RESEARCH ARTICLE

Development and Evaluation of Vasoactive Intestinal Peptide Freeze-Dried Injection

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ABSTRACT

Introduction: Vasoactive intestinal peptide (VIP), a ubiquitous, naturally synthesized human peptide is extensively documented to have diverse physiological effects like anti-inflammatory, immune-modulatory, anti-hypertensive, stimulation of contractility in the heart, vasodilation, and promoting neuroendocrine-immune communication. The synthetic form of VIP is called aviptadil (AVP). The main objective of this research was to develop a novel stable lyophilized dosage of VIP (Aviptadil) using sucrose as a bulking agent.

AVP is a peptide with known concern for aqueous stability, which seems to be challenging for storing finished drug products and supply chain management. The VIP injection was developed using the lyophilization technique in the presence of bulking agent and some other pH-adjusting agent. The bulking agent and solvent system selection depends upon the solubility, stability of the drug substance, and feasibility during manufacturing. During product formulation development, the bulk solution was evaluated for processing time and temperature impact. The lyophilization cycle was developed using the most advanced freeze-drying technology.

Result and discussion: With the usage of bulking agent (sucrose) as may act as a cryoprotectant for peptide, the formulated bulk solution was freeze-dried, and primary drying was done at-25°C (below than critical product temperature) followed by secondary drying at 25°C. The critical quality attributes of lyophilized drug products like the description of lyophilized cake/powder, moisture content, reconstitution time, active drug content and color of the solution were evaluated. The developed formulation bulk solution was stable and compatible with contact materials like SS vessels when hold up to 24 hours at 2 to 8°C. The optimized freeze-dried product meets the predefined acceptance criteria as part of the quality target product profile.

Conclusions: It can be concluded from the research work carried out that a stable lyophilized parenteral formulation containing VIP (AVP) was developed using sucrose as a bulking agent. These findings show that the freeze-dried formulation is an appropriate technological remedy for stabilizing VIP in lyophilized injectable dosage form.

Keywords: Vasoactive intestinal peptide, Aviptadil, sucrose, quality by design, Freeze dried microscope, lyophilization.

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INTRODUCTION

Vasoactive intestinal peptide (VIP), a ubiquitous, naturally synthesized human peptide, is extensively documented to have diverse physiological effects like anti-inflammatory, immune-modulatory, anti-hypertensive, stimulation of contractility in the heart, vasodilation, and promoting neuroendocrine-immune communication.[1] VIP is the synthetic form of VIP that increases adenosine cyclase activity with consequent smooth muscle relaxation. Relief Therapeutics has been granted investigational new drug (IND) status in the US and Europe, along with orphan drug designation for the use of VIP in acute respiratory distress syndrome (ARDS), acute lung injury (ALI), pulmonary fibrosis, and sarcoidosis.[2]

The male genital tract naturally contains the 28-amino acid neurotransmitter known as the VIP (VIP: International non-proprietary name, Aviptadil), which is thought to play a part in the local neurological control of smooth muscle activity and penile erection.[3] VIP appears to play a specialized role in smooth muscle relaxation, which results in systemic vasodilation, enhanced cardiac output, and bronchodilation.

VIP has a variety of physiological effects, including smooth muscle relaxation that causes systemic vasodilation, increased cardiac output, bronchodilation, some variations in the effects on gastric motility and secretory processes, hyperglycemia, inhibition of smooth muscle cell proliferation, hormonal regulation, analgesia, hyperthermia, neurotropic effects,

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learning and behavior, and bone metabolism.[4] Localized acute respiratory distress syndrome is a severe pulmonary parenchymal injury to most or both lungs, and VIP is one of the signal molecules of the neuroendocrine-immune network inducing anti-proliferative, anti-inflammatory, and immune-regulatory features, particularly in the lung where it is predominantly found.[5] ARDS is the rapid onset of progressive malfunction of the lungs, especially about the ability to take in oxygen, usually associated with the malfunction of other organs.[6] The condition is associated with extensive lung inflammation and accumulation of fluid in the alveoli (air sacs) that leads to low oxygen levels in the lungs.[7] ARDS is characterized by diffuse pulmonary microvascular injury resulting in increased permeability and, thus, non-cardiogenic pulmonary edema.[8]

The pre-formulation study also shows that drug substances have limited solution stability in an aqueous solution. Hence based on available information, the freeze-dried formulation was selected for development and characterization. VIP is sensitive to temperature and light. Based on the literature review and laboratory trial it is observed that the lyophilization technique will be the most suitable, economical, convenient, and safe technique to stabilize the proposed injectable drug product.[9]

MATERIALS AND METHODS

Chemical and Reagent

A VIP drug substance gift sample was provided by MSN Laboratories. Sucrose, hydrochloric acid, and glacial acetic acid were received from Merk.

Methods

VIP is freely soluble in water, soluble in methanol, and practically insoluble in acetonitrile. The VIP is hygroscopic and must be stored in well-closed containers. The molecular formula of VIP is $C_{147}H_{238}N_{44}0_{42}S$ and its molecular weight is 3325.8 Daltons. The molecular structure of VIP is depicted in Figure 1.

The proposed drug product is selected to develop a novel stable injectable formulation looking at its proposed line of treatment for managing acute respiratory distress syndrome. Developed formulation was evaluated for the requirement of general injectable quality attributes. VIP is an active pharmaceutical ingredient in the formulation in the presence of other stabilizing excipients. Based on pre-formulation study results, the quantity of each ingredient was finalized. The proposed formulation is a freezing-dried lyophilized injectable drug product filled in glass vials with a rubbers stopper and aluminium flip-off seal.

Selection of excipients in formulation

In the proposed novel injectable formulation sucrose was selected as bulking agent/cry-protectant in the presence of other excipients like pH adjusting agents (hydrochloric acid and sodium hydroxide) and water for injection was selected as solvent before lyophilization for evaluation. The risk

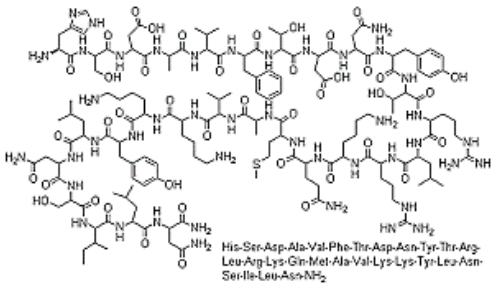

Figure 1: The chemical structure of the Synthetic form of VIP

assessment for all the listed excipients was performed as part of quality by design activity. The quantity of all excipients was optimized during the developmental study. The critical material attributes for all the excipients were identified based on the literature and scientific knowledge.

Selection of excipient grades

Excipients used in the proposed drug product are mentioned in Table 1. Excipients used in the formulation meet the inactive Ingredients Database (IID) limits. The excipients selected for the product were complying with global pharmacopeial standards.

Selection of suitable solvent system

VIP is freely soluble in water, soluble in methanol, and practically insoluble in acetonitrile. Considering the proposed formulation is a parenteral drug product for intravenous/intramuscular administration and solubility of active drug substance, water was selected as the choice of a solvent system for the drug formulation.

Selection of suitable manufacturing process

This section describes the development of process parameters for the drug product. A risk assessment of the overall drug product manufacturing process was performed to identify the high-risk steps or process variables that may affect the CQAs of final drug product. These factors were afterward examined to better comprehend the production process and create a control strategy to lower the chance of batch failure. The solubility of the active drug was determined in the proposed formulation composition at different pH conditions to demonstrate adequate solubility and stability in the presence of other excipients. The dissolution rate of the drug substance was evaluated to recommend the stirring conditions (stirring speed and time) to be maintained during the manufacturing of the formulation bulk. Bulk solution stability at different temperature conditions over a period was evaluated to define the processing conditions. The preparation of bulk solution involves the addition of excipients followed by drug substance to the aqueous solvent system (processed at a defined temperature) and followed by volume makeup.

Solubility study: Solubility of VIP in an aqueous vehicle in the presence of other excipients

The solubility of the drug substance was evaluated in the water containing sucrose at a temperature of 2 to 8°C which is recommended storage condition for a bulk solution and pH

Table 1: The selected excipients grade evaluated for proposed generic formulation

Ingredients	*Manufacturer*	*Remarks*
Sucrose Hydrochloric acid Sodium hydroxide	Merck	Propose excipients have multi-compendial standards as well as having microbial specification as part of the release specification

5.0 and 7.0 (lower and upper limits of the proposed pH range considering physiological body pH). The study results are summarized in Table 3.

