Vacuum Foam Drying for Preservation of LaSota Virus: Effect of Additives

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ABSTRACT

The purpose of this research was to apply vacuum foam drying (VFD) for processing of LaSota virus and to screen formulation additives for its stability. The aqueous dispersion of harvest containing sucrose or trehalose in combination with additive (monosaccharides, polymers, N-Z-amine) was prepared. The diluted dispersions in vials were vacuum concentrated, foamed to form a continuous structure, and vacuum dried. The products were evaluated for foam characteristics, residual moisture, virus titer, x-ray diffraction pattern, and stability profile. The foamability increased with solid content in solutions. The foamability of sucrose was enhanced with incorporation of N-Z-amine (10% and 15% wt/vol) and polyvinyl pyrrolidone (PVP K30, 3% wt/vol). The fructose- or galactose-containing mixtures were deposited irregularly on the vial surface. The virus titer increased with disaccharides in the formulation. Sucrose provided better protection than trehalose. Unlike lyophilization, N-Z-amine with sucrose protected the virus from Millard's Browning. Amino acids do not have a catalytic effect on hydrolysis of sucrose during VFD. Monosaccharides were ineffective. A synergistic effect of PVP K30 or polyethylene glycol 6000 (3% wt/vol) with N-Z-amine provided the maximum virus titer (6.97 and 7.15, respectively). This formulation retained the desired virus potency at 5°, 25°, and 40°C. The diffraction pattern revealed that a threshold concentration of N-Z-amine was required for inhibiting crystallization of sucrose during VFD. VFD was successfully applied to produce a solid LaSota formulation. The products were amorphous and did not devitrify on storage.

KEYWORDS: Foam drying, LaSota virus, additives, stabilization.

INTRODUCTION

Lyophilization for solid protein pharmaceuticals involves freezing the solutions or suspensions of sensitive biomaterials, followed by primary and secondary drying.[1,2] The technique is based on sublimation of water at subzero temperature under vacuum. Lyophilization is a key process for manufacturing solid protein pharmaceuticals. However, the rate of water vapor diffusion from solid cake is very low. Hence, the process is time-consuming. This process generates stresses capable of unfolding or denaturing the proteins. Cold denaturation is evident after incubation of frozen ovalbumin solution. The structural changes increased with decreasing temperature from −10 to −40°C.[3] Interferon (IFN-γ) aggregation in liquid mannitol is more severe at −20°C than at −15°C.[4] Freeze-thawing causes loss of activity of lactate dehydrogenase and recombinant hemoglobin, and 60% loss of L-asparaginase activity.[5-7] Studies have been mostly reported on screening the protein protectants during lyophilization.[8,9] Very few researchers have proposed an alternative process for lyophilization of proteins.

The foam drying is scalable technology for preservation of sensitive biotherapeutics in the dry state. Therapeutic biomolecules stable at moderate temperatures and pressures, such as erythropoeitin, enzymes, and vaccines, can be stabilized in the presence of common protectants using vacuum foam drying (VFD).[10] The process can be performed using the commonly available lyophilizers. The suspension or solution of biologicals is transformed into foam by boiling under vacuum, above freezing point, but significantly below 100°C. The foam consists of thin films of material from which water can be efficiently removed at an elevated temperature. The process is based on the principle of evaporation under vacuum at low temperatures. The additives for VFD stresses need to be individually optimized for a given protein.

LaSota, a lyophilized Newcastle disease vaccine, is produced by lyophilization over 72 hours. Lyophilization causes significant virus denaturation. The dried products show puffing, product collapse, and/or partial meltback. These products necessitate cold chain transport and storage for acceptable shelf stability. Pisal et al have optimized the lyophilization cycle for manufacturing LaSota vaccine.[11] A mixture of trehalose with polyvinyl pyrrolidone (PVP) provided some protection for the virus during lyophilization (optimum virus titer 5.25).[12] However, the result revealed significant vaccine denaturation during freezing and dehydration. The purpose of this research was to apply VFD for processing of the LaSota virus and to screen protective additives. Our laboratory has screened pluronic foaming

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agents and conditions of VFD for the LaSota virus.[13] The suitability of sugars, polymers, and N-Z-amine as single components and in combination is reported. The LaSota products with optimum virus titer were subjected to short-term stability analysis.

