Effect of excipients on product characteristics and structure of lyophilized 
lasota vaccine

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The research was aimed to minimize product defects and lyophilization-induced denaturation of lasota vaccine. Sugars alone produced coherent cake but failed to protect virus during lyophilization. Maillard cake browning due to denaturation was evident in N,Z amine products. Polymers, PF-127 and PVP K-90 were able to produce porous cake structure and thus showed efficient water removal. DSC curves reveal endothermic melting corresponding to partial crystallization of PEG-6000 and PF-127, while remaining plugs were amorphous. The X-ray diffraction confirms the DSC findings. Aqueous IR of harvest revealed symmetrical $\alpha$-helix of virus. Loss of alpha helix in lasota products is indicated by the decreased absorbance of 1654 cm$^{-1}$ band. The qualitative comparisons of $\alpha$-helix region in aqueous IR spectra are in correlation with antibody titer. Trehalose with most excipients gave better titer than sucrose. The stable glass matrix with arrested molecular mobility prevented unfolding of lasota. PF-127 was better stabilizer than PEG-6000 but both crystallized during freezing. Trehalose-PVP K-90 showed optimum product characteristics and maximum protection to antigen.

Keywords: additives, antibody titer, DSC, FTIR, lasota, lyophilization, XRPD
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Introduction

Biotechnological advances have resulted in availability of large number of peptide drugs and vaccines as therapeutics in prevention and treatment of diseases. Recombinant DNA and hybridoma technology have successfully produced pharmaceutical biomolecules, e.g. tissue plasminogen activator (Activase), human growth hormone (Protropin), sargramostim GMM-CSF (Leukine), cytochrome c and hemoglobin$^{1,2}$. Unfortunately, the art of turning these proteins into stable formulations has been developed at a much slower pace than the technology of producing these therapeutically important proteins. Therapeutic proteins are large complex molecules. Their aqueous solutions are prone to loss of activity due to interaction involving covalent bonding and denaturation due to conformational changes. Aggregation and adsorption during processing has been reported for sensitive biologicals$^3$. To optimize protein stability during storage and shipping, lyophilized dehydrated protein products are preferred. However, lyophilization itself induces stresses capable of denaturation. Stresses those routinely recognized are cold temperature, exposure to concentrated solutes due to crystallization of water, pH changes and generation of ice-water interface$^4$. Freezing induced denaturation of proteins is a major concern in the development of protein pharmaceuticals. The stability of peptide formulations cannot only be evaluated by potency test only and requires advanced analytical techniques$^5$.

Lasota vaccine consists of Newcastle Disease Virus (NDV), which is a paramyxovirus$^6$. The spherical virus particles range 100-300 nm in diam. The envelope contains the myxovirus fringe consisting of haemagglutinin and neuraminidase protein. A layer of lipid is present below the projections on the envelope. The use of lipid solvent causes the disaggregation of the lipid layer and hence disruption of the virion. Ribonucleoprotein, an internal component, forms a symmetrical helix, which has a periodicity of 17 nm. This single-stranded RNA virus is genetically diploid or polyploid in nature and has a molecular weight of $10^{10}$. It is a thermolabile virus and can be fully inactivated by incubation at 60$^\circ$C for 30 min$^7$.

The present investigation was aimed to prevent vaccine denaturation during lyophilization and

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remove common product defects (puffing, product collapse, partial melt back etc.) for adequate shelf stability and improved antibody titer. NDV strain grows in embryonating eggs in the allantoic sac without requiring adaptation for isolation. Lasota is a live veterinary vaccine. Excipients that stabilize protein need to be individually optimized for a given protein (lasota vaccine) and stress. The optimized lyophilization cycle from our previous study was utilized in evaluating the protection offered by additives. The ability of sucrose or trehalose to prevent protein unfolding during freeze drying by increasing free energy for protein unfolding is tested. The consequent growth of current investigation also includes the study of pluronic PF-127, polyvinyl pyrrolidone (PVP K-90), PEG-6000 and N,Z amine as stabilizer against lyophilization induced cryoinjury. The effect of these additives, as single component and in combination, on antibody titer is reported and the stability of lasota vaccine during lyophilization is critically analyzed using aqueous IR, X-ray powder diffractometry (XRPD) and DSC techniques.

Materials and Methods

The harvest of lasota and specific pathogen free (SPF) eggs were kindly provided by Ventri Biological Pvt. Ltd. (Vaccine division), Pune, India. Pluronic PF-127 (Lutrol) was procured from BASF Corporation, Mount Olive, New Jersey. Trehalose dihydrate was a gift sample from British Sugar Plc, Peterborough, UK. N,Z amine gift sample was supplied by Quest International, Norwich, New York. Potassium phosphate monobasic (J T Baker, Phillipsburg, USA), sucrose, PEG-6000 and PVP K-90 of analytical grade were purchased from Space Chemicals, Nashik, India.

Stabilizer Composition and Lyophilization of Lasota Vaccine

SPF eggs were inoculated and incubated for 72 hrs for harvesting NDV as per Cottral technique. Eggs chilled overnight at 4°C were surface sterilized with 70% alcohol. Vacuum (12.5 cm of water) aspiration of the allantoic fluids in harvesting bottles was performed aseptically. The fluids were harvested into containers of 500 ml capacity. The bottles were removed, closed, and placed immediately in a refrigerator at 4°C. Bacteriological sterility of harvest, antibody titer and second derivative aqueous IR were performed.

The effect of additives was studied as single components and as binary mixtures in stabilizer separately. The stabilizer composition (stock diluent, 25 ml) for 100 doses of vaccine (1.7 ml fill volume) includes 22.5 ml distilled water, 0.5 ml potassium phosphate monobasic (KH2PO4) buffer (pH 4.4), 2 ml di-sodium hydrogen orthophosphate (Na2HPO4) buffer (pH 9.2) and 16% (w/v) each of sucrose and N,Z amine. The stabilizers for different single component systems were prepared by inclusion of sucrose (6.4%, w/v), trehalose (6.4%, w/v), PVP K-90 (4%, w/v) and pluronic PF-127 (4%, w/v) separately. For a binary mixture of stabilizers, sucrose (6.4%, w/v) was kept constant and the effect of PVP K-90 (3.2%, w/v), pluronic PF-127 (3.2%, w/v), N,Z amine (3.2%, w/v) and PEG-6000 (3.2%, w/v) was studied separately. Similar studies were carried out replacing sucrose with 6.4% (w/v) trehalose, keeping the excipients and their strength constant.

The stabilizer was autoclaved for 20 min at 121°C and mixed aseptically with harvest (1:4 ratio) and the mixture was filled in 2 ml vials. Vials were then placed in tray with half-closed rubber closures and lyophilized using Virtis Advantage lyophilizer with identical cycle conditions for both types of formulations (Fig. 1).

Evaluation of Lyophilized Product

Analysis of Product Characteristic—Lyophilized cakes were observed for product features and photographed. The cake height and thickness was measured. Reconstitution time was determined by injecting 2 ml potassium phosphate buffer (pH 7.4) in the vial and measuring the solution time without visible aggregates. The residual moisture was determined by Karl-Fisher titration. The product vials
were reconstituted in 30 ml of distilled water and pH of the reconstituted solution was measured.

**Thermal Behaviour of Products**—Samples (1.5-7 mg) were analyzed in crimped, vented aluminum pans under a dry nitrogen purge using DSC 821° (Mettler Toledo) with an automated liquid nitrogen cooling accessory. Samples were heated from 25 to 220°C with scanning rate of 20°C/min. DSC thermograms were analyzed for thermal behaviour.

**XRPD of Products**—The XRPD analysis of all the colyophilized product batches was performed using Philips PW1729 X-Ray diffractometer. Powdered product cakes were mounted on sample holder and scanned between 20 range of 2-100° at a scan speed of 0.1° 20/sec with radiation of 1.524 Å. The XRPD analysis of pure PEG-6000 and plutonic PF-127 was also performed.

**FTIR Analysis of Colyophilized Products**—Second derivative aqueous IR of colyophilized cakes were recorded (1620-1690 cm⁻¹, 20 scans in each run) at room temperature with Perkin-Elmer spectrum 2000 and analyzed with Origin software. Aqueous solution of trehalose and sucrose containing mixtures, separately (reconstituted with 2 ml of distilled water) in CaF₂ window with 20 μm path length were subjected to IR analysis.

**Antibody Titer of Lyophilized Products**—The cakes were reconstituted to 1.7 ml solution with stock diluent. Dilutions of 0.5 ml of reconstituted solution with 4.5 ml of stock diluent in 10⁻¹-10⁻¹⁰ range were prepared aseptically to be inoculated into susceptible hosts (SPF egg embryo). The non-specific deaths within 24 hrs were eliminated. Host embryos were examined daily (for 7 days) and categorized as infected or dead. The 50% end point is determined by Reed and Muench method.

**Results and Discussion**

**Harvest Characteristics**
The antibody titer (potency) of the present batch, used for formulation optimization, was found to be 9.13. The titer of this potency indicates that 10⁸-10⁹ of active virus antigen are present in 1 ml of the harvest. The harvest of all the batches was allowed to reach the same titer to maintain uniformity of potency throughout the studies. The absence of microbial contamination of harvest was confirmed, thus assuring the aseptic processing of the harvesting technique.

The aqueous IR of isolated harvest, diluted suitably (4:1) with water for injection, revealed the peak at 1653-1654 cm⁻¹ favouring α-helix structure of paramyxovirus strain. Lower intensity peaks for turns or bends at 1670 cm⁻¹ and 1685 cm⁻¹, disordered structure at characteristic 1645 cm⁻¹ and β-sheet structure at 1637 cm⁻¹ were also observed. The secondary protein structure is derived from the steric relations of amino acids that are close to one another. The periodic secondary structures, α-helix and β-sheet are held together by a delicate balance of non-covalent forces (hydrophobic, ionic, van der Waals interactions and hydrogen bonding). The disulphide covalent linkage between sulfur containing amino acids contributes substantially to maintain the protein conformation. The water soluble proteins has a hydrophobic core of non-polar amino acids surrounded by a hydrophilic shell of polar-solvated amino acids, e.g. γ interferon, granulocyte colony stimulating factor etc. The structural features of lasota virus showed a symmetrical helix in the internal component of ribonucleoprotein form. However, the α-helix native like structure of virus could be attributed to haemagglutinin-neuraminidase protein (HN) and fusion protein (F). Although other peaks were observed, the symmetrical helix of the antigen was predominant and the existence was confirmed in aqueous infrared spectrum.

**Effect of Additives on Product Characteristics**
The effect of single component, i.e. sucrose (6.4% w/v), trehalose (6.4% w/v), pluronic PF-127 (4% w/v) or PVP K-90 (4% w/v), on product characteristic and dimension was observed. With PVP K-90 and PF-127, the product remained as intact cake of uniform dimension without puffing, which could be due to establishment of mechanically stable three-dimensional cake structures in presence of the polymers. The solid plug seemed more porous with PVP K-90 as compared to PF-127, which might be attributed to higher swelling capacity of PVP. However, a total collapsed product was observed with sucrose and trehalose, indicating that sugars alone is not sufficient to build up the cake structure. Sugars used in concentrations less than 20% (w/v) have earlier been also resulted in collapsed product. The colyophilized product batches containing sucrose and N,Z amine showed higher moisture content and highly friable, disintegrated (or disappeared) product (Fig. 2, Table 1). In both cases, the higher residual moisture content could be responsible for hydrolyzing the low volume cake. Although, insignificant puffing was observed but a brownish discolouration...
indicated denaturation of antigen as well as N,Z amine.

Sucrose in single component system failed to build up 3-dimensional structure but in a binary system with PVP K-90, the product formed as intact cake with uniform consistency and without puffing or collapse. PVP K-90 in very low concentration has the property of holding the solid plug. However, product shrinkage, puffing at surface and dense cake was obtained with sucrose and PF-127 additives. The hydrophobic association of polyoxypropylene chains in PF-127 during dehydration could have caused the shrinking of cake. PEG-6000 along with sucrose gave granular cake with larger irregular air spaces.

Trehalose in combination with N,Z amine showed non-homogenous cake browning with partial meltback at the bottom. The observed browning indicated significant protein degradation via Maillard reaction of both the antigen and N, Z-amine. The presence of trehalose prevented puffing that was seen with its sucrose combination. Trehalose with PVP K-90 formulation gave highly porous, white cylindrical coherent cake without any product defect. However, effect of trehalose with PF-127 on the product was found similar to sucrose with PF-127. Pitfalls in the solid plug characteristic and dimension might be reflected in titer of the product. Therefore, an elegant, highly porous cake with optimum dimensions is desired for efficient water removal during lyophilization and shelf life stability.

The residual moisture content is the critical parameter responsible for product characteristics, potency and stability. Increased moisture initiates hydrolysis and degradative chemical pathways and imparts undesirable product characteristics. The lowest moisture content was observed with PF-127 (0.74±0.25), followed by PVP K-90 (2.61±0.72), which suggests that the rate of water vapour diffusion is favoured through the larger surface area retained due to higher porosity of cake. The initial polymer hydration in aqueous system helps to retain high residual cake porosity. Sugars are inadequate in producing uniform cake as increased resistance to water vapour removal gives product with higher moisture content (6.72±1.03), which is responsible for partial product collapse. Sucrose and trehalose batches containing N,Z amine also retained higher moisture (Table 1); hence, showed shrinking and produced high density and drum shaped cakes.

Reconstitution of lyophilized cake is likely to affect protein unfolding and/or refolding. The time for

<table>
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<tr>
<th>Table 1—Effect of additives on product characteristics</th>
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<tr>
<td><strong>Stabilizer components</strong></td>
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<tr>
<td></td>
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<tr>
<td>Sucrose-N,Z amine</td>
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<tr>
<td>Sucrose-PVP K-90</td>
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<td>Sucrose-PF-127</td>
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<tr>
<td>Sucrose-PEG-6000</td>
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<tr>
<td>Trehalose-N,Z amine</td>
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<td>Trehalose-PVP K-90</td>
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<tr>
<td>Trehalose-PF-127</td>
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<td>Trehalose-PEG-6000</td>
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Each reading is an average of three measurements
complete rehydration of the solid plug (without visible aggregate) can be altered by the product nature. Also the lower reconstitution time is indicative of the uniform drying and cake structure. The differences for reconstitution time in different treatments were insignificant and found to be in the range of 60-90 sec. Formulations containing mixture of trehalose-PF-127 and sucrose-PF-127 and all formulation as such containing polymers with sugar additives interestingly showed shorter reconstitution time because of higher water absorption and penetration through porous cake. The pH changes in the formulation during freezing or drying affect the activity of protein, stability and its conformation. The denaturation due to pH change is characteristic of an individual protein and its native protein structure. The changes in pH of various formulations were found insignificant, which might be due to neutral additives.

**Thermal Behaviour of Colyophilized Products**

The characteristic melting endotherms were observed in sucrose as well as trehalose batches containing PF-127 or PEG-6000 (Fig. 3). Whereas, all other batches did not show melting endotherm at about 54-65°C, revealing amorphous nature of the colyophilized products and suitability for long-term shelf stability. PF-127 and PEG-6000 have melting points of 52-57 and 55-63°C, respectively, which correspond to the melting endotherms of formulations where they were present. These findings reveal that both PEG-6000 and PF-127 are partly crystallized out during lyophilization. Further, the identical behaviour of PF-127 and PEG-6000 could be due to the existence of similar polyoxyethylene chain in both the polymer. The glass transition temperature (Tg) of sucrose is 75°C for which no endotherm is seen; therefore, sucrose is retained in amorphous form22,23. However, continuous decomposition of product is seen after melting. The transition point of trehalose is 120°C and was not obtained in any formulation similar to sucrose.

N,Z amine formulations with sucrose and trehalose showed diffused endothermic energy changes at 80-120 and 80-140°C, respectively. This might be due to the gradual loss of higher water retained in batches. Small exothermic crystallization of sucrose-N,Z amine batch around 180°C followed by endothermic melting was observed as reported earlier24. Whereas, trehalose-N,Z amine batch showed second endotherm initiated product decomposition at around 200°C. Sucrose-PVP K-90 batch showed broad diffused endotherm, assigned to gradual loss of moisture, and small exotherm around 150°C, indicating residual crystallization and decomposition of sucrose. However, trehalose-PVP K-90 showed only small endothermic transition for moisture loss at around 100-130°C. Thus, the results of DSC study reveal that PEG-6000 and PF-127 containing formulations show endothermic melting corresponding to partial crystallization.