Dissolution rate study

The study was performed at a minimum concentration of 100 mcg/mL of the VIP at a fixed paddle speed of 100 rpm and a temperature of 2 to 8°C. The volume of water used for the study was about 80% of the total batch volume. The completion of dissolution was verified by visual observation and samples were withdrawn and analyzed for clarity of solution and active drug content. The study results are summarized in Table 4.

Formulation bulk solution stability

Formulation bulk solution stability was evaluated at a concentration of 100 mcg/mL using two different concentrations of bulking agent (sucrose 25 and 50 mg/mL) when stored at 2 to 8°C and ambient room temperature (RT), respectively. The solution stability study samples were collected after specified intervals of 0 hours (initial), 8 and 24 hours and tested for critical quality attributes in comparison with the initial sample results. The study results are summarized in Tables 5 and 6.

Development and optimization of lyophilization cycles

Lyophilization is a process in which water is removed from the product after it is frozen and placed under a vacuum, allowing the ice to sublime without passing through a liquid phase. The lyophilization process consists of three phases and the process parameters of relevance are summarized in Table 2.

VIP injection contains VIP as an active pharmaceutical ingredient, sucrose as a bulking agent, and sodium hydroxide and hydrochloric acid as alkalizing/acidic agents. The bulk solution is proposed to be filled into glass vials and half-stoppered with lyostoppers for lyophilization.

Lyophilization Process Design Criteria

The selection of the process parameters for the freezing step during the manufacture of VIP injection was based on the thermal characteristics and stability of the formulation bulk solution.

Freezing temperature

The freeze-drying microscope (FDM) is used for the evaluation of the freezing point of the proposed bulk formulation. The images are presented result section in Figures 2 and 3

The bulk solution stability study indicates that VIP bulk solution is stable at 2 to 8°C for 24 hours. Therefore, the partially stoppered filled vials are loaded into the lyophilizer. The solution is frozen, allowing uniformity of the vials across the lyophilizer.

Primary drying

The primary drying phase, called the sublimation phase, is optimized based on the theoretical considerations and observations drawn from the critical temperature evaluation using FDM. This phase's temperature and pressure set points are such that the product sublimes without undergoing collapse. The critical temperature through FDM is presented in the result section (Figures 2 and 3).

Secondary drying

In secondary drying the chamber pressure was maintained at 0.050 mbar. During secondary drying, there will be a rapid loss of free moisture from the product. Hence, a low chamber vacuum would ensure that moisture is removed efficiently.

Process Step: stoppering

After the completion of freeze drying, it is recommended to stopper the freeze-dried vials under partial pressure (when there is low pressure in the chamber than atmospheric pressure) Stoppering of vials under partial pressure allows to maintain vacuum over the product and prevents moisture absorption from the headspace of product.

Lyophilization cycle optimization

A lab batch of VIP injection bulk at 100 mcg/mL concentration The filled vials were loaded into a lyophilizer and released at the end of the lyophilization cycle vacuum. The vials were completely stoppered under partial vacuum and sealed. The details of lyophilization optimization trials and observation are provided in the result section.

Additional Characterization Study

PXRD Data

PXRD studies were performed on lyophilized finished formulation and physical mixture before lyophilization. This study helps us know the finished formulation's polymorphic form after lyophilization. The X-ray diffractogram of various samples is presented in the result and discussion section (Figures 4 and 5).

Table 2: Lyophilization process parameter considered during process development

Stage of Process	*Freezing*	*Primary drying*	*Secondary drying*
Critical parameter	Freezing set temperature	Primary drying set temperature Vacuum applied during primary drying	Secondary drying set temperature Vacuum applied during secondary drying
Monitoring parameters during the lyophilization process	Applied self-temperature. Actual product temperature during the freezing process	Applied self-temperature and vacuum. Actual product temperature during the primary process	Applied self-temperature and vacuum. Actual product temperature during the primary process

