitor, are driving the need for insulins with increasingly faster time action. To this end, numerous approaches are being explored, including modification of the subcutaneous tissue using permeation enhancers to increase insulin dispersion and accelerate absorption

(Muchmore and Vaughn 2010), disruption of the hexameric state of insulin and masking surface charges to facilitate more rapid absorption of monomers (Heinemann et al. 2012), and the use of "biochaperones" to assist transport of insulin across the capillary membrane (Soula et al. 2010). None of these approaches have produced commercially available products at this time.

■ Intermediate-Acting Insulin Preparations

The only intermediate-acting insulin preparation available is NPH. This formulation achieves extended time action by necessitating the dissolution of a crystalline form of insulin. This dissolution is presumed to be the rate-limiting step in the absorption of intermediate insulin. Consequently, the time action of the formulation is prolonged by further delaying the dissociation of the hexamer into dimers and monomers.

NPH refers to neutral protamine Hagedorn, named after its inventor H. C. Hagedorn (1936), and is a neutral crystalline suspension that is prepared by the cocrystallization of insulin with protamine. Protamine consists of a closely related group of very basic peptides that are isolated from fish sperm. Protamine is heterogeneous in composition; however, four primary components have been identified and show a high degree of sequence homology (Hoffmann et al. 1990). In general, protamine is ~30 amino acids in length and has an amino acid composition that is primarily composed of arginine, 65–70 %. Using crystallization conditions identified by Krayenbuhl and Rosenberg (1946), oblong tetragonal NPH insulin crystals with volumes between 1 and 20 μm^3 can be consistently prepared from protamine and insulin (Deckert 1980). These formulations, by design, have very minimal levels of soluble insulin in solution. The condition at which no measurable protamine or insulin exists in solution after crystallization is referred to as the isophane point.

NPH has an onset of action from 1 to 2 h, peak activity from 6 to 12 h, and duration of activity from 18 to 24 h (Table 12.2). As with other formulations, the variations in time action are due to factors such as dose, site of injection, temperature, and the patient's physical activity. In T2DM patients, NPH can be used as either once-daily or twice-daily therapy; however, in T1DM patients, NPH is predominately used as a twice-daily therapy. NPH can be readily mixed with regular insulin either extemporaneously by the patient or as obtained from the manufacturer in a premixed formulation (Table 12.2). Premixed insulin, e.g., 70/30 or 50/50 NPH/regular, has been shown to provide the patient with improved dose accuracy and consequently improved glycemic control (Bell et al. 1991). In these preparations, a portion of the soluble regular insulin will reversibly adsorb to the surface of the NPH crystals through an electrostatically mediated interaction under formulation conditions (Dodd et al. 1995); however, this adsorption is reversible under physiological conditions and consequently has no clinical significance (Galloway et al. 1982; Hamaguchi et al. 1990; Davis et al. 1991). Due, in part, to the reversibility of the adsorption process, NPH/regular mixtures are uniquely stable and have a 3-year shelf life.

The rapid-acting insulin analog, insulin lispro, can be extemporaneously mixed with NPH; however, such mixtures must be injected immediately upon preparation due to the potential for exchange between the soluble and suspension components upon long-term storage. Exchange refers to the release of human insulin from the NPH crystals into the solution phase and concomitant loss of the analog into the crystalline phase. The presence of human insulin in solution could diminish the rapid time-action effect of the analog. One way to overcome the problem of exchange is to prepare mixtures containing the same insulin species in both the suspension and the solution phases, analogous to human insulin regular/NPH preparations. However, this approach requires an NPH-like preparation of the rapid-acting analog.

An NPH-like suspension of insulin lispro has been prepared, and its physicochemical properties relative to human insulin NPH have been described (DeFelippis et al. 1998). In order to prepare the appropriate crystalline form of the analog, significant modifications to the NPH crystallization procedure are required. The differences between the crystallization conditions have been proposed to result from the reduced self-association properties of insulin lispro.

Pharmacological studies reported for the insulin lispro NPH-like suspension, formerly referred to as neutral protamine lispro (NPL) (DeFelippis et al. 1998; Janssen et al. 1997), indicate that the PK and PD properties of this analog suspension are analogous to human insulin NPH (Table 12.3). Clinical trials of insulin lispro protamine suspension (ILPS) alone in T2DM and in combination with insulin lispro in T1DM have been reported (Strojek et al. 2010; Fogelfeld et al. 2010; Chacra et al. 2010). In T2DM patients, the PK/PD profile of ILPS can support a once-daily therapy regimen (Hompesch et al. 2009), In addition, studies with ILPS in T1DM patients have shown a more predictable response than insulin glargine due to reduced intrasubject variability (Ocheltree et al. 2010). Moreover, the availability of ILPS allows for the preparation of homogeneous, biphasic mixture preparations containing intermediate-acting ILPS and rapid-acting solutions of insulin lispro that are not impacted by exchange between solution and crystalline forms. ILPS is also available as a stand-alone basal analog in several EU countries and Japan.