Materials and Methods

Materials

The harvest of LaSota, specific pathogen–free eggs, and N-Z-amine (Quest International, Norwich, NY) were provided by Ventri Biological Pvt Ltd (Vaccine Division, Pune, India). N-Z-amine is a high-quality source of peptides produced by the enzymatic digestion of casein. Its average molecular weight is 250 Da, and it predominantly contains glutamate, proline, lysine, and aspartate. Pluronic polymers, namely, F-108, F-68, and F-87, were gift samples from BASF Corporation (Mount Olive, NJ). Trehalose dihydrate was a gift sample from British Sugar Plc (Peterborough, UK). Sucrose, galactose, and fructose were purchased from MB Sugar Ltd (Pune, India). Polyethylene glycol 6000 (PEG, EP grade) and PVP K30 were obtained from Space Chemicals (Nashik, India).

Methods

Design of Experiments

Aqueous solutions (30 mL) for 200 doses of vaccine with 2-mL fills were prepared containing 2 mL of potassium phosphate buffer, 0.6% wt/vol of PF108, and 20 mL La-Sota harvest. Sucrose or trehalose at 20%, 30%, 40%, and 50% wt/vol strength, each separately, was dissolved in the harvest. Similarly, a solution containing 25% wt/vol each of sucrose and trehalose was prepared. The additive compositions were prepared as shown in Table 1.

Two milliliters of aqueous solution (each separately) was filled in a 10-mL glass vial, and rubber stoppers were positioned. The vials were kept at –10ºC for 10 minutes. Ten vials of each composition were placed in the glass chamber of a VirTis Lyophilizer (Advantage Model, Sl 210655, VirTis Co, Gardiner, NY). The aqueous solutions were vacuum-concentrated for 2 hours (10ºC, 1000-800 mTorr vacuum). The product temperature was raised gradually from 10ºC to 28ºC (2ºC each increment, with holding time of 120 minutes). Simultaneously, the vacuum was reduced to 25 mT over 240 minutes and kept constant for 18 hours. The foam structure formed was dried using a temperature-vacuum cycle as shown in Table 2.

Table 1. Additive Composition of Various LaSota Formulations*

	Additive Composition in Product				
Product Code	Sucrose (% wt/vol)	Trehalose or N-Z-amine (% wt/vol)	Fructose (% wt/vol)	Galactose (% wt/vol)	PEG or PVP (% wt/vol)
SF-5	50	—	5	—	—
SF-10 (F10)	50	—	10	—	—
SF-15	50	—	15	—	—
SGL-5	50	—	—	5	—
SGL-10	50	—	—	10	—
SGL-15	50	—	—	15	—
TF-5	—	50	5	—	—
TF-10	—	50	10	—	—
TF-15	—	50	15	—	—
TGL-5	—	50	—	5	—
TGL-10	—	50	—	10	—
TGL-15	—	50	—	15	—
SFP-30	30	—	20	—	3 PEG
SFP-40	40	—	20	—	3 PEG
SFP-50	50	—	20	—	3 PEG
SGP-30	30	—	—	20	3 PEG
SGP-40	40	—	—	20	3 PEG
SGP-50	50	—	—	20	3 PEG
NZ-10	50	10 NZA	—	—	—
NZ-15	50	15 NZA	—	—	—
NZ-20	50	20 NZA	—	—	—
NZ/PV	50	10 NZA	—	—	3 PVP
NZ/PE	50	10 NZA	—	—	3 PEG

*PEG indicates polyethylene glycol; PVP, polyvinyl pyrrolidone; SF, sucrose + fructose; SGL, sucrose + galactose; TF, trehalose + fructose; TGL, trehalose + galactose; SFP, sucrose + fructose + polyethylene glycol; SGP, sucrose + galactose + polyethylene glycol; NZ, N-Z-amine; PV, polyvinyl pyrrolidone; PE, polyethylene glycol.