Table 3: Solubility of VIP

Active drug (mcg)	*Sucrose (mg)*	*Water (mL)*	*Solubility observation at pH 5.0*		
			Initial	*48 hrs/ 2°C-8°C at pH 5.0*	*48 hrs/ 2°C-8°C at pH 7.0*
25	25	1	soluble clear solution	soluble clear solution	soluble clear solution
25	50	1	soluble clear solution	soluble clear solution	soluble clear solution
50	25	1	soluble clear solution	soluble clear solution	soluble clear solution
50	50	1	soluble clear solution	soluble clear solution	soluble clear solution
75	25	1	soluble clear solution	soluble clear solution	soluble clear solution
75	50	1	soluble clear solution	soluble clear solution	soluble clear solution
100	25	1	soluble clear solution	soluble clear solution	soluble clear solution
100	50	1	soluble clear solution	soluble clear solution	soluble clear solution

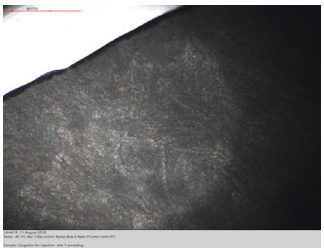

Figure 2: Freezing point for bulk solution at -4.5°C

Figure 3: First sign of collapse observed as the product reaches -29°C

Fourier-transform infrared spectroscopy

The lyophilized drug product charged for the FTIR study. Here for a better understanding of the formation of complexation of drug substance with sucrose, FTIR spectra were generated (Figures 6-8) using individual drug substance, sucrose, and complex form of the lyophilized product of VIP with sucrose.

RESULTS AND DISCUSSION

The drug substance was found to be easily soluble in water. However, preliminary experimental study results showed that bulk solution was stable when stored at 2 to 8°C up to 24 hours but limited solution stability when stored at ambient room temperature.

Experimental Study and Observation

Solubility and stability evaluation of VIP

Based on the above data, it is confirmed that VIP bulk solution is physically stable at the proposed pH range (pH 5.0 and 7.0) at a temperature of 2 to 8°C when dissolved at a concentration from 25 to 100 mcg/mL in the presence of sucrose at 25 to 50 mg/ mL concentration. Hence all further developmental trials were carried out using sucrose at a concentration of 25 mg/mL in the presence of 100 mcg/mL concentration of active drug substance in summarised in Table 3.[10]

Dissolution rate study

The study showed the dissolution of the VIP at a concentration of 100 mcg/mL at a pH range from 5.0 to 7.0. Complete drug dissolution was observed within 10 minutes of stirring when bulk solution temperature was kept at 2 to 8°C and a stirring speed of about 100 rpm by visual observation shown in Table 4.

There is no significant difference in drug content between 10 and 15 minutes of stirring indicating that VIP drug is easily soluble within 10 minutes of stirring at specified experimental parameters.[11]

Formulation Bulk Solution Stability Study

The results of the formulation bulk solution stability study using sucrose at concentrations of 25 and 50 mg/mL at 2 to 8°C did not show any significant change in the results of all the evaluated parameters at the end of 24 hours and results are comparable with the initial sample. Bulk storage at ambient temperature for up to 24 hrs shows a slight drop in active drug content.[12] Hence it is concluded that VIP injection bulk formulation can be stored at 2 to 8°C and the maximum hold time of the formulation bulk can be restricted to 24 hours from the start of the addition of API till the start of the lyophilization cycle to have a better process control were shown in Tables 5 and 6.

The above experimental study revealed that VIP was easily soluble in the presence of sucrose as bulking/cryoprotectant at concentrations of 25 to 50 mg/mL. All ingredient is easily soluble in water so the sequence of addition will not impact on the formulation manufacturing process. Developed bulk solutions of VIP were found to be stable at 2 to 8°C up to 24 hours.

Lyophilization Cycle Optimization

For successful lyophilization of drug prodrug, it is important to know the bulk solution freezing point and onset of the collapse. FDM techniques were used[13] to get information on the freezing point and onset of collapse. FDM studies revealed that the critical product temperature was found in a range of -25 to -29°C. The complete collapse was observed as -20°C. The FDM images of the bulk solution are presented in Figures 2 and 3.

