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Insulin for Implantable Pumps

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Abstract— **Implantable Devices require biotechnology drugs with a variety of properties. In this paper we discuss some of the more challenging issues with implantable devices.**

I. INTRODUCTION

THE purpose of this document is to describe one possible approach to formulation of a chemically and physically stable, high concentration insulin formulation suitable for use in implantable devices. In addition to the formulation itself, we describe some manufacturing and process control variations and we describe some of the requisite analytical testing required to validate the stability of the formulation..

II. THE COMPONENTS

The formulation has several components which include:

a. Water for injection
b. Insulin
c. Exogenous Zinc
d. An isotonicity agent
e. A buffer system
f. pH control
g. A preservative system
h. A surfactant system

III. THE INSULIN

The Insulin: Most of the world's experience in making physically stable insulin formulations has been with semi-synthetic human insulin, which is porcine insulin that has been enzymatically converted to human insulin. Some additional level of experience has been obtained with recombinant human insulin from e-coli based sources as well as experience with recombinant insulin lispro (Humalog). For the purposes of this formulation, only recombinant human insulin will be considered since it is commercially available and has the requisite stability profile. Key considerations of the human insulin to be used for the formulation are the relative purity the insulin as well as the identity of any impurities (a21 and b3 desamido insulin for example) as well as the native zinc content of the insulin. The final formulation requires that the total zinc content of the formulation be optimized and controlled. The chemical and physical stability of batches of insulin varies significantly between manufacturers and also between different lots from the same manufacturer so incoming inspection of a given lot must be performed as described below in the section on analytical tests.

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IV. ZINC

Zinc is a natural component of mammalian insulin production by the beta cell and is present in varying levels in recombinant human insulin. Zinc is a stabilization factor for insulin in the hexameric form and is often added to insulin formulations to increase stability. In the early 1980's there was a significant effort by Jen Brange at Novo to understand the various factors that influence insulin stability in implantable and external devices. Some of this work led to the observation that while too much zinc precipitates insulin from physiological pH solutions, the correct amounts of zinc can stabilize the insulin formulation against denaturation, particularly against the formation of aggregates. Figure 1 below shows the results of an investigation of physical stability of a given insulin formulation with various amounts of added zinc.

As is seen in the graph, native insulin as manufactured has a zinc content of about 2.5 zinc atoms per insulin hexamer. In this formulation, it has a relative stability in an aggregation test of 1. Adding zinc significantly increases the relative stability of the formulation until we reach the solubility limit. At more than 5 zinc atoms per insulin hexamer, insulin precipitates even without agitation. This work has led to U.S. patent 4,476,118 to Brange and Havelund, issued in 1984 and now expired. In the patent, the first claim is for insulin formulations that have more than 4 atoms of zinc per hexamer with an upper limit of an amount of zinc that leads to precipitation. In their ideal formulation there are 4.8 atoms of zinc per hexamer.

We have found that zinc indeed plays a very important role in stabilizing insulin formulations particularly at high insulin concentrations and particularly with respect to aggregation in pumps. There are three important considerations that need to be addressed with respect to zinc. The first is the description of "zinc atoms per hexamer". Human recombinant insulin in a formulation of U500 is predominantly but not totally in the hexameric form, and for exactness, the relative proportions of monomer, dimer and hexamer need to be calculated using the equilibrium constants for insulin in the presence of excess zinc. Unfortunately, adding zinc to the formulation moves the equilibrium towards the hexamer and there are no published reports of the relative equilibria at high zinc concentration. For practical purposes, we assume in our formulations that 96% of the insulin in the formulation is hexameric and use

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this for calculating the appropriate zinc content. More importantly than the ratio of zinc/insulin is the concept of zinc availability to bind to the exposed histidine residues of the insulin hexamer. The conceptmeans that we need to have a measure of free zinc. Zinc complexes with many other potential components of insulin formulations, particularly with buffers and we need to understand the zinc complexation chemistry to make stable formulations. For example, zinc binds very well to both phosphate and glycine and this complex chemistry can be used to control the relative amounts of zinc that are available for the formulation. This will be addressed more fully in the section on buffers.

The third important consideration with regards to zinc is the form of salt that is used in the formulation. There are a wide variety of zinc salts that could be used ranging from zinc chloride to zinc acetate to zinc oxide. In our experience we prefer to start with zinc oxide and dissolve it along with the insulin in the HCl along with the insulin (see below).

The reasons for using zinc oxide are that it is available in very pure form compared to other salts. Zinc chloride has the formula $ZnCl_2$ but in reality is a very complex mixture of zinc and chloride higher cations and anions and is difficult to get in very high quality with consistent stoichiometry and zinc acetate adds both buffering and complexing capacity to the formulation that needs to be controlled.

