

## II. LITERATURE REVIEWS

Various quality-control materials, which used in clinical chemistry laboratory and for interlaboratory principally differed in their origins (human, various animal systems, or synthetic) or their physical states (liquid or dry). The stability is a prime requirement for such specimens, therefore specimens are distributed in lyophilized form (Grannis and Miller, 1976, Fraser, et al., 1978, Stamm, 1974). It was reported that freeze-drying process altered certain physical-chemical properties of the components (Pichel, 1965, MacKenzie and Luyet, 1967). For example when human  $\beta$ -lipoproteins are irreversibly denatured, serum viscosity is changed and its turbidity increased (Atwood and Marshall, 1973, Proksch and Bonderman, 1976). If  $\beta$ -lipoprotein is removed before lyophilization, to minimize specimen turbidity (Proksch and Bonderman, 1976), this also results in a material that is fundamentally altered in physiological and chemical characteristics. Other typical modifications of quality-control sera include dialysis and addition of matrix expanders, preservatives, antimicrobial agents, and (or) clarifying agents were also reported (Klein and Weissman, 1958, Frajola and Maurukas, 1976, Rush and Vlastelica, 1974, Schultz and Gates, 1978).

Concentrations of constituents may also be made abnormal in attempts to simulate specimens from patients with certain characterized diseases. Such specimens, prepared by dilution or addition of pure materials to a normal specimen (Bowers, et al., 1977) may differ from the corresponding abnormal fresh patients' sera because metabolites or other substances are lacking. Therefore, a result by a nonspecific method that is influenced by one or several metabolites will not be affected in the case of such a supplemented quality-control material in the same way as it would by a freshly collected patient's serum. Similarly, if enzymes or peptide hormones purified from animal origin are added to a control serum, the resulting mixture may behave differently from human sera (Fasce, et al., 1973, Rej, et al., 1975, Bowers, et al., 1967, Yalow, 1978). The composition of the matrix such as albumin, enzyme concentrations and physical characteristic etc., may differ from native serum and among sources. Finally, the pH of lyophilized control materials differs significantly from that of normal or patients' sera (Louderback and Anido, 1975).

Lyophilization is commonly used to prepare protein products such as control serum or protein pharmaceuticals in dry form to extend shelf life. Operating cycles may be 24 hours to several days and typically

involve three process segments: freezing, primary drying, and secondary drying. Freezing transforms the protein-excipient solution in a vial into two or more phases, usually crystalline ice and an amorphous freeze-concentrate containing the protein, lyoprotectant, and water. Additional phases may also form if the excipient precipitates or crystallizes out during freezing. Primary drying is the sublimation of ice from the frozen vial content under vacuum. Secondary drying, also under vacuum, involves the removal of water from the freeze-concentrate, reducing the residual moisture content to a level (e.g.  $\leq 3$  wt %/wt) suitable for long-term storage (Pikal, et al., 1991).

In lyophilization, the longest process segment is usually the sublimation of ice from frozen vials. The rate of ice removal is a function of the lyophilizer shelf temperature and chamber pressure (Nail, 1980, Pikal, et al., 1984, Livesey and Rowe, 1987, Pikal and Shah, 1990, Chang and Fischer, 1995). The product temperature, which is also dependent on the shelf temperature and the pressure, is often 20-30<sup>0</sup>C below that of the shelf as a result of the energy consumed by the transformation of ice to water vapor. The product temperature during primary drying is critical, because too high a temperature can result in product meltback or collapse (Pikal, 1985) (potentially degradative events), whereas too low a temperature will result in lengthy lyophilization cycles. The product temperature accompanying a given temperature-pressure combination may vary between formulations.

The most common used control material in clinical chemistry laboratory is the lyophilized serum in which the preparative process can denature protein and lipoprotein. Lin, et al. (2000) found that secondary structure of human serum albumin denatured after lyophilization (Lin, et al., 2000). Reconstitution of the lyophilized serum in different laboratory may cause vial-to-vial variation. The turbid effect obtained after reconstitution of the product changed the characteristic of serum being used as a control material.

Proksch and Bonderman (1976) suggested the precipitation of pre-beta and beta-lipoproteins with dextran sulfate and divalent metal ions for preparing an optically clear human serum but serum is removed lipoproteins did not favor in clinical chemistry laboratory.

Recent study reported that some stabilizers or lyoprotectants for protect protein and lipid denaturation mostly are disaccharides, which are one type of excipients. There are many excipients, which used to add in lyophilized samples to maintain stability of the products during lyophilized process. These excipients are also referenced as stabilizers,

chemical additives, co-solutes, co-solvents. They are classified as followed (Wang, 2000).

1. Sugars/polyols : saccharose, trehalose, mannitol, sorbitol, glucose, maltose, galactose, etc.
2. Polymers : serum albumin, dextran, polyvinylpyrrolidone (PVP), hydroxypropyl methylcellulose (HPMC), hydroxyethyl cellulose (HEC), gelatin, etc.
3. Non-aqueous solvents : ethylene glycol, polyethyleneglycol, glycerol, dimethylsulphoxide (DMSO), etc.
4. Surfactants : Tween 80, Triton X-10, Sodium dodecyl sulfate (SDS), etc.
5. Amino acids : glycine, proline, serine, alanine, phynylalanine, etc.
6. Miscellaneous : salts (potassium phosphate, ammonium sulfate, magnesium sulfate, sodium acetate, etc.), metal ions ( $Zn^{2+}$ ,  $Cu^{2+}$ ,  $Ca^{2+}$ ,  $Mn^{2+}$ ,  $Mg^{2+}$ , etc.)

The effect of excipients in stabilizing of protein and enzyme were reported by several researchers. In 1998, Sampedro described effect of carbohydrates to protect plasma membrane  $H^+$ -ATPase from *Kluyveromyces lactis* during freeze-during and rehydration. The protective efficiency of carbohydrates was as trehalose > maltose > sucrose > glucose > galactose which suggested that these carbohydrates might protect other membrane enzymes (Sampedro, et al., 1998).

Heller, et al. (1999) showed that phase separation and protein damage during freeze-dried process might also be avoided by addition of mannitol at concentrations sufficient to cause crystallization. Allison, et al. (2000) reported that addition of dextran to sucrose or trehalose inhibit protein unfolding during lyophilization and improved storage stability. These results demonstrated that hydrogen bonding between carbohydrate and protein is necessary to prevent dehydration-induced protein damage (Allison, et al., 1999).

Disaccharides, such as sucrose and trehalose, are capable of maintaining high levels of native protein conformation during freeze-dried process (Duddu and Monte, 1997, Prestrelski, et al., 1993, Chang, et al., 1996, Kreilgaard, et al., 1999, Allison, et al., 1998, 1999). The use of trehalose as a stabilizer has been extensively studied (Kreilgaard, et al., 1999). However, it was demonstrated that trehalose was less effective than sucrose at inhibiting lyophilization-induced unfolding of lysozyme, especially at high initial concentrations of protein and sugars (Allison, et al., 1999). This effect may be due to the greater propensity of trehalose to

phase separate from polymers during freeze concentration (Izutsu, et al., 1996).

As described above that carbohydrate can preserve protein (enzymes) conformation (inhibit protein unfolding) during lyophilization and improve product stability during storage. Therefore it is important to investigate such these freezing and drying effects of lyophilization on protein, enzymes and lipoprotein formulated in control serum, which was widely used all over the world for quality control in clinical chemistry laboratory. The stability, the turbidity of the products and advantages of the lyophilized process to produce a stabilized bovine serum matrix quality control serum are also evaluated.

## OBJECTIVES

1. To examine the effect of lyophilized process on protein, enzymes and lipid components in two level bovine control sera.
2. To compare the effects of saccharose, trehalose, mannitol and dextran on the stabilization of protein, enzymes and lipoprotein during lyophilized process of a bovine-based control material.
3. To investigate the effect of selected disaccharide in maintaining the storage shelf life of the control products in dried solid form.
4. To prepare and evaluate the stability of two level bovine-based control serum stabilized with the selected disaccharide for use in quality control work in clinical chemistry laboratory.