By changing the different temperatures and vacuum, various lyophilization cycles were assessed to optimize the required cycle to provide a consistent outcome. A summary

Table 4: Dissolution rate study data for VIP injection

Test parameters	*After 10 min of stirring*		*After 15 min of stirring*	
	pH 5.0	*pH 7.0*	*pH 5.0*	*pH 7.0*
Description	The clear, colorless solution		The clear, colorless solution	
pH of the bulk solution	5.70	6.91	5.78	6.98
Color absorption	0.00	0.01	0.02	0.01
% Active drug content	99.8	99.6	100.1	99.7

Values represented as mean ± SD (n = 3)

Table 5: Evaluation of bulk solution stability using 25 mg/mL sucrose at 2–8°C and ambient room temperature (RT)

Test parameters	*Initial*	*8 Hours*		*24 Hours*	
		2–8°C	*RT*	*2–8°C*	*RT*
Description	The clear, colorless solution	The clear, colorless solution		The clear, colorless solution	
pH of the bulk solution	5.70	5.70	5.71	5.78	5.68
Color absorption	0.01	0.00	0.01	0.02	0.02
% Active drug content	104.9	103.2	104.5	103.8	98.5

Values represented as mean ± SD (n = 3)

Table 6: Evaluation of bulk solution stability using 50mg/mL sucrose at 2°C-8°C and ambient room temperature (RT)

Test parameters	*Initial*	*8 Hours*		*24 Hours*	
		2–8°C	*20–25°C*	*2–8°C*	*20– 25°C*
Description	The clear, colorless solution	The clear, colorless solution		The clear, colorless solution	
pH of the bulk solution	5.50	5.60	5.71	5.48	5.51
Color absorption	0.01	0.00	0.01	0.02	0.02
% Active drug content	103.8	107.3	105.5	106.5	96.9

Values represented as mean ± SD (n = 3)

Table 7: Lyophilization cycle used for Trial 01

Step	*Temperature (°C)*	*Time (minutes)*	*Vacuum (mbar)*
Freezing	-40	240	Off
Primary drying	-30	720	0.2
Secondary drying	25	720	0.05

Table 8: Physical parameter of lyophilized drug product of trial 01

S. No	*Parameter*	*Observation after lyophilization*
1	Description	White to off-white powered/ cack without any melt back
2	Reconstitution time	30 seconds
3	Water content	4 to 5%

Values represented as mean ± SD (n = 3)

of the lyophilization trials performed along with the study conclusions that lead to the final lyophilization cycle is presented in the following section. The samples from these development cycles were evaluated for the physicochemical parameter to further assess the suitability of the cycle in manufacturing a stable product.

Trial 1: Trial has taken using -40°C for freezing and -20°C for primary drying

Based on available information from the FDM study, a batch of VIP bulk at a concentration of 100 mcg/mL with sucrose with 25 mg/mL and pH adjusted to 6.0 was prepared and bulk solution filled in glass vials with partially stopper vial (Table 7 and 8).

The sampling for residual moisture content analysis results from different locations confirms the moisture content was slightly higher.

Trial 2: Trial taken with increase primary drying time

The lyophilization cycle parameters employed during trial 01 indicate a lyocycle that results in a product with higher water content. Manufacturing this trial batch aimed to increase the primary drying temperature and modify intermittent phases to further boost the sublimation process. The temperature of the primary drying was set to -25°C and the duration of the primary drying temperature reduce from 12 to 10 hours and the intermittent temperature was included as -15°C for a period of 10 hours considering the safer side of the lyophilization process and scale factor to yield the product with better results (Table 9).

The results of trial 02 lyophilization for description, pH, water content, reconstitution characteristics, and solution color were within expectation. The product vials showed good structure and did not show signs of shrinkage or melt back at the end of the lyophilization cycle and after storage in ambient room temperature conditions. The physicochemical data (Table 10) shows that the lyophilization cycle yielded a stable product that can be easily reconstituted. The samples from the batch were evaluated for X-ray diffraction by comparing them with a physical mixture of standard drug substance and sucrose and lyophilized drug product (Figures 4 and 5). Also, samples tested for FTIR study to establish the suitability of the cycle to achieve a stable product complexation in its physicochemical characteristics and show the expected product profile.

The successful execution of the drug product by employing the lyocyle at a lab-scale lyophilizer indicates the suitability of the cycle for VIP injection.