Table 2. Vacuum Foam Drying Process Specifications

Step No	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Temperature (°C)	–10	10	15	20	22	24	26	28	28	28	28	30	40	26
Vacuum (mTorr)	1200	1000	800	600	200	100	25	25	25	25	25	25	25	25
Time (min)	15	60	60	120	120	120	120	120	120	120	120	120	120	120

Evaluation of Foam-Dried Products

The foam-dried LaSota products were analyzed for foam characteristics (the extent, structure, and uniformity of foam). The products were observed and photographed. Residual moisture content was determined using Karl-Fisher titration. Twenty milliliters of anhydrous methanol was transferred to a titration vessel, and titrations were performed to the electromagnetic endpoint. Ten microliters of water was titrated. The minimum water equivalent of reagent was 3.5 mg/mL of reagent. The effective inhibitory dose (EID_{50}) titer of vaccine was determined using a method described previously.[14]

X-Ray Powder Diffraction Studies

The optimized batches with maximum virus titer were subjected to x-ray diffraction analysis using an x-ray diffractometer (Philips PW 1729 X-Ray Diffractometer, Amsterdam, The Netherlands). The powdered foams were mounted on a sample holder. The samples were scanned in a 2θ range of 2° to 100° at a scan speed of 0.1° 2θ /sec. The radiation used was of (λ) 1.524 A^o wavelength. The x-ray powder diffraction pattern was recorded.

Stability of Vacuum-Foam-Dried Products

The foam-dried products of LaSota vaccine with optimum titer were subjected to short-term stability studies. Twenty vials of each product were vacuum-sealed and stored at 5°C, 25°C, and 40°C for 21 days. The products were analyzed periodically, each after 7, 14, and 21 days, for virus titer. The x-ray diffraction pattern of stability-study products kept at 25°C was examined at the end of 21 days.

Results and Discussion

Screening of Virus Protectants During VFD

Product Characteristics

Foam formation was effective in sucrose products (Figure 1, S-series). Uniform- and closed-foam structures were developed. The foam volume increased with sucrose concentration. A product containing 50% sucrose occupied three fourths of vial volume. However, the dry sucrose foam collapsed during the handling of products. Although drying was efficient, the powder form was not suitable for protein formulation.

The trehalose products were in the form of porous discs (Figure 1, T-series). Although foamability increased with trehalose concentration, sucrose foams were stable during the initial stages of VFD.[12,15] The foamability of sucrose was further enhanced by N-Z-amine. The dried foams were glossy and retained their structure during evaluation. The addition of amino acids (from N-Z-amine) might have contributed to the amorphous glassy state; this was subsequently confirmed in x-ray powder diffractometry analysis. The formation of a similar glassy matrix has been reported when bovine serum albumin was incorporated in protein pharmaceuticals.[16] The uniform foam formation and stability in the initial stages as well as after drying was obtained with a mixture of sucrose, N-Z-amine, and PVP. The solid matrix of fructose- or galactose-containing mixtures was deposited irregularly on the inner surface of the vial. These mixtures did not foam, even in the presence of PVP or PEG. The results confirmed that foam formation was influenced by the nature of the additive. The foam-drying process involves vacuum concentration of solution followed by foam

Figure 1. Effect of sucrose (S-series) and trehalose (T-series) concentration on foam characteristics.

formation and drying of the foam. The formation of stable foam during drying is a prerequisite for increasing the surface area for effective diffusion of water vapor. The solid content of the solutions affected the foamability.