As with insulin lispro, premixed formulations of the insulin aspart have been prepared in which rapid-acting soluble insulin aspart has been combined with a protamine-retarded crystalline preparation of insulin aspart (Balschmidt 1996). Clinical data on insulin lispro mixtures and those composed of insulin aspart have been reported in the literature (Weyer et al. 1997; Heise et al. 1998). The pharmacological properties of the rapid-acting analogs are preserved in these stable mixtures (Table 12.3). Premixed formulations of both rapid-acting analogs are now commercially available in many countries.

Immunogenicity issues with protamine have been documented in a small percentage of diabetic patients (Kurtz et al. 1983; Nell and Thomas 1988). Individuals who show sensitivity to the protamine in NPH formulations (or premixed formulations of insulins lispro and aspart) are routinely switched to other long-acting insulin formulations, e.g., Lantus® or Levemir®, to control their basal glucose levels.

■ Long-Acting Insulin Formulations

The normal human pancreas secretes approximately 1 unit of insulin (0.035 mg) per hour to maintain basal glycemic control (Waldhäusl et al. 1979). Adequate basal insulin levels are a critical component of diabetes therapy because they regulate hepatic glucose output, which is essential for proper maintenance of glucose homeostasis during the diurnal cycling of the body. Consequently, long-acting insulin formulation must provide a very different PK profile than "mealtime" insulin formulation.

There are two long-acting insulin analog preparations currently commercially available, Lantus® (insulin glargine) and Levemir® (insulin detemir), which were approved in the 2000s (Table 12.1; Fig. 12.1). The approval of these solution-based analog preparations made the zinc-insulin crystalline Ultralente obsolete, and it was subsequently removed from the marketplace. Lantus® derives its protracted time-action profiles from the slow and relatively constant dissolution of solid particles that form as result of a pH shift of the acidic formulation to neutral pH in the subcutaneous tissue. This slow dissolution precedes the dissociation of insulin into absorbable units, and thus the *rate of absorption* (units per hour) into the bloodstream is significantly decreased in comparison to that of prandial or bolus (mealtime) formulations. Levemir®, on the other hand, achieves its protracted effect by a combination of structural interactions and physiological binding events (Havelund et al. 2004).

Insulin glargine (Gly^{A21}, Arg^{B31}, Arg^{B32}-human insulin; CAS Number 160337-95-1; Lantus®; Sanofi-Aventis) is a long-acting insulin analog, whose amino acid sequence modifications are highlighted in Table 12.1 and Fig. 12.1. This analog differs from human insulin in that the amino acid asparagine is replaced with glycine at position Asn^{A21} and two arginine residues have been added to the C-terminus of the B-chain. The impact of the additional arginine residues is to shift the isoelectric point from a pH of 5.4–6.7, thereby producing an insulin analog that is soluble at acidic pH values, but is less soluble at the neutral pH of subcutaneous tissue. Lantus® is a solution formulation prepared under acidic conditions, pH 4.0. The introduction of glycine at position Asn^{A21} yields a protein with acceptable chemical stability under acidic formulation conditions, since the native asparagine is susceptible to acid-mediated degradation and reduced potency. Thus, the changes to the molecular sequence of insulin have been made to improve chemical stability and to modulate absorption from the subcutaneous tissue, resulting in an analog that has approximately the same potency as human insulin. The Lantus® formulation is a clear solution that incorporates zinc and m-cresol (preservative) at a pH value of 4. Consequently, Lantus® does not need to be resuspended prior to dosing. Immediately following injection into the subcutaneous tissue, the insulin glargine precipitates due to the pH change, forming a slowly dissolving precipitate. This results in a relatively constant rate of absorption over 24 h with no pronounced peak (Table 12.3). This profile allows once-daily dosing as a patient's basal insulin. As with all insulin preparations, the time course of Lantus® may vary in different individuals or at different times in the same individual, and the rate of absorption is dependent on blood supply, temperature, and the patient's physical activity. Lantus® should not be diluted or mixed with any other solution or insulin, as will be discussed below.

Insulin detemir (Lys^{B29}(*N*-tetradecanoyl)des(B30) human insulin; CAS Number 169148-63-4; Levemir®; Novo Nordisk A/S) utilizes acylation of insulin with a fatty acid moiety as a means to achieve a protracted pharmacological effect. As shown in Table 12.1 and Fig. 12.1, the B30 threonine residue of human insulin is eliminated in insulin detemir, and a 14-carbon, myristoyl fatty acid is covalently attached to the ε-amino group of Lys^{B29}. The analog forms a zinc hexamer at neutral pH in a preserved solution. Clinical studies have reported that insulin detemir displays lower PK and PD variability than NPH and/or insulin glargine (Hermansen et al. 2001; Vague et al. 2003; Heise et al. 2004; Porcellati et al. 2011). An approximate description of the PD profile of Levemir® is listed in Table 12.3. This analog appears to display a slower onset of action than NPH without a pronounced peak (Heinemann et al. 1999). However, whether the duration of the protracted effect can truly be considered sufficient to warrant classification of insulin detemir as a long-acting

insulin remains a subject of debate since published clinical studies of this insulin analog are typically referenced to intermediate-acting NPH.

Binding of the tetradecanoyl-acylated insulin to albumin was originally proposed as the underlying mechanism behind the observed prolonged effect for insulin detemir analog; however, recent investigations on insulin detemir have determined that the mechanism is more complex (Havelund et al. 2004). It has been proposed that subcutaneous absorption is initially delayed as a result of hexamer stability and dihexamerization. Such interactions between hexamers are likely a consequence of the symmetrical arrangement of fatty acid moieties around the outside of the hexamers (Whittingham et al. 2004), as shown by X-ray crystallographic studies. These associated forms further bind to albumin within the injection site depot. Additional prolongation may result due to albumin binding.

Although Lantus® and Levemir® have improved basal insulin therapy, both products fail to achieve the goal of a once-daily administered basal insulin product with both full 24-h coverage and low variability. Moreover, the desire to eliminate or minimize nocturnal hypoglycemia has driven the exploration of improved basal insulin therapies. Consequently, there are five basal insulin programs of note in Phase III clinical testing, according to ClinicalTrials.gov (http://clinicaltrial.gov). As of March 2012, Sanofi-Aventis is testing a new formulation of insulin glargine (ClinicalTrials.gov Identifier: NCT01499082); although no peer-reviewed literature is available, Sanofi-Aventis disclosed at the 32nd Cowen Annual Health Care Conference in Boston that the new glargine formulation provides a unique flat PK/PD profile with lower injection volume (Zerhouni 2012). Eli Lilly and Company is testing two basal insulin candidates, LY2605541 and LY2963016. The company has yet to disclose the nature of these basal insulin candidates; however, as of March 2012, LY2605541 is slated for six Phase III trials*, and LY2963016 is slated for two Phase III studies. Novo Nordisk, as of March 2012, had filed with regulatory agencies, insulin degludec (NN1250), an ultra-long basal insulin, and insulin degludec plus (NN5401), a soluble basal insulin derivative combined with a bolus insulin. Insulin degludec is a new acylated insulin, wherein desB30 human insulin is modified at position Lys^{B29} with a derivatized fatty acid moiety defined as 29B-[N^{6}-[N-(15-carboxy-1-oxopentadecyl)-L-γ-glutamyl]-L-lysine] (CAS Number 844439-96-9). The protracted time action of degludec is derived from the utilization of a depot release strategy specific to the derivatized insulin wherein, after injection of the soluble insulin degludec formulation, di-hexamers agglomerate to form multi-hexamers to add an additional rate-limiting step to the release of absorbable insulin monomers and dimers from the subcutaneous tissue. The preliminary data indicate that insulin degludec had comparable glycemic control to insulin glargine with a reduced hypoglycemia profile in T1DM patients (Birkeland et al. 2011) and T2DM patients (Zinman et al. 2011). Moreover, in the latter study the protracted time action of insulin degludec was exemplified by showing the product could be administered every 2 days with efficacy and safety.

■ Concentrated Insulin Formulations

Concentrated U-500 beef regular insulin (500 U/mL) first became available in the USA in 1952 (Iletin®, Eli Lilly and Company). It was initially used to manage very high insulin requirements of diabetes patients with insulin antibody insulin resistance. Pork U-500 regular insulin (Iletin II®, Lilly) replaced the beef formulation in 1980. Over time, with progressive improvements in insulin formulations and purity, severe insulin resistance (insulin requirements of >200 U/day or ≥2 U/kg/day) became associated with T2DM and severe obesity, parallel epidemics currently in the USA and worldwide. Recombinant human U-500 regular insulin was introduced in 1997 (Humulin® R U-500, Eli Lilly and Company in the USA, Actrapid® U-500, Novo Nordisk in the UK [voluntarily withdrawn in 2008]). Providing an appropriate amount of insulin for these patients using U-100 insulins may be logistically difficult and may require eight or more separate syringes or pen injections daily, making patient adherence to therapy and attaining glycemic control difficult (Lane et al. 2009; Segal et al. 2010).