PXRD Data

A quick analytical method called X-ray powder diffraction (XRD) can reveal information on the dimensions of unit cells and is mostly used to identify the phase of crystalline materials. As per the literature, most formulation was found to be more stable after lyophilization if it is in amorphous form than crystalline form.[14] Hence the proposed drug product was evaluated for its polymorphic form by doing a PXRD study for the finished formulation of the VIP against a physical mixture of drug substance and sucrose.

Table 9: Optimized lyophilization cycle for VIP injection

Step	*Temperature (°C)*	*Time (minutes)*	*Vacuum (mbar)*
Freezing	-50	240	Off
Primary drying	-25	600	0.2
	-15	600	0.2
Secondary drying	25	600	0.05

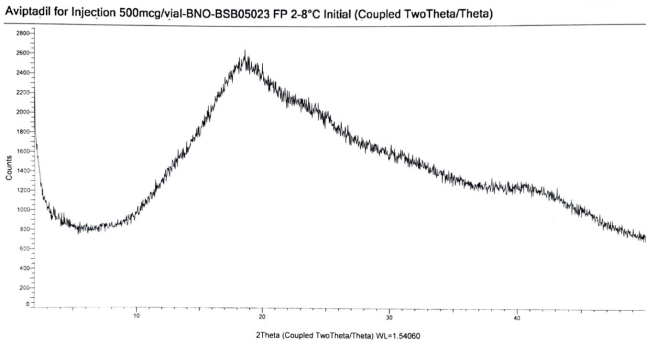

Figure 4: PXRD graph of lyophilized formulation of VIP

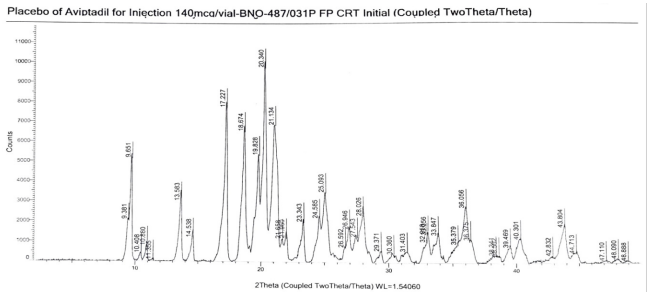

Figure 5: PXRD graph of the physical mixture before lyophilization

Figure 6: FTIR of active drug substance

Below is a graphical presentation of PXRD for lyophilized drug products and placebo.

XRD Data of Finished Formulation of Lyophilized VIP Injection

PXRD of physical mixture before lyophilization

According to Figure 5, the physical mixture of standard drug substance and sucrose showed a crystalline structure. The sucrose has distinct peaks at 9.6, 13.1, 18.7, 19.8, 20.3, and 21.1°. Whereas the absence of all characteristic peaks of sucrose in freeze-dried assayed samples of finished drug of vasoactive intestinal injection (Figure 4) confirms the formation of a stable complex between sucrose and standard drug after lyophilization and forms stable amorphous lyophilized drug product