Protective Efficiency of Additives

LaSota killed poultry vaccine is used in the treatment of Newcastle disease. Earlier research on optimizing virus stability during lyophilization using various protectants did not increase the virus titer.[12] In addition, the processing time was 72 hours. The virus titer of 9 (pure harvest) during commercial lyophilization was reduced to 5.25 ± 14. VFD was intended to improve the processing stability of the LaSota virus. The effect of sucrose/trehalose concentration in the range of 20% to 50% wt/vol and a batch containing 25% wt/vol each of sucrose and trehalose on EID_{50} of LaSota harvest is shown in Figure 2. The virus titer increased with disaccharide concentration. Sucrose provided better protection than did trehalose.[17] Sucrose formed a greater number of hydrogen bonds with lysozyme than did trehalose.[18] Bronshtein has reported that 50% sucrose was required to protect protein during processing and storage.[10,19,20] A protective monolayer of saccharides on the protein surface enhances the stability. The EID_{50} results in the present study revealed that 50% sugar concentration provided better virus protection than did lower concentrations.

The effect of formulation additives on moisture content in the product and on virus titer is shown in Table 3. N-Z-amine in the formulation range of 10% to 20% wt/vol provided virus protection. During drying, proteins are adsorbed at the air-water interface. N-Z-amine has an average molecular weight of 250 Da. This predominantly contains glutamate, proline, lysine, and aspartate. Hence, these small molecules are adsorbed prior to LaSota virus at the solid-air interface. When N-Z-amine was used with sucrose in a previous lyophilization study, a nonuniform, brown-colored (Millard degradation) product with half the virus titer was obtained. The browning intensified with storage for 4 weeks. The virus titer was below the detectable limit, indicating significant inactivation of the virus. Such a phenomenon was not evident during VFD. Reducing sugars react with amino acid (lysine and arginine) residues of protein to form carbohydrate via the Millard reaction. Sucrose is hydrolyzed into reducing sugars in liquid and also in the solid state (evident during lyophilization). However, the amino acids did not have a catalytic effect on hydrolysis of sucrose during VFD. Protein aggregation is generally concentration-dependent. The higher N-Z-amine concentration decreased protein aggregation. The virus titer of monosaccharide batches was less than that of the plain sucrose product (50% wt/wt). Sucrose with 10% wt/wt fructose had a slightly higher titer—6.47.

Table 3. Moisture Content and Virus Titer of LaSota Compositions*

Additives (% wt/vol)	Residual Moisture (% wt/wt)	EID_{50} Titer
SF-5	3.51 ± 0.03	5.97 ± 0.02
SF-10	3.22 ± 0.02	6.47 ± 0.04
SF-15	3.81 ± 0.04	6.28 ± 0.01
SGL-5	3.92 ± 0.06	6.30 ± 0.05
SGL-10	6.13 ± 0.01	6.10 ± 0.07
SGL-15	5.97 ± 0.04	5.97 ± 0.03
TF-5	4.66 ± 0.04	5.97 ± 0.1
TF-10	4.71 ± 0.07	5.97 ± 0.09
TF-15	4.06 ± 0.09	5.47 ± 0.15
TGL-5	4.91 ± 0.04	5.97 ± 0.05
TGL-10	4.60 ± 0.10	5.30 ± 0.05
TGL-15	4.72 ± 0.05	6.28 ± 0.03
SPF-30	3.37 ± 0.14	6.25 ± 0.05
SPF-40	3.66 ± 0.01	5.64 ± 0.11
SPF-50	3.00 ± 0.02	5.30 ± 0.11
SGP-30	3.34 ± 0.04	5.64 ± 0.07
SGP-40	3.50 ± 0.04	5.30 ± 0.14
SGP-50	3.51 ± 0.05	5.30 ± 0.09
NZ-10	1.90 ± 0.01	6.82 ± 0.06
NZ-15	1.90 ± 0.01	6.93 ± 0.11
NZ-20	2.10 ± 0.02	6.82 ± 0.08
NZ/PV	3.00 ± 0.09	6.97 ± 0.12
NZ/PE	2.51 ± 0.01	7.15 ± 0.11