**XRPD Pattern of Products**

Binary co-lyophilized mixtures of both sucrose and trehalose with PVP K-90 and N,Z amine were found to be amorphous in nature as shown by diffused maxima in 2θ range of 16-26° (Fig. 4). XRPD patterns of PEG-6000 and PF-127 alone, and each in combination with sucrose and trehalose showed characteristic peaks of crystalline material. Coincidentally, peaks were observed around the same 20 as that of crystalline sucrose23. However, both the sugars at very low concentration (3.2%, w/v) are available in amorphous glassy state in lyophilized
products. Carpenter et al\textsuperscript{25} has developed a two-component system for stress specific stabilization during lyophilization, where PEG-6000 was used as a cryoprotectant. Other carbohydrates could also be used as protectant during dehydration. PEG-6000 alone completely stabilizes proteins during freeze thawing. However, it provides little or no protection during dehydration because it crystallizes during lyophilization. Pluronic PF-127, a polyoxyethylene-poloxylpropylene (70:30) block co-polymer with molecular weight of 12,500, may crystallize out similar to PEG-6000 in presence of carbohydrates. Characteristic peaks observed in 2\(\theta\) range of 17.4-21.8° and corresponding melting endotherms confirm the crystallization of PEG-6000 and PF-127 (Figs 3 & 4).

Second Derivative Aqueous IR Analysis

Aqueous IR spectroscopy in amide I region has been used for analysis of secondary protein structure and stress-induced alterations in protein conformation\textsuperscript{26-28}. Structural information is obtained from conformationally sensitive amide I band located between 1620-1690 cm\(^{-1}\). This sensitive technique is extensively used in resolution enhancement of lyophilization induced structural transitions (Fig. 5). Amide I band is for the in plane C=O stretching vibration, weakly coupled with C–N stretching, and in-plane N–H bending. Each type of secondary structure (i.e. \(\alpha\)-helix, \(\beta\)-sheet structure and unordered segment) give rises to a different C=O stretching frequency. The FTIR spectra of 21 globular proteins, including the components revealing the amide I band, and assignments for helical segments, extended \(\beta\)-segments, unordered segments as well as turns has been reported extensively \textsuperscript{29}. Loss of \(\alpha\)-helix in \textit{lasota} formulations is indicated by the decreased absorbance of 1654 cm\(^{-1}\) band, which is compensated by increased absorbance in \(\beta\)-sheet, turns and bends. The amide I frequencies and assignments (cm\(^{-1}\)) in case of \textit{lasota} formulations were made as per Byler method\textsuperscript{29} (Table 2). These changes were attenuated when the

![Fig. 4—XRPD pattern of products: a. PEG-600, b. PF-127, c. Sucrose-PEG-6000, d. Sucrose-PF-127, e. Sucrose-PVP K-90, f. Sucrose-N,Z Amine, g. Trehalose-PEG-6000, h. Trehalose-PF-127, i. Trehalose-PVP K-90 & j. Trehalose-N,Z Amine](image)

protein was lyophilized in the presence of stabilizer, documenting an increased retention of native structure in the molecular population. A qualitative visual comparison of second-derivative spectra reveals the influence of additives on protein structure during lyophilization. Formulations with high titer, i.e. trehalose-PVP K-90, showed significant protection of α-helix as indicated by a sharp peak at around 1654 cm⁻¹, indicating significant retention of native structure (α-helix). Antibody titer reflects the potency of vaccine and hence ultimately the stability of α-helix structure.

Antibody Titre of Products

The qualitative comparisons of α-helix region in aqueous IR correlate well with the observed antibody titer of products. The low titer formulations showed increased intensities for β-sheet structure and turns and bends (sucrose-PF-127, trehalose-PF-127, trehalose-N,Z amine, Sucrose-PVP K-90). The retention of higher percentage of helix conformation indicates stabilization of antigen structure due to additives (trehalose-PVP K-90). Antibody titers of the single component product differentiate by insignificant log margin of 0.18 (5.06±0.09). This confirms that single components have not provided effective protection during freeze-drying. PF-127 and trehalose showed less protection. While being very bulky, the hydrogen bonding ability of PVP K-90 to the charged and polar groups on the dried protein was minimized due to steric hindrance. Trehalose with all the additives gave better titer than sucrose except in case of N,Z amine. Trehalose offered protection by both the water substitution and glassy state mechanisms. Moreover, trehalose formed stable glass and arrested molecular mobility of proteins more effectively than sucrose and hence less unfolding of α-helix structure. Similar results have also been reported on human growth hormones. Trehalose-PF-127 and trehalose-PEG-6000 compared to sucrose batches reveal better protection in the presence of sucrose (1 or 0.125%), native form of anti-L selectin has substantially been retained (as revealed by FTIR analysis) during lyophilization. However, in the present case sucrose did not show the desired protection. Sucrose with PEG-6000 proved its protectiveness during freezing but crystallized out during dehydration, hence showed the lower titer. PF-127 contains higher fraction of polyoxyethylene chains and therefore expected to give more protection than PEG-6000 during freezing state (higher titer) but crystallized during drying and hence, afforded lesser protection than expected. N,Z Amine possesses protein dilution effect required for protecting native like structure of lasota vaccine. Although, improved titers were obtained with N,Z amine but the batch retains 7% of moisture and undesirable cake characteristics. However, similar ability of heparin has been reported in protecting fibroblast growth factors. Sucrose-PVP K-90 gave necessary plug structure but its inefficient hydrogen bonding resulted in low titer.

### Table 2—Second derivative amide-I aqueous IR frequencies and assignment

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Extended chains (β)</th>
<th>Helix</th>
<th>Unordered</th>
<th>Turns and bends</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvest</td>
<td>1637±3, 1631±3, 1624±3</td>
<td>1654±3</td>
<td>1645±2, 1663±4</td>
<td>1670±4, 1683±2, 1688±2</td>
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<tr>
<td>Sucrose-N,Z amine</td>
<td>1637.5, 1625.2, 1654.6</td>
<td>1664.7, 1659.3</td>
<td>1673.8, 1680.1, 1689.9</td>
<td></td>
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<tr>
<td>Sucrose-PVP K-90</td>
<td>1638.1, 1629.1, 1619</td>
<td>1654, 1648.4</td>
<td>1669.8, 1679.5, 1689.5</td>
<td></td>
</tr>
<tr>
<td>Sucrose-PF-127</td>
<td>1637.9, 1630.9, 1624.7</td>
<td>1643.6, 1661.8</td>
<td>1668.1, 1684.8, 1690.7</td>
<td></td>
</tr>
<tr>
<td>Trehalase-N,Z amine</td>
<td>1638.9, 1629.6, 1623.6</td>
<td>1649.8, 1643.9, 1662.9</td>
<td>1669.7, 1684.8, 1689.8</td>
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<tr>
<td>Trehalose-PVP K-90</td>
<td>1638, 1629.9, 1623.3</td>
<td>1654, 1619.2, 1648.9</td>
<td>1661, 1668.7, 1691</td>
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<tr>
<td>Trehalose-PF-127</td>
<td>1638.1, 1630.9, 1623.9</td>
<td>1653, 1619.2, 1648.5</td>
<td>1644.2, 1659.1, 1673.9, 1673.9, 1684.7, 1691.9</td>
<td></td>
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<tr>
<td>Trehalose-PEG-6000</td>
<td>1638.9, 1631.5, 1624.7</td>
<td>1649.1, 1664.7, 1669.7</td>
<td>1663.6, 1674.5, 1682.5, 1688.2</td>
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</table>

Most protein pharmaceuticals are multicomponent systems. Sucrose offers protection through water substitution and glassy state mechanisms.
against cold denaturation. Uniform and homogenous plug of low moisture with trehalose and PVP K-90 formed stable glass and arrested molecular mobility; hence, prevented unfolding of higher order protein structure. PVP K-90 has been reported to inhibit hence, prevented unfolding of higher order protein formed stable glass and arrested molecular mobility; plug of low moisture with trehalose and PVP K-90 against cold denaturation. Uniform and homogenous three-dimensional structures to the lyophilized cake 33. Therefore, the maximum titer of trehalose-PVP K-90 was reported. The restriction of translational and relaxational molecular movements prevented protein unfolding and subsequent dilution of protein within glass matrix led to spatial separation of proteins to prevent protein aggregation.

Conclusion

On the basis of above results, it may be conclude that single components used, viz. sucrose (6.4% w/v), trehalose (6.4% w/v), PF–127 (4% w/v) and PVP K-90 (4% w/v), does not minimize product defects and lyophilization-induced conformational changes in symmetrical α-helix of lasota virus. Sugars alone are inadequate in building uniform cake and with non-polymer excipients retain higher moisture. The thermal behaviour and XRD pattern reveal partial crystallization of PEG-6000 and PF-127 in combination with sugars. The qualitative comparisons of amide I region (α-helix) in aqueous IR spectra are found in correlation with antibody titer. Trehalose with most excipients gives better titer than sucrose. The trehalose-PVP K-90 gives stable glass matrix with arrested molecular mobility of α-helix and minimizes lasota denaturation during lyophilization. The study reveals that stabilizer combinations should be optimized for a given protein and process stresses.

Acknowledgement

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