Early pharmacological studies demonstrated reduced absorption associated with increasing concentrations of insulin (Binder 1969; Binder et al. 1984). Galloway et al. (1981) showed no statistically significant differences in PK serum insulin levels with increasing concentrations of pork regular insulin (at 0.25 U/kg) from U-40 to U-500; however, time to peak glucose responses were mildly delayed, and peak effect was variably reduced as concentration increased. The first PK/PD study of human U-500 vs. U-100 regular insulin in healthy obese subjects was recently published (de la Peña et al. 2011). Overall insulin exposure and overall effect were similar at both 50- and 100-U doses (0.5 and 1.0 U/kg) with both formulations. However, the two formulations were not bioequivalent: peak insulin concentration (C_{max}) and effect (R_{max}) were significantly prolonged for U-500 vs. U-100 for both doses. Time to peak concentration (t_{max}) and time to maximal effect (tR_{max}) were significantly longer for U-500 vs. U-100 only at the 100-U dose. Duration of action (tR_{last}) was prolonged for U-500 at both doses vs. U-100 (50 U: 19.7 vs. 18.3 h; 100 U: 21.5 vs. 18.3 h; $p < 0.05$ for both). The

*During the preparation of this book chapter, the structure of LY2605541 was disclosed at conference proceedings as insulin lispro PEGylated at LysB28 with a 20kDa PEG (Hansen et al. 2012).

onset of action (t_{onset}) was within 20 min for both formulations and supports the clinical use of human U-500 regular 30 min before meals to leverage the prandial effect. Basal insulin needs are expected to be covered by the long "tail" of action of the U-500 formulation (de la Peña et al. 2011).

Although no randomized controlled trials of U-500 insulin have been completed (A randomized controlled trial comparing twice-daily and thrice-daily U-500 in insulin-resistant T2DM was initiated in the USA in 2013 [CT.gov NCT 01774968]), case series (review by Lane et al. 2009; Ziesmer et al. 2012; Boldo and Comi 2012) have generally demonstrated reductions in HbA1c (glycated hemoglobin) of 1.0–1.7 % over 3–98 months of use. Paradoxically, insulin dose generally did not statistically increase after conversion to human U-500 regular insulin, although one large case series did report an increase in total daily dose by 0.44 U/kg (Boldo and Comi 2012). Weight gain with treatment was variable, up to 4.2–6.8 kg (Lane et al. 2009; Boldo and Comi 2012). Reports of severe hypoglycemia have been infrequent, although an increase in non-severe hypoglycemia was reported in one large series (Boldo and Comi 2012). Most series have used twice-daily or thrice-daily regimens (Lane et al. 2009; Ziesmer et al. 2012; Boldo and Comi 2012). A simplified dosing algorithm was published by Segal et al. (2010).

Safety concerns with concentrated insulin therapy in diabetes patients, besides hypoglycemia and weight gain, mainly relate to the risk of dose confusion due to lack of a dedicated injection device for U-500 insulin. Thus, U-100 insulin syringes or tuberculin (volumetric) syringes have to be used; careful notation of unit markings (e.g., a 100-unit dose would be drawn to the 20 unit marking on a U-100 insulin syringe) or volume markings in mL (e.g., a 100-unit dose would be drawn to 0.2 mL on a tuberculin syringe), respectively, is required. Dosing conversion tables and formulas are useful, as have been included in the revised product label (March 2011) and recent clinical reviews (Lane et al. 2009; Segal et al. 2010). Pharmacists need to ensure that patients have had appropriate education on how to measure and administer doses (Segal et al. 2010). The U-500 insulin vial and labeling of vial and box are distinctive from U-100 insulins, with black-and-white lettering, brown diagonal stripes, and larger size (20 mL containing 10,000 U).

PHARMACEUTICAL CONCERNS

■ Chemical Stability of Insulin Formulations

Insulin has two primary routes of chemical degradation upon storage and use: hydrolytic transformation of amide to acid groups and formation of covalent dimers and higher-order polymers. Primarily the pH, the storage temperature, and the components of the specific formulation influence the rate of formation of these degradation products. The purity of insulin formulations is typically assessed by high-performance liquid chromatography using reversed-phase and size exclusion separation modes (USP Monographs: Insulin 2012). In acidic solution, the main degradation reaction is the transformation of asparagine (Asn) at the terminal 21 position of the A-chain to aspartic acid. This reaction is relatively facile at low pH, but is extremely slow at neutral pH (Brange et al. 1992b). This was the primary degradation route in early soluble (acidic) insulin formulations. However, the development of neutral solutions and suspensions has diminished the importance of this degradation route. Stability studies of neutral solutions indicate that the amount of A21 desamido insulin does not change upon storage. Thus, the relatively small amounts of this bioactive material present in the formulation arise either from the source of insulin or from pharmaceutical process operations.