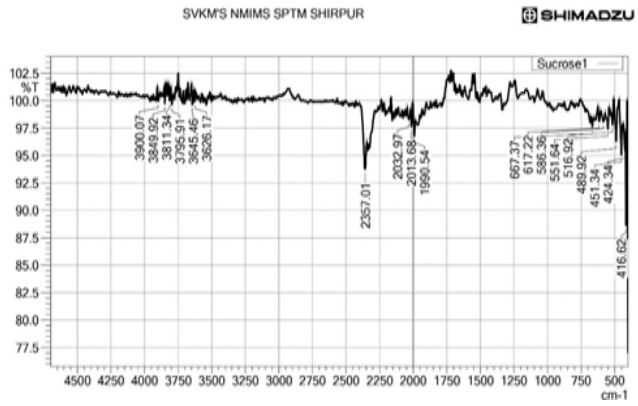

Figure 7: FTIR of Sucrose

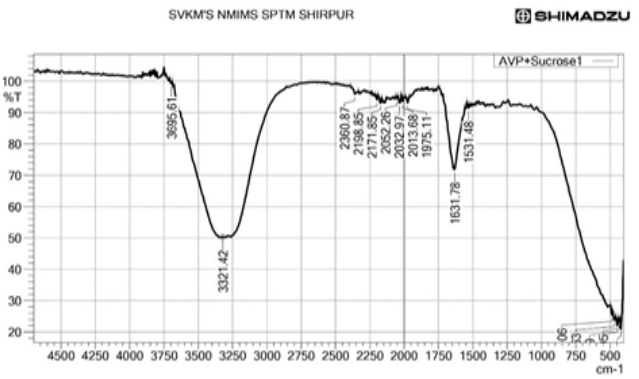

Figure 8: FTIR for the finished formulation of the VIP

Table 10: Physicochemical data of finished drug product for VIP injection

Drug Product Name: VIP injection				
Sr No	*Parameter*	*Results*		
		*Initial**	*2–8°C (6M)*	*RT (6M)*
1	Description before reconstitution	WCLC	WCLC	WCLC
2	Description after reconstitution with water	CCS	CCS	CCS
3	Reconstitution time	30 sec	35 sec	52 sec
4	pH of reconstituted drug solution	6.4	6.4	6.5
5	Colour absorption of reconstituted drug product	0.00	0.01	0.05
6	Active drug content (% of the labelled amount)	104	103.4	93.7
7	Water content (%)	1.51	1.2	2.3

* WCLC: White to off-white lyophilized cack / powder; CCLS: clear colourless solution; values represented as mean ± SD (n = 3)

Fourier-transform infrared spectroscopy

The lyophilized drug product charged for the FTIR study. For a better understanding of the formation of complexation of drug substance (VIP) with sucrose, FTIR spectra were generated using individual drug substance (Figure 6), sucrose (Figure 7), and complex form of the lyophilized product of VIP with sucrose (Figure 8).

The IR spectrum of sucrose was characterized by a peak at 3800 to 3500 cm^{-1} due to O-H stretching, a peak at 2357 cm^{-1} due to C-H stretching, and peaks at 1900 to 2050 cm^{-1} due to C-O stretching. A distinct peak of sucrose was observed at 3795 cm^{-1} (O-H stretching). This peak was shifted to a lower frequency of 3321 cm^{-1} in the lyophilized drug sample. Additionally, active drug product was characterized by a peak at 1647 and 1535 cm^{-1} due to C=O stretching. This peak was shifted to a lower frequency at 1631 and 1531 cm^{-1} in the final lyophilized drug sample.[15] The reason for this observation is interpreted as a consequence of hydrogen bonding between hydrogen and oxygen molecules of VIP and sucrose.

Physico-chemical Characterization of Lyophilized Drug Product Manufactured Using Optimized Formula and Lyophilization Cycle

After lyophilization, the sample was analyzed for its physicochemical characterization, and the same batch was evaluated for shelf-life parameters of up to 6 months when stored at 2 to 8°C and accelerated storage condition by keeping at ambient room temperature.

Details of physicochemical testing results and stability parameters are presented below in Table 12

From the above stability data, it is observed that the initial result of the finished lyophilized drug product meets all expected quality target product profiles for VIP injection and general injectable requirements.[16] The finished drug product does not show any melt-back in lyophilized cake, reconstitution of the cake was very fast, and moisture content was found in control. All these results confirmed the suitability of the proposed lyophilization cycle. On stability, the drug content for the sample held at ambient room temperature significantly dropped from 104 to 93.7% (10.3%) which concluded here that the developed lyophilized formulation is still heat sensitive but does not show any significant variation when the lyophilized sample store at 2 to 8°C up to 6 months. Hence, the developed lyophilized drug product is sensitive to temperature and can be stored at 2 to 8°C for long-term storage.

CONCLUSION

The aim was to develop a novel stable freeze-dried parenteral formulation of VIP injection which can be evaluated for the management of acute respiratory distress syndrome. As the drug has poor aqueous stability, therefore stability was improved by using a lyophilization process using cryoprotectants like sucrose in the developed novel formulation. The selected excipient shows no interaction with the drug and is more compatible with all parts of contact. An efficient, consistent product with the necessary water content is produced using an optimized lyophilization cycle. From additional analytical characterization studies like FTIR and PXRD, it can be concluded that a stable complex drug product is formed that helps stabilize VIP in lyophilized form in the presence of cryoprotectant sucrose. The prepared VIP (VIP) injection satisfies all quality target product profiles. Finally, it can be concluded that a novel lyophilized formulation was developed in the presence of sucrose-stabilized VIP.