*SF-5, sucrose (50) + fructose (5); SF-10, sucrose (50) + fructose (10); SF-15, sucrose (50) + fructose (15); SGL-5, sucrose (50) + galactose (5); SGL-10, sucrose (50) + galactose (10); SGL-15, sucrose (50) + galactose (15); TF-5, trehalose (50) + fructose (5); TF-10, trehalose (50) + fructose (10); TF-15, trehalose (50) + fructose (15); TGL-5, trehalose (50) + galactose (5); TGL-10, trehalose (50) + galactose (10); TGL-15, trehalose (50) + galactose (15); SPF-30, sucrose (30) + fructose (20) + polyethylene glycol (3); SPF-40, sucrose (40) + fructose (20) + polyethylene glycol (3); SPF-50, sucrose (50) + fructose (20) + polyethylene glycol (3); SGP-30, sucrose (30) + galactose (20) + polyethylene glycol (3); SGP-40, sucrose (40) + galactose (20) + polyethylene glycol (3); SGP-50, sucrose (50) + galactose (20) + polyethylene glycol (3); NZ-10, sucrose (50) + N-2-amine (10); NZ-15, sucrose (50) + N-2-amine (15); NZ-20, sucrose (50) + N-2-amine (20); NZ/PV, sucrose (50) + N-Z-amine (10) + polyvinyl pyrrolidone (3); NZ/PE, sucrose (50) + N-Z-amine (10) + polyethylene glycol (3).

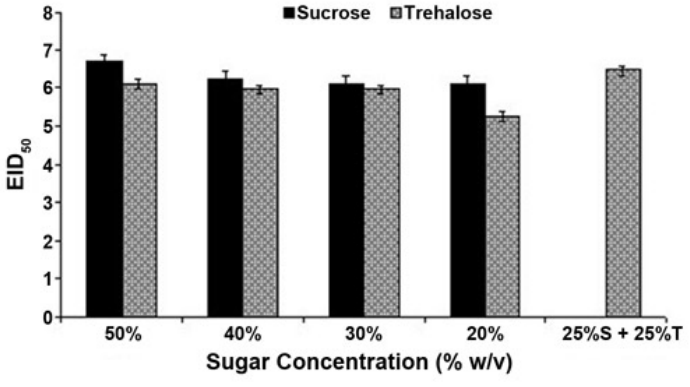

Figure 2. Effect of sugar concentration on EID_{50} value of vaccine.

The results revealed that the virus titer of sucrose-containing monosaccharide mixtures was more than that of mixtures with trehalose. In addition, the residual moisture was less than that of trehalose-containing products. Disaccharides better stabilized the virus than did monosaccharides. However, disaccharides in combination with monosaccharides did not show a significant increase in the virus titer. The formulations containing 30% to 50% wt/vol sucrose, 20% of monosaccharides (fructose, glucose, and galactose, each separately), and 3% wt/vol PEG 6000 did not show a higher virus titer as compared with plain product containing 50% wt/vol sucrose. However, PVP K30 and PEG 6000 at 3% wt/vol strength in combination with N-Z-amine (10% wt/vol) showed improved virus titers of 6.97 and 7.15, respectively. The foam features of products with optimum virus titer are shown in Figure 3.

Arakawa et al have used polymers to stabilize proteins during dehydration.[21] The levels of protein stabilization afforded by these polymers depend on the structure and concentration of both the polymer and the protein. Polymers can be used in combination with monosaccharides and disaccharides to stabilize proteins during dehydration. PVP and PEG (3%), in combination with N-Z-amine, retained the highest virus titer. The hydrogen bonding ability of PVP with protein molecules has been well established. A similar increase in stability was observed with the incorporation of PEG. Polymers (eg, dextran) have been reported to stabilize proteins by raising the glass transition temperature of a protein formulation and by inhibiting crystallization of stabilizing excipients (sucrose).[18] PVP might contribute to inhibition of crystallization in a similar fashion as dextran does. PEG has a surface-active property that inhibits aggregation of proteins in solid protein pharmaceuticals.

Figure 3. Foam characteristics of optimized LaSota formulations containing 50% sucrose. NZ10 indicates 10% N-Z-amine; NZ/PV, 10% N-Z-amine + 3% PVP; NZ/PE, 10% N-Z-amine + 3% PEG; NZ15, 15% N-Z-amine; F10, 10% fructose.

PEG has effectively stabilized lyophilized invertase at 90°C.[19] A synergistic protective effect of N-Z-amine with PVP was observed.

Stability Profile of LaSota Products

LaSota products with optimum titer (Figure 3) were subjected to short-term stability studies. The virus stability of different LaSota products is shown in Table 4. The LaSota virus titer of the batch containing plain sucrose (S50) reduced even when the batch was stored at 5°C, and maximum denaturation was evident at higher temperatures. In NZ10, significant virus potency was retained at 5°C for 21 days. However, the virus titer of the mixture at 25°C reduced significantly, from 6.82 to 5.19, at 40°C. No denaturation of the virus was observed with NZ15 at 5°C, and the product remained stable at 25°C. However, this product completely denatured at 40°C after 7 days. In the case of the NZ/PE batch, vaccine potency was retained at 5°C for 21 days. Gradual denaturation was observed at 25°C. Significant denaturation of the virus was evident at 40°C. Minimum denaturation of vaccine was observed with the NZ/PV product at all temperatures and times. The storage stability of the LaSota harvest is explained below with reference to the x-ray powder diffraction patterns of products.

X-Ray Powder Diffraction Analysis Products

The diffraction pattern of the plain sucrose product (S50) is shown in Figure 4. Peaks corresponding to pure crystalline sucrose were observed.[21] In the absence of any monosaccharide or polymer, sucrose crystallizes out during dehydration drying. The x-ray powder diffraction of LaSota products containing N-Z-amine (plain), N-Z-amine with PVP or PEG, and fructose is shown in Figure 5. The diffraction pattern of N-Z-amine 15% and that of the fructose-containing mixture was amorphous. Similarly, the product containing 10% of N-Z-amine and polymers (PVP/PEG 3%) did not show the characteristic lines of sucrose. The x-ray diffraction pattern of 10% and 15% N-Z-amine products revealed that a threshold concentration of N-Z-amine was required; below that, sucrose tended to crystallize, as indicated by small peaks in the 2θ range of 20° to 24°.

Figure 6 shows the x-ray diffraction pattern of products after the stability period (21 days, 25°C). NZ10 and the NZ/PV product did not show alterations in the diffraction pattern. The NZ/PE product showed small crystalline peaks of 2×10^3 intensity at 20 to 24θ. The diffraction patterns were not altered in the case of NZ15, indicating an absence of devitrification during storage. The pre–stability study diffraction pattern of F10 was amorphous, but after storage, crystalline peaks characteristic of sucrose were observed. Hence, the product was unstable at higher temperatures.[22]

Table 4. Virus Titer of Stability-Study LaSota Products*

Additives (% wt/vol)	Zero Time	EID_{50} of Products								
		After 7 Days			After 14 Days			After 21 Days		
		5°C ± 2°C	25°C ± 2°C	40°C ± 2°C	5°C ± 3°C	25°C ± 2°C	40°C ± 2°C	5°C ± 3°C	25°C ± 2°C	40°C ± 2°C
S-50	6.64 ± 0.12	6.29 ± 0.01	5.35 ± 0.09	†	6.20 ± 0.05	†	†	6.01 ± 0.07	†	†
NZ-10	6.82 ± 0.04	6.66 ± 0.05	6.29 ± 0.01	6.03 ± 0.01	6.52 ± 0.09	5.88 ± 0.05	5.41 ± 0.09	6.32 ± 0.13	5.43 ± 0.22	5.19 ± 0.25
NZ-15	6.93 ± 0.06	6.93 ± 0.06	6.61 ± 0.05	5.36 ± 0.18	6.82 ± 0.04	6.32 ± 0.04	†	6.82 ± 0.04	6.32 ± 0.13	†
NZ/PE	7.10 ± 0.22	7.10 ± 0.22	6.71 ± 0.08	6.0 ± 0.03	6.98 ± 0.05	6.46 ± 0.16	4.90 ± 0.09	6.89 ± 0.06	6.34 ± 0.27	†
NZ/PV	6.71 ± 0.12	6.71 ± 0.08	6.71 ± 0.12	6.66 ± 0.51	6.60 ± 0.12	6.57 ± 0.05	6.35 ± 0.10	6.52 ± 0.09	6.47 ± 0.17	6.14 ± 0.16
F-10	6.63 ± 0.12	6.19 ± 0.04	6.35 ± 0.09	5.19 ± 0.25	5.40 ± 0.18	6.35 ± 0.09	†	5.36 ± 0.25	5.17 ± 0.09	†

*S-50, sucrose (50% wt/vol); NZ-10, sucrose (50% wt/vol) + N-Z-amine (10% wt/vol); NZ-15, sucrose (50% wt/vol) + N-Z-amine (15% wt/vol); NZ/PE, sucrose (50% wt/vol) + N-Z-amine (10% wt/vol) + polyethylene glycol (3% wt/vol); NZ/PV, sucrose (50% wt/vol) + N-Z-amine (10% wt/vol) + polyvinyl pyrrolidone (3% wt/vol); F-10, sucrose (50% wt/vol) + fructose (10% wt/vol).
†Virus titer below 5, that is, practically undetected, and hence significant virus denaturation.

Bronshtein has successfully inhibited the crystallization of sucrose by incorporating fructose.[10] The prevention of crystallization by fructose was observed in its x-ray diffraction pattern. The relative concentration of N-Z-amine below which sucrose crystallized was 15% wt/vol. N-Z-amine and polymers significantly inhibited the crystallization of sucrose during VFD. PVP and PEG are reported to inhibit the partial crystallization of sucrose and thus prevent protein mobility. PVP, like maltodextrin, reduced the crystallization of sucrose. The virus titer of stability mixtures confirmed these findings. The EID_{50} of PEG- and PVP-containing batches was retained during stability at low and room temperatures, owing to retention of the amorphous nature of the product. The initial virus titer of the fructose-containing batch was acceptable, but the mixture failed to protect the virus during storage owing to devitrification of sucrose. Hence, a combination of N-Z-amine with PVP provided optimum dehydration and storage stability (even at 40ºC) for the LaSota virus.

Process Efficiency of Lyophilization and VFD

The present research was a continuation of previous research on protecting the secondary structure of LaSota virus during lyophilization.[12] Sucrose in concentrations of 20% to 50% wt/vol, with PEG or PVP separately, was evaluated as a

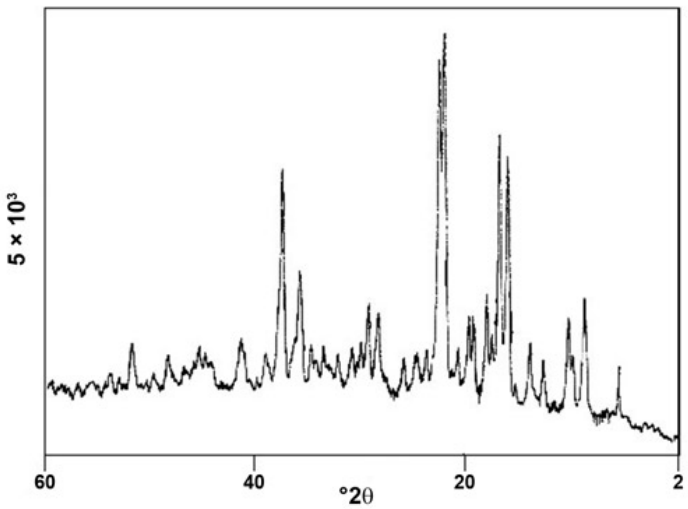

Figure 4. X-ray diffraction pattern of S50 product.