The deamidation of the AsnB3 of the B-chain is the primary degradation mechanism at neutral pH. The reaction proceeds through the formation of a cyclic imide that results in two products, aspartic acid (Asp) and iso-aspartic acid (iso-Asp) (Brennan and Clarke 1994). This reaction occurs relatively slowly in neutral solution (approximately 1/12 the rate of A21 desamido formation in acid solution) (Brange et al. 1992b). The relative amounts of these products are influenced by the flexibility of the B-chain, with approximate ratios of Asp:iso-Asp of 1:2 and 2:1 for solution and crystalline formulations, respectively. As noted earlier, the use of phenolic preservatives provides a stabilizing effect on the insulin hexamer that reduces the formation of the cyclic imide, as evidenced by reduced deamidation. The rate of formation also depends on temperature; typical rates of formation are approximately 2 % per year at 5 °C. Studies have shown B3 deamidated insulin to be essentially fully potent (R.E. Chance, personal communication).

High molecular weight protein (HMWP) products form at both refrigerated and room temperature storage conditions. Covalent dimers that form between two insulin molecules are the primary condensation products in marketed insulin products. There is evidence that insulin-protamine heterodimers also form in NPH suspensions (Brange et al. 1992a). At higher temperatures, the probability of forming higher-order insulin oligomers increases. The rate of formation of HMWP is less than that of hydrolytic reactions; typical rates are less than 0.5 % per year for soluble neutral regular insulin formulations at 5 °C. The rate of formation can be affected by the strength of the insulin formulation or by the addition of glycerol as an isotonicity agent. The latter increases the rate of HMWP formation presumably

by introducing impurities such as glyceraldehyde. HMWP formation is believed to also occur as a result of a reaction between the N-terminal B1 phenylalanine amino group and the C-terminal A21 asparagine of a second insulin molecule via a cyclic anhydride (or succinimide, based on unpublished results of the authors) intermediate (Darrington and Anderson 1995). Reaction with the intermediate may also occur via the N-terminus of the A-chain or side-chain epsilon amine of the lysine residue located near the C-terminus of the B-chain. Disulfide exchange leading to polymer formation is also possible at basic pH; however, the rate for these reactions is very slow under neutral pH formulation conditions. The quality of excipients such as glycerol is also critical because small amounts of aldehyde and other glycerol-related chemical impurities can accelerate the formation of HMWP. The biopotency of HMWP is significantly less (1/12–1/5 of insulin) than monomeric species (Brange 1987c).

Only limited chemical stability data has been published in the scientific literature for the insulin analog formulations containing insulin lispro, insulin aspart, insulin glulisine, insulin glargine, or insulin detemir; however, it is reasonable to presume that similar chemical degradation pathways are present to varying extents in these compounds. Nevertheless, since some analogs are formulated under acidic conditions, e.g., Lantus® is formulated at pH 4.0, or have been modified with hydrophobic moieties, e.g., Levemir®, it is reasonable to presume that alternate chemical degradation pathways may be operable. It should be noted that the amino acid substitution of glycine for asparagine at position 21 of the insulin glargine A-chain is expected to effectively eliminate the potential for deamidation that would occur under the acidic pH conditions used in the Lantus® formulation.

■ Physical Stability of Insulin formulations

The physical stability of insulin formulations is mediated by noncovalent aggregation of insulin. Hydrophobic forces typically drive the aggregation, although electrostatics plays a subtle but important role. Aggregation typically leads to a loss in potency of the formulation, and therefore conditions promoting this type of physical degradation (i.e., extreme mechanical agitation or exposure to air-liquid interfaces often in combination with elevated temperatures) should be avoided for all insulin products. A particularly severe type of nonreversible aggregation results in the formation of insulin fibrils. The mechanism of insulin fibrillation is widely believed to result from destabilization of hexamers (i.e., the predominant self-associated form of most insulin solution preparations) causing an increase in the population of monomers that can partially unfold and initiate the aggregation process (Jansen et al. 2005). Physical attributes of insulin formulations are readily assessed by visual observation for macroscopic characteristics as well as by instrumental methods such as light and differential phase contrast microscopy. Insulin fibrillation can be confirmed using atomic force microscopy (Jansen et al. 2005). Various particle-sizing techniques also may be used to characterize physical degradation phenomena. Fluorescence spectroscopy using specific dyes has proven useful in monitoring the time course of insulin fibrillation process (Nielsen et al. 2001).

In general, insulin solutions have good physical stability. Physical changes in soluble formulations may be manifested as color or clarity change or, in extreme situations, increases in solution viscosity, a phenomenon referred to as gelation, or the formation of a precipitate that could be an indication of fibrillation. Insulin suspensions, such as NPH, are the most susceptible to changes in physical stability. Such physical instability typically occurs as a result of both elevated temperature and mechanical stress to the insulin preparation. The increase in temperature favors hydrophobic interactions, while mechanical agitation serves to provide mixing and stress across interfacial boundaries. Nucleation and higher-order forms of aggregation in suspensions can lead to conditions described as visible clumping of the insulin microcrystalline particles or adherence of the aggregates to the inner wall of the glass storage container. The latter phenomenon is referred to as frosting. In severe cases, resuspension may be nearly impossible because of caking of the suspension in the vial. Temperatures above ambient (>25 °C) can accelerate the aggregation process, especially those at or above body temperature (37 °C). Normal mechanical mixing of suspensions to achieve dispersion of the microcrystalline insulin particles prior to administration is not deleterious to physical stability. However, vigorous shaking or mixing should be avoided. Consequently, this latter constraint has, in part, led to the observation that patients do not place enough effort into resuspension. Thus, proper emphasis must be placed on training the patient in resuspension of crystalline, amorphous, and premixed suspension formulations of insulin and insulin analogs. The necessity of rigorous resuspension may be the first sign of aggregation and should prompt a careful examination of the formulation to verify its suitability for use.

As with the chemical stability data, published information regarding the physical stability of the newer insulin analog formulations containing insulin lispro, insulin aspart, insulin glulisine, insulin glargine, or insulin detemir is limited. However, it is reasonable to assume that similar controls are practiced for preventing exposures to extreme agitation and thermal excursions to minimize undesirable

physical transformations such as precipitation, aggregation, gelation, or fibrillation.

CLINICAL AND PRACTICE ASPECTS

■ Vial Presentations

Insulin is commonly available in 10-mL vials. In the United States, a strength of U-100 (100 U/mL) is the standard, whereas outside the USA both U-100 and U-40 (40 U/mL) are commonly used. Recent introduction of U-100 insulins (Humalog®, Humulin® N, R, and 70/30) in 5-mL vials (filled to 3 mL: 300 U) has met a need for smaller volumes and less waste in hospital usage. It is essential to obtain the proper strength and formulation of insulin in order to maintain glycemic control. In addition, brand/method of manufacture is important. Any change in insulin should be made cautiously and only under medical supervision (Galloway 1988; Brackenridge 1994). Common formulations, such as regular and NPH, are listed in Table 12.2, and the newer insulin analog formulations are listed in Table 12.3. Mixtures of rapid- or fast-acting with intermediate-acting insulin formulations are popular choices for glycemic control. The ratio is defined as ratio of protamine-containing fraction/rapid- or fast-acting fraction, e.g., Humalog Mix 75/25 where 75 % of a dose is available as 75 % ILPS and 25 % insulin lispro for injection. With regard to NPH regular mixtures, caution must be used in the nomenclature because it may vary depending on the country of sale and the governing regulatory body. In the USA, for example, the predominant species is listed first as in N/R 70/30, but in Europe the same formulation is described as R/N 30/70 (Soluble/Isophane) where the base ("normal") ingredient is listed first. Currently, an effort is being made to standardize worldwide to the European nomenclature. Human insulin mixtures available in the USA include N/R 70/30 and 50/50, while Europe has R/N 15/85, 25/75, 30/70, and 50/50 available from Eli Lilly and Company, Novo Nordisk, and Sanofi-Aventis.

■ Injection Devices

Insulin syringes should be purchased to match the strength of the insulin that is to be administered (e.g., for U-100 strength use 30-, 50-, or 100-unit syringes designated for U-100). The gauge of needles available for insulin administration has been reduced to very fine gauges (30–32 G) in order to minimize pain during injection. In addition to finer gauge needles, the length of needles has shortened to a minimum of 5 mm, in part, to prevent unintended IM injection. Recently, studies have shown that skin thickness is rarely >3 mm and that needles of 4–5 mm consistently deliver insulin into the subcutaneous adipose tissue (Gibney et al. 2010). The use of a new needle for each dose maintains the sharp point of the needle and ensures a sterile needle for the injection.

In recent years, the availability of insulin pen devices has made dosing and compliance easier for the patient with diabetes. The first pen injector used a 1.5-mL cartridge of U-100 insulin (NovoPen® by Novo Nordisk in 1985). A needle was attached to the end of the pen, and the proper dose was selected and then injected by the patient. The cartridge was replaced when the contents were exhausted, typically 3–7 days. Currently, 3.0-mL cartridges in U-100 strength for regular, NPH, and the range of R/N mixtures, as well as the various rapid- and long-acting insulin analogs, have become the market standard, particularly disposable pen devices with prefilled insulin reservoirs, with regard to size and strength. The advantages of the pen devices are primarily better compliance for the patient through a variety of factors including more accurate and reproducible dose control, easier transport of the drug, more discrete dose administration, timelier dose administration, and greater convenience.