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REFERENCES

1. Delgado M, Pozo D, Ganea D. The significance of vasoactive intestinal peptide in immunomodulation. Pharmacol Rev. 2004;56(2):249–90.
2. Suri R, Beg S, Kohli K. Target strategies for drug delivery bypassing ocular barriers [Internet]. Vol. 55, Journal of Drug Delivery Science and Technology. Elsevier B.V.; 2020. 101389 p. Available from: https://doi.org/10.1016/j.jddst.2019.101389
3. Juenemann KP, Lue TF, Luo JA, Jadallah SA, Nunes LL, Tanagho EA. The role of vasoactive intestinal polypeptide as a neurotransmitter in canine penile erection: A combined in vivo and immunohistochemical study. J Urol. 1987;138(4):871–7.
4. Onoue S, Misaka S, Yamada S. Structure-activity relationship of vasoactive intestinal peptide (VIP): Potent agonists and potential clinical applications. Naunyn Schmiedebergs Arch Pharmacol. 2008;377(4–6):579–90.
5. B. Taylor Thompson, M.D., Rachel C. Chambers, Ph.D., and Kathleen D. Liu, M.D. PD. Ards Nejm 2017. Nejm. 2017;
6. Beal AL, Cerra FB. Multiple Organ Failure Syndrome in the 1990s: Systemic Inflammatory Response and Organ Dysfunction. JAMA J Am Med Assoc. 1994;271(3):226–33.
7. The inernational non-Hodgkins lymphoma prognostic factors project. The New England Journal of Medicine Downloaded from nejm.org at UNIV OF CAPE TOWN LIBRARIES on September 18, 2015. For personal use only. No other uses without permission. Copyright © 1993 Massachusetts Medical Society. All rights reserved. N Engl J Med. 1993;329:987–94.
8. Glasser F, Fein AM, Feinsilver SH, Cotton E, Niederman MS. Non-cardiogenic pulmonary edema after pericardial drainage for cardiac tamponade. Chest. 1988;94(4):869–70.
9. Misra A, Jinturkar K, Patel D, Lalani J, Chougule M. Recent advances in liposomal dry powder formulations: Preparation and evaluation. Expert Opin Drug Deliv. 2009;6(1):71–89.
10. Xu C, Guo Y, Qiao X, Shang X, Niu W, Jin M. Design, recombinant fusion expression and biological evaluation of vasoactive intestinal peptide analogue as novel antimicrobial agent. Molecules. 2017;22(11).
11. EKLUND S, JODAL M, LUNDGREN O, SJOQVIST A. Effects of vasoactive intestinal polypeptide on blood flow, motility and fluid transport in the gastrointestinal tract of the cat. Acta Physiol

Scand. 1979;105(4):461–8.
12. Maggio RM, Vignaduzzo SE, Kaufman TS. Practical and regulatory considerations for stability-indicating methods for the assay of bulk drugs and drug formulations. TrAC - Trends Anal Chem [Internet]. 2013;49:57–70. Available from: http://dx.doi.org/10.1016/j.trac.2013.05.008
13. Kawasaki H, Shimanouchi T, Kimura Y. Recent Development of Optimization of Lyophilization Process. J Chem. 2019;2019.
14. Lundberg P, Boström I, Mukohyama H, Bjurholm A, Smans K, Lerner UH. Neuro-hormonal control of bone metabolism: Vasoactive intestinal peptide stimulates alkaline phosphatase activity and mRNA expression in mouse calvarial osteoblasts as well as calcium accumulation mineralized bone nodules. Regul Pept. 1999;85(1):47–58.
15. Athari SS, Pourpak Z, Folkerts G, Garssen J, Moin M, Adcock IM, et al. Conjugated Alpha-Alumina nanoparticle with vasoactive intestinal peptide as a Nano-drug in treatment of allergic asthma in mice. Eur J Pharmacol [Internet]. 2016;791:811–20. Available from: http://dx.doi.org/10.1016/j.ejphar.2016.10.014
16. Wernig K, Griesbacher M, Andreae F, Hajos F, Wagner J, Mosgoeller W, et al. Depot formulation of vasoactive intestinal peptide by protamine-based biodegradable nanoparticles. J Control Release [Internet]. 2008;130(2):192–8. Available from: http://dx.doi.org/10.1016/j.jconrel.2008.06.005