We recommend that the zinc salt for formulation be zinc oxide (USP grade) and that it be added to the formulation at between 4.5 and 4.8 atoms of zinc per hexamer of insulin based on insulin being 96% hexamer at U 500 concentration. In terms of formulation, the endogenous zinc concentration is measured (typically between 2 and 3 zinc atoms per hexamer or 0.3% to 0.4% by weight) then the appropriate zinc is added as zinc oxide. The appropriate final concentration of zinc is between 0.25 and 0.4 mg per 100 Units of insulin.

V. Tonicity Agents

The role of the tonicity (osmotic pressure control, osmolality control) agent in an injectable formulation for subcutaneous or intraperitoneal injection is to control the local reaction of the tissue to the formulation. Solutions that are hypotonic or hypertonic will cause osmotic pressure changes in the local tissue that is in contact with the formulation and can lead to significant local irritation and potential discomfort to the patient. Long-term (hours to days) exposure to such hypo or hyper tonic solutions can also exacerbate local immune system activation, potentially leading to site loss. Site loss is a poorly understood phenomenon where continuously infused insulin from a pump stops controlling blood glucose in spite of no loss of biological potency of the insulin in the reservoir. Historically, there has been a wide array of osmotic control agents used in insulin formulations and are broken into ionic salt based systems and small non-ionic organic molecules like sugars. The advantages of the ionic systems like sodium chloride are that only half as much total material is necessary for osmotic control since the salts ionize. Further, sodium chloride is the common ion system in-vivo. The disadvantages of NaCl based tonicity have to do with the fact that salt based systems traditionally have somewhat reduced physical stability and the presence of varying levels of other ionic impurities in USP grade NaCl. Most modern insulin formulations use small organic molecules for tonicity control, predominantly glycerin (glycerol). Glycerol solutions are isotonic at 16 mg/ml in the final formulation of insulin, and we recommend this as the general approach for tonicity control.

Glycerol is available in USP grade from two types of sources. The first and by far most common is animal derived glycerin. The second and recommended version is synthetic glycerin. Totally synthetic glycerin is a bit more costly than the animal derived material, however in our experience, synthetic glycerin leads to formulations with much better chemical and physical stability.

VI. Buffer System

The buffer system for insulin is important for both chemical and physical stability. In non-buffered insulin formulations, atmospheric CO_2 can diffuse into the formulation, lower the pH of the solution and lead to insulin precipitation. This is a well documented phenomenon in the early days of insulin pump therapy where precipitation of the insulin was a common problem in infusion sets. There are many possible acceptable buffer systems for insulin formulation, however phosphate buffer is the predominant buffer used in commercial insulin formulations today. While phosphate is the current buffer of choice for discrete injection formulations, it is not the most appropriate buffer for high concentration insulins that require exceptional chemical and physical stability. The role of zinc in helping preserve both chemical and physical stability is described above and zinc complexation with phosphate is well known. In a phosphate buffer system where zinc is present in high concentration, small changes in pH lead to correspondingly larger changes in the ratio of $H_2PO_4^-$ and HPO_4^{2-} which can significantly change the ratio of free/bound zinc thus affecting the relative stability of the formulation. We have studied a relatively large number of buffer systems and found that only TRIS and Arginine buffers can lead to sufficiently stable formulations for high concentration insulin formulations. Some data for some representative lots of starting insulin and buffer system are shown in Figure 2, below.

In this experiment, different lots of starting insulin were formulated at U400 with 4.5 zinc atoms per hexamer (calculated as the amount of free zinc, not total zinc) were formulated in different buffers. All buffer systems were at 50 mM. Aliquots of the insulin were put into sealed glass vials with Teflon beads to provide a hydrophobic surface. The vials were shaken at 100 Hz at a 1 inch stroke and turbidity was measured at intervals. Several points are

evident from the data. Different batches of starting material had significantly different stability with lot 1 showing the least stability. Secondly, phosphate buffer does not sufficiently stabilize insulin at high concentration in this system while both TRIS and arginine at 50 mM did the job well.

With this data in hand, we recommend the use of TRIS buffer in the insulin formulation. Investigations of buffer strength have been inconclusive. In some experiments higher buffer concentration improved stability, while in others there was no appreciable effect of buffer strength. For practical purposes we recommend that the total TRIS concentration be between 1.5 and 3 mg/ml. The final buffer concentration depends significantly on the expected permeability of the infusion device and the placement. For glass or other rigid reservoirs, minimal buffer capacity is sufficient, however for polymeric systems, especially for systems with significant silicone rubber components higher concentrations of buffer are required since CO2 permeability is higher in these devices. Similarly, intraperitoneal delivery will require higher buffer capacity if permeable catheters are used due to the higher local concentration of CO2 in the intraperitoneal space. A significant advantage of TRIS as a buffer is that it has a large, negative temperature coefficient of pH. Practically, this means that formulations can be obtained at relatively high pH (7.7 to 7.9) at refrigerator storage temperatures leading to excellent chemical stability due to the low temperature, while once it is put into the body and the temperature rises, the pH lowers to near physiological pH and the chemical degradation rate increases. However, once the pH is a bit lower, the chemical degradation pathway is well known, leading to mostly desamido products that have 100 percent bioactivity. More simply, TRIS allows for high pH at formulation and low temperature storage with good stability and for controlled chemical reactivity with known, benign chemical changes at body pH. As mentioned above, the exact TRIS concentration has a minimal effect on stability of the formulation, but needs to be carefully evaluated in the context of both the design of the device and the site of drug delivery; either subcutaneous or intraperitoneal.