■ Continuous Subcutaneous Insulin Infusion: External Pumps

As previously mentioned, solution formulations of human insulin specifically designed for continuous subcutaneous insulin infusion (CSII) are commercially available. CSII systems were traditionally used by a small population of diabetic patients but have become more popular with the recent introduction of rapid-acting insulin analogs. Currently, all three rapid-acting insulin analog formulations have received regulatory approval for this mode of delivery. Specific in vitro data demonstrating physicochemical stability for CSII has been reported for Humalog® (DeFelippis et al. 2006; Sharrow et al. 2012), Novolog®, and Apidra® (Senstius et al. 2007a, b). Pump devices contain glass or plastic reservoirs that must be hand filled from vial presentations by the patient. Some pumps have been specifically designed to accept the same glass 3-mL cartridges used in pen injector systems. Due to concerns over the impact of elevated temperature exposure and mechanical stress on the integrity of the insulin molecule along with the potential increased risk of microbial contamination, the patient information leaflets for the rapid-acting insulin analog products specify time intervals for changing the CSII infusion set as well as the infusion site. The package information leaflets should be consulted for the maximum duration each product may remain in the CSII reservoir. This time period varies with 7, 6, or 2 days listed for Humalog®, Novolog®, and Apidra®, respectively. As always, the patient information leaflets supplied with these products should be consulted for the most current information related to in-use periods.

■ Noninvasive Delivery

Since the discovery of insulin, there has been a strong desire to overcome the need for injection-based therapy (cf. Chap. 4). Progress has been made in the form of needle-free injector systems (Robertson et al. 2000), but these devices have not gained widespread acceptance presumably because administration is not entirely pain-free, device costs are high, and other factors make it less desirable than traditional injection. Extensive research efforts have also focused on noninvasive routes of administration with attempts made to demonstrate the feasibility of transdermal, nasal, buccal, ocular, pulmonary, oral, and even rectal delivery of insulin (Heinemann et al. 2001). Unfortunately, most attempts failed to progress beyond the proof of concept stage because low bioavailability, dose–response variability, and other adverse factors seriously called into question commercial viability. This situation has changed to some extent for pulmonary and buccal delivery of insulin. Several pulmonary delivery systems specifically aimed at insulin administration have advanced sufficiently through development to enable more extensive studies in human clinical trials, and comprehensive reviews examining this work in detail are available (Patton et al. 1999, 2004; Cefalu 2004). One of these insulin pulmonary delivery system, referred to as Exubera®, received regulatory approval in both Europe and the United States (White et al. 2005). Exubera® consisted of a dry powder insulin formulation composed of small geometric diameter particles produced by spray drying (Eljamal et al. 2003). The powder formulation was packaged into individual blisters and combined with an active device that incorporates a mechanical energy source to achieve dispersion and aerosolization of the particles. While the pharmacological properties reported for this Exubera® were deemed appropriate to meet prandial insulin requirements, the product was ultimately withdrawn from the market shortly after being introduced. Several reasons for the limited use by providers and patients that prompted this action include (1) need for follow-up of pulmonary function tests, (2) large delivery device, (3) insulin dose in capsule marked in mg rather than the traditional units of insulin, and (4) cost and lack of payers for reimbursement or a higher tier (co-pay) reimbursement (Garg and Kelly 2009). Consequently, the pulmonary insulin development programs of other major insulin manufacturers were terminated prior to seeking evaluation by regulatory authorities for potential marketing approval. Only one pulmonary delivery technology from Mannkind Corp. currently remains as an active development program (Pfützner et al. 2002; Richardson and Boss 2007; Peyrot and Rubin 2010; Heinemann 2012).

In addition to pulmonary insulin, a buccal insulin product, referred to as Oralin™, has been developed consisting of a solution formulation of insulin containing various absorption enhancers needed to achieve mucosal absorption (Modi et al. 2002), and a metered-dose inhaler is used to administer a fine mist into the oral cavity. Clinical study results evaluating this buccal delivery system in healthy subjects as well as patients with T1DM and T2DM have been reported (Modi et al. 2002; Cernea et al. 2004). The regulatory approval status of Oralin™ is still limited to only a few countries; however, clinical investigations are continuing presumably to acquire data needed to support additional marketing authorizations in other locations.