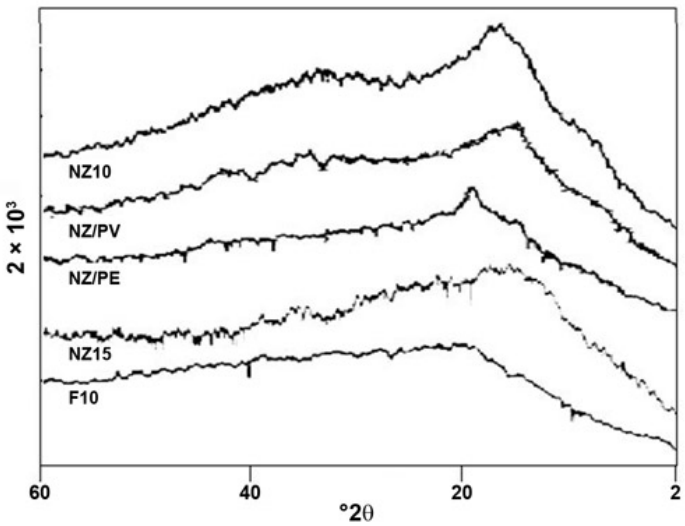

Figure 5. X-ray diffraction pattern of stability product.

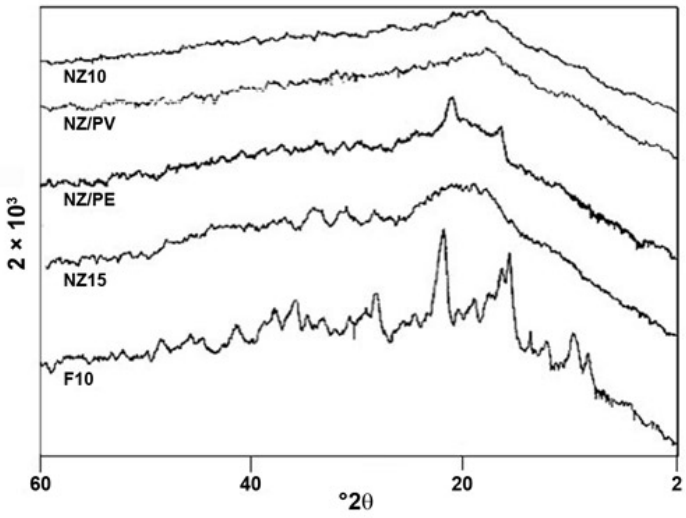

Figure 6. X-ray diffraction pattern of optimized product after stability.

protectant during freeze-drying. The products with optimum stability in lyophilization (containing 20% wt/vol sucrose) were compared with the VFD products to measure protection of LaSota virus during dehydration. Sucrose (20%) with either PEG 6000 (3%), PVP (3%), or N-Z-amine (10%) during lyophilization produced solid cakes with virus titers of 5.17 ± 0.09, 5.35 ± 0.1, and 5.88 ± 0.05, respectively.[11] The virus titers of sucrose (50%, VFD) containing PEG 6000 (3%), PVP (3%), or N-Z-amine (10%-15%) were found to be 7.15 ± 0.22, 6.71 ± 0.12, and 6.97 ± 0.21, respectively. Lyophilization itself induces stresses at 3 different stages: freezing, drying, and reconstitution. Virus denaturation occurred at all 3 stages. Dehydration-induced aggregation reduced the EID_{50} value during lyophilization. In VFD the convection of heat between shelf and product temperature was more effective under vacuum.[23] The surface area available was greater because of foam formation, and hence there was effective removal of moisture in a short period. The results suggest that for an α-helical LaSota structure, the highest proportions of sucrose in combination with N-Z-amine and polymers during VFD provided better protection than did lyophilization. In addition, VFD was a simple and short process.

Conclusion

VFD was applied successfully to produce a solid LaSota formulation retaining a protective titer. The foam characteristics were significantly altered because of the nature and amount of additive. The water removal was rapid. A mixture of sucrose, N-Z-amine, and PVP K30 significantly inhibited the inactivation of virus. The products were amorphous and did not show devitrification on storage. The VFD-processed products were stable at higher temperatures.

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