VII. pH CONTROL

The control of pH in the formulation is of considerable concern. The same issues of tolerability and tissue irritation that are important in tonicity control are important in the control of pH. In addition, pH is important since physiological pH (7.3 to 7.5) is close to the solubility limits of insulin. Insulin is very soluble at low pH (<4) and high pH (>10) with an isoelectric point near 5.5. If the formulation pH is too close to 7.0, adventitious CO2 adsorption can lead to precipitation of insulin near pH 6.4. The chemical stability of insulin is also pH dependent as described by Jens Brange in "The Galenics of Insulin" and other publications. At high pH, several chemical degradation pathways become important including disulfide shuffling and the formation of covalent dimers. At lower pH, deamidation reactions predominate. The choice of Tris as a buffer is important due to its relatively large negative coefficient of pH with temperature as described above. It is our recommendation that insulin be formulated to have a pH of 7.4 at 37oC which means nearly 7.8 at refrigerator temperatures. It is critical to use pH meters and electrodes specifically designed (Ross electrodes) for measuring the pH of protein based solutions fro reliable results.

VIII. PRESERVATIVE SYSTEMS

The preservative system in insulin formulations serves two purposes. For any container system (vial, reservoir etc.) that can be addressed more than a single time by the user, a preservative is required to protect the formulation from adventitious bacterial contamination. Such systems need to pass the Preservative Effectiveness Test (PET) where at the end of shelf-life the container is challenged with 5 organisms and all organisms need to be killed in the formulation. In insulin formulations, phenolic preservatives (phenol and m-cresol) serve a second purpose. Together with zinc, they stabilize insulin from chemical and physical degradation by stabilizing the hexameric conformation, particularly at high insulin concentrations. This is a well known phenomenon described in the literature. The choice of preservative system depends a bit on the device. Devices that contain reservoirs that are not addressed more than once will require significantly less preservative than multi-dose vials or reservoirs since they will not have to pass the PET. In our experience, a mixed preservative system leads to optimal stability of insulin formulation. In any mixed system of phenol and m-cresol, the total preservative concentration should be between 2.7 and 3.0 mg/ml. In our experience, the preservative system that leads to optimal stability is one where the m-cresol concentration is approximately 1.6 mg/ml and the phenol concentration is approximately 1.4 mg/ml.

IX. SURFACTANT SYSTEMS

Surfactants, particularly non-ionic surfactants near their critical micelle concentration are remarkably effective at stabilizing insulin formulations. The pioneering work of Horst Thurow at Hoechst in the development of surfactant systems for insulin is encapsulated in U.S. patents 4,885,164 and 4,783,441. In those patents, Thurow identifies Genapol PF10 as an ideal surfactant for insulin formulations. Genapol is a block copolymer of ethylene oxide and propylene oxide and there are many compounds in this family. The preferred compound at this time is Pluronic F-68 which is chemically identical to Genapol. The concentration of Pluronic or Genapol is adjusted to between 80% and 90% of the critical micelle concentration which for pure water is about 10ppm. The exact concentration for the surfactant needs to be established empirically by measuring the CMC in the formulation then adjusting to 85%. One critical issue with the surfactant is the analytical method used for testing and

qualification. There are to our knowledge, no good analytical methods for this surfactant at this concentration. Compendial methods use starch iodine techniques and are semi-quantitative at best.

X. MANUFACTURING

The process for manufacturing the solution is important. In general, protein formulations can be made by dissolving the insulin at high pH then adjusting downward, or at low pH and adjusting upward through the PI and then to physiological pH. The latter method leads to far more stable formulations in our experience. Typically, one makes a suspension of the insulin, zinc oxide, glycerin, phenol and m-cresol at the required concentration, adds HCl to the mix to get to pH 2 to 2.5 then stirs to get the insulin crystals to dissolve. The pH is then adjusted slowly (20 to 30 minutes) to physiological pH, then the appropriate amount of buffer, already adjusted to pH 7.4 is added to the formulation.

XI. TESTING

The testing of the formulation is based on HPLC methods (insulin content, insulin related proteins, high molecular weight, and purity) and physical methods for estimation of physical stability and aggregation. The aggregation testing is best tested using physical insult (shaking) in the presence of a hydrophobic interface (Teflon) using Thioflavin T fluorescence as the output.