The future of noninvasive insulin administration is presently uncertain. Withdrawal of Exubera® was clearly a major setback for pulmonary delivery, and the situation for Oralin™ suggests a rather challenging path to regulatory approval. The lack of any significant developments in other noninvasive routes of delivery may reflect a general realization of the limited practicality of such products. Indeed, a recent examination of the scientific literature suggests there is an apparent decline in research efforts focusing on noninvasive insulin delivery (Heinemann 2012).

■ Storage

Insulin formulations should be stored in a cool place that avoids sunlight. Vials or cartridges that are not in active use should be stored under refrigerated (2–8 °C) conditions. Vials or cartridges in active use may be stored at ambient temperature. The in-use period for insulin formulations ranges from 28 to 42 days depending upon the product and its chemical, physical, and microbiological stability during use. High temperatures, such as those found in non-air-conditioned vehicles in the summer or other non-climate-controlled conditions, should be avoided due to the potential for chemical and/or physical changes to the formulation properties. Insulin formulations should not be frozen; if this occurs, the product should be disposed of immediately, since either the formulation or the container-closure integrity may be compromised. Insulin formulations should never be purchased or used past the expiration date on the package. Further information on storage and use of specific insulin products are contained in their respective patient information leaflets.

■ Usage

Resuspension

Insulin suspensions (e.g., NPH, ILPS, premixtures) should be resuspended by gentle back-and-forth mixing and rolling of the vial between the palms to obtain a uniform, milky suspension. The patient should be advised of the resuspension technique for specific insoluble insulin and insulin analog formulations,

which is detailed in the package insert. The homogeneity of suspensions is critical to obtaining an accurate dose. Any suspension that fails to provide a homogeneous dispersion of particles should not be used. Insulin formulations contained in cartridges in pen injectors may be suspended in a similar manner; however, the smaller size of the container and shape of the pen injector may require slight modification of the resuspension method to ensure complete resuspension. A bead (glass or metal) is typically added to cartridges to aid in the resuspension of suspension formulations.

Dosing

Dose withdrawal should immediately follow the resuspension of any insulin suspension. The patient should be instructed by their doctor, pharmacist, or nurse educator in proper procedures for dose administration. Of particular importance are procedures for disinfecting the container top and injection site. The patient is also advised to use a new needle and syringe for each injection. Reuse of these components, even after cleaning, may lead to contamination of the insulin formulation by microorganisms or by other materials, such as cleaning agents.

Extemporaneous Mixing

As discussed above in the section on "Intermediate-Acting Insulin," regular insulin can be mixed in the syringe with NPH and is stable enough to be stored for extended periods of time.

With regard to extemporaneous mixing of the newer insulin analogs, caution must be used. Lantus®, due to its acidic pH, should not be mixed with other fast- or rapid-acting insulin formulations which are formulated at neutral pH. If Lantus® is mixed with other insulin formulations, the solution may become cloudy due to isoelectric point (pI) precipitation of both the insulin glargine and the fast- or rapid-acting insulin resulting from pH changes. Consequently, the PK/PD profile, e.g., onset of action and time to peak effect, of Lantus® and/or the mixed insulin may be altered in an unpredictable manner. With regard to rapid-acting insulin analogs, extemporaneous mixing with human insulin NPH formulations is acceptable if used immediately. Under no circumstances should these formulations be stored as mixtures, as human insulin and insulin analog exchange can occur between solution and the crystalline matter, thereby potentially altering time-action profiles of the solution insulin analog. With regard to Levemir®, the human prescription drug label states that the product should not be diluted or mixed with any other insulin or solution to avoid altered and unpredictable changes in PK or PD profile (e.g., onset of action, time to peak effect).

SELF-ASSESSMENT QUESTIONS

■ Questions

1. Which insulin analog formulations cannot be mixed and stored? Why?
2. What are the primary chemical and physical stability issues with human insulin formulations?

■ Answers

1. Lantus®, a long-acting insulin formulation which is formulated at pH 4.0, should not be mixed with rapid- or fast-acting insulin, which are formulated under neutral pH. If Lantus® is mixed with other insulin formulations, the solution may become cloudy due to pI precipitation of both the insulin glargine and the fast- or rapid-acting insulin resulting from pH changes. Consequently, the PK/PD profile, e.g., onset of action and time to peak effect, of Lantus® and/or the mixed insulin may be altered in an unpredictable manner.
2. The two primary modes of chemical degradation are deamidation and HMWP formation. These routes of chemical degradation occur in all formulations. However, they are generally slower in suspension formulations. Physical instability is most often observed in insulin suspension formulations and pump formulations. In suspension formulations, particle agglomeration can occur resulting in the visible clumping of the crystalline and/or amorphous insulin. The soluble insulin in pump formulations can also precipitate or aggregate.

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