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ORIGINAL ARTICLE

Studies On Lyophilization of Injectable Formulations Containing Model Synthetic Nucleoside Analogs

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ABSTRACT

Objective: Synthetic nucleoside analogue drugs are effective antiviral agents that inhibit polymerase. However, long-term storage without freezing remains a challenge. In this study, we aimed to develop an intravenous injection of acyclovir sodium, a compound known for its instability in aqueous solutions, by using lyophilisation technology to extend its shelf life and improve its stability.
Methods: Lyophilization was performed on multiple batches with variations in total cycle time, freezing and holding time, and primary and secondary drying times, while keeping the quantities of active pharmaceutical ingredients constant. The process was optimised to achieve a total lyophilisation cycle of 51.75 hours. The resulting lyophilised product was evaluated for several in vitro parameters including cake appearance, reconstitution time, pH, assay, related substances, particulate matter, water content, colour value, percentage light transmittance, and Differential Scanning Calorimetry (DSC).
Findings: Batch-V was identified as the optimised formulation based on its compliance with all the USP specifications and evaluation parameters. Accelerated stability studies confirmed that this formulation remained stable under the tested conditions, demonstrating the effective preservation of product quality over time. This study presents a significant improvement in stabilising acyclovir sodium at a concentration of 1 gram per vial, demonstrating the efficacy of lyophilisation in maintaining drug stability.
Novelty: This study demonstrates that lyophilisation enhances the stability of acyclovir sodium injections, extending their shelf life beyond aqueous solutions, offering a potential solution to overcome the long-term storage limitations of nucleoside analogues.

Keywords: Acyclovir sodium; lyophilization; Differential Scanning Calorimetry

INTRODUCTION

Lyophilisation is the most commonly used method for parenteral manufacturing when aqueous solution stability is a major issue. It is central to the protection of materials, which require low moisture content (less than 1%) to ensure stability and a sterile and gentle preservation process [1–6]. During the past decade, there has been dramatic progress in our understanding of the intracellular uptake, drug metabolism, interaction with cellular targets, and pharmacokinetics of many nucleoside analogues. These compounds are antimetabolites that interfere with nucleic acid synthesis either by being incorporated into viral or cellular DNA or RNA or by modifying the metabolism of physiological nucleosides [7]. In freeze-dried form, mRNA-LNP (lipid nanoparticle) vaccines can be conveniently shipped worldwide without the need for cooling or freezing [8–10]. Lyophilisation is essential for thermolabile and solution-unstable substances. This study aimed to develop a stable injectable dosage form using lyophilisation. It involves pre-formulation studies on the drug, selecting suitable excipients and cryoprotectants, and formulating the injectable dosage. The lyophilisation process was performed and optimised, followed by a thorough evaluation of the formulation and stability to ensure long-term stability and effectiveness.

METHODOLOGY

Material and sources

Acyclovir sodium (source: Sequent Life Science), water for injection, chloroform, potassium bromide, acetonitrile, acetone, and dilute acetic acid. Potassium dihydrogen

phosphate and sodium hydroxide were procured from the Strides Technology and Research Centre (STAR), Strides Arco Lab, Bangalore.

Pre-formulation study on active pharmaceutical ingredients

The main aim of pre-formulation study is to ascertain that the drug substance complies with the pharmacopoeia standards. The formulation details are listed in Table 1 .

Table 1: Formulation details of acyclovir sodium for injection

Sr. No.	Ingredients	Formulation Trial Batches				
		1	2	3	4	5
		Lyo cycle 1	Lyo cycle 2	Lyo cycle 3	Lyo cycle 4	Lyo cycle 5
1	Acyclovir sodium	1gm/vial	1gm/vial	1gm/vial	1gm/vial	1gm/vial
2	Water for injection	q.s to 10 ml	q.s to 10 ml	q.s to 10 ml	q.s to 10 ml	q.s to 10ml

The API was analyzed during the pre-formulation stage for its description, solubility, melting point, identification, pH, water content, and assay.

Description

The powder was a white crystalline powder, odourless or almost odourless, tasteless, and less hygroscopic in nature.

Solubility

Freely soluble in water and in solutions of alkali hydroxides and carbonates, slightly soluble in alcohol, practically insoluble in chloroform and in ether.

Melting Point

The Melting point was determined using a Campbell melting point apparatus.

Identification

A small amount of potassium bromide was triturated into an agate mortar and used as a blank disc, while 1% acyclovir sodium was triturated into powder KBr and again used as a blank disc. Chromatograms were recorded using a Jasco FTIR spectrometer.

The sample spectrum of acyclovir sodium was compared to the standard spectrum of acyclovir sodium.

PH

The pH of the acyclovir sodium solution was measured and recorded.

Water content (by Karl Fisher

Karl Fisher titration was used as an analytical method for quantifying the water content in the drug, and methanol was used as a solvent. Water content was calculated using the following formula:

$$Formula = \frac{Volume\ Consumed \times KF \times 100}{Weight\ taken\ (mg)}$$

Assay

Assay and related substances were analysed by Agilent 1100 series HPLC. Potassium dehydrogenate orthophosphate, acetonitrile, and orthophosphoric acid were used and USP Acyclovir sodium was used as the working standard.

Chromatographic Conditions. The chromatographic conditions for the analysis were as follows: a YMC C18 column with dimensions of 250 × 4.6 mm and a particle size of 5 μm was used. The column temperature was maintained at 25 °C. A sample volume of 10 μL was injected and the flow rate was set to 1 ml/min. Detection was carried out at a wavelength of 254 nm using a Variance Wavelength Detector. The total run time for the analysis was 30 minutes and water was used as the diluent.

A buffer solution. 6.0 gm of potassium dihydrogen orthophosphate ($KH2PO_4$) was dissolved in a 1000 ml volumetric flask with distilled water. The pH of orthophosphoric acid ($H3PO_4$) was adjusted to 3.0, and the final volume was made up with distilled water to 1000 ml, mixed well, and filtered.

Mobile Phase. Buffer solution and acetonitrile (95:5) were prepared and filtered.

Standard Preparation. Acyclovir sodium (25 mg) was weighed into a 25 ml volumetric flask with distilled water which served as the stock solution. Five milliliters were transferred from the stock solution into a 50 ml volumetric flask and made up to the final volume with distilled water to 50 mL which served as the standard.

Sample preparation. The lyophilised vial was reconstituted with 10 ml of water for injection. Subsequently, 1 ml of this solution was transferred to 50 ml of water. From this solution, 10 ml was transferred and made up to 100 ml with water (the sample solution).

Related substances. The related substances were determined by HPLC. The unknown impurities and total impurities were determined and recorded.

- **The unknown impurities were calculated as follows:**

$$\frac{\text{Impurity Area} \times \text{Std. Weight} \times \text{Sample Dilution} \times \text{Potency of Std.} \times 100}{\text{Avg. Std. Area} \times \text{Std. dilution} \times \text{Sample taken} \times 100 \times \text{L C}}$$

- **The total Impurity content was calculated as follows:**

$$\frac{\text{Total Impurity Area} \times \text{Std. Weight} \times \text{Sample Dilution} \times \text{Potency of Std.} \times 100}{\text{Avg. Std. Area} \times \text{Std. dilution} \times \text{Sample taken} \times 100 \times \text{L C}}$$

Method of preparation

The manufacturing process involved washing and sterilizing the vials, lyo stoppers, and aluminum seals under aseptic conditions. The drug was weighed and fresh water for injection was collected, cooled, and purged with nitrogen. The drug was then added to water, stirred, and filtered. The solution was then filled into 10 ml USP Type I tubular flint glass vials, partially stoppered, and loaded into a lyophilizer. After lyophilization, the rubber plugs were stoppered under nitrogen, sealed with aluminum seals, and stored at a suitable temperature.

Potency of API was calculated as follows:

$$Conversion\ factor = \frac{Mol.\ Weight\ of\ Acyclovir\ Sodium}{Mol.\ Weight\ of\ Acyclovir}$$
$$= \frac{247.2}{225.2}$$
$$= 1.097$$
$$Quantity\ required\ for\ batch = Actual\ quantity \times 1.097$$
$$\frac{Quantity\ for\ batch = Strength \times 100 \times 100}{Assay\ value \times (100 - Water\ content)}$$
$$= \frac{109.8 \times 100 \times 100}{99.5\ x\ (100 - 5.18)}$$
$$= 117.59\ mg/ml$$

Evaluation of lyophilized product

The lyophilized product was evaluated for several formulation characteristics, including description, reconstitution time, pH, water content, identification by IR, particulate matter, colour value, light transmission, and assay.

Description

The samples were viewed under visible light.

Reconstitution time

The lyophilised vial of the formulation was reconstituted with 10 ml of water for injection. The reconstitution time was recorded.

PH of the reconstituted solutions

The lyophilised vial of the formulation was reconstituted with 10 ml of water for injection. The pH values of the reconstituted solutions were recorded.

Water content

Karl Fischer titration was used to determine the moisture content of the drug using methanol as a solvent.

Identification by IR

Transfer small quantities of KBr into agate mortar, triturate evenly, and perform as the blank disc. Triturated about 1% Acyclovir sodium (batch-V) was then added to the powder. KBr evenly to again a blank disc. Scan blank flakes and sample flakes and record the chromatogram. The identification was performed using a Jasco FTIR spectrometer. The sample spectrum of acyclovir sodium was compared to the standard spectrum of acyclovir sodium.

Particulate matter[11]

The correct syringe size was selected and rinsed with the rinsing solvent, and 30% IPA was used for rinsing. After rinsing, the vials were reconstituted with 10 ml of water for injection. Approximately 50 ml of the reconstituted sample was collected and introduced into a particulate matter counter. The counter displays the particulate count three times, and the average is taken as no. of the particles present in the sample. As per the USP, particulate matter in injections is allowed up to 600 and 6000 particles of 25 μm and 10 μm per container, respectively.

Colour value

The colour value of the reconstituted solutions was observed at 430 nm using a UV spectrophotometer and recorded.

Light Transmission

The light transmission of the reconstituted solutions was observed at 650 nm using a UV spectrophotometer, and the light transmission value was recorded.

Assay

Buffer solution. About 6.0 gm of potassium dihydrogen orthophosphate (KH2PO4) was dissolved in 1000 mL volumetric flask with distilled water. The pH of orthophosphoric acid (H3PO4) was adjusted to 3.0, and the final volume was made up to 1000 mL with distilled water, mixed well, and filtered.

Mobile Phase. Buffer solution and acetonitrile at a ratio of 95:5 were prepared and filtered.

Standard Preparation. Acyclovir sodium (25 mg) was accurately weighed in a 25 ml volumetric flask with distilled water (Stock Solution). The stock solution (5 mL) was transferred into a 50 mL volumetric flask and made up to the final volume with distilled water (standard).

Sample preparation. The lyophilised vial was reconstituted with 10 ml of water for injection. From this solution, 1 mL was transferred and diluted with 50 ml of water. From this solution, 10 mL was transferred to 100 ml of water (sample solution).

Chromatographic Conditions. A YMC C18 column with dimensions of 250 × 4.6 mm and a particle size of 5 μm was used, and the column temperature was maintained at 25 °C. An injected volume of 10 μL was used at a flow rate of 1 ml/min. Detection was carried out at a wavelength of 254 nm using a Variance Wavelength Detector. The run time for the analysis was 30 minutes, and water was used as the diluent.

- **(Acyclovir sodium) Assay percentage was calculated using the formula:**

$$\text{Formula} = \frac{\text{Avg. sample Area x Std. weight x Std. Dilution x Sample Conc. x Std. potency}}{\text{Avg. std. Area x Dilution Factor x 100 x LC}}$$

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was used to determine the difference in the amount of heat required to increase the temperature of the sample, and the reference was measured as a function of time. DSC was performed at a ramp rate 5°C/min to -60°C and 5°C/min to 60 °C. Both the sample and the reference were maintained at the same temperature throughout the experiment.

Stability study [12]

Accelerated testing studies were designed to increase the rate of chemical or physical degradation of the drug substance/product by using exaggerated storage conditions as per USFDA, and rapid detection of deterioration of the drug in different formulations can be achieved in a short time. Stability studies were performed according to the ICH guidelines. The accelerated study was carried out at temperatures of 40 °C±2 °C/75%±5% RH, and the sample was withdrawn at one-month intervals and analysed for evaluation parameters, such as assay, pH, loss on drying, reconstitution time, colour value, light transmission, and particulate matter.

RESULTS AND DISCUSSION

As a primary step, the drug was subjected to pre-formulation studies to check compliance with the USP specification. The drug was found to be a white crystalline powder, which complies with the standard. Solubility was determined, and it was found that the drug was freely soluble in water, slightly soluble in alcohol, and practically soluble in chloroform and ether. The drug was identified by the IR spectrum, which complied with the standard drug spectrum. The assay was carried out by HPLC, which was found to be 99.50%. The related substances guanine and unknown impurities were found to be 0.14% and 0.02% respectively and the total impurity was found to be 0.16%. The water content of the drug was analyzed by the Karl Fischer titration method and was found to be 4.78%. The pH of the solution was found to be 11.3 and the melting point was 2570C. These results are within the USP limits (Table 2).

The lyophilization technique was adopted to formulate the parenteral dosage form of acyclovir sodium, with water for injection as a vehicle. The lyophilization was carried out in five different batches by varying the total cycle time, freezing and holding time, primary drying and secondary drying times Acyclovir sodium strength was maintained at 1gm/vial without any cryoprotectant added.

In batch-I, a cycle duration of 41.33 hours was carried out which resulted in bulging of the cake, which is not desirable for a stable and elegant product. The cycle was altered in batch-II by reducing the primary drying duration and increasing the secondary drying duration of the total cycle to 43.33 hours which resulted in melt back, the condition that was characterized by the extreme shrinkage of material that looked like a sticky mass in the container. This results in incomplete drying, a high moisture content, and decreased solubility. Due to melt back in batch-II, the freezing and primary drying duration increased in batch-III with a total cycle duration of 44.91 hours which resulted in layers of cake.

The cycle was further modified in batch IV with a total duration of 46.25 hours. The cake structure was good, but the moisture content was 6.23%, which was above the limits (4% - 6%) Hence, in batch-V the secondary drying duration was increased 6.5 hours to 14.5 hours, with total duration of 51.75 hours. The cake structure was good, and the moisture content was within the specification range (4% -6%). All other evaluation parameters were found to be within the limits and hence the lyo cycle of batch-V with duration of 51.75 hours was selected as optimized cycle with freezing- 9.25 hours, primary drying- 28.00 hours and secondary drying- 14.50 hours (Table 2).

Evaluation of the lyophilized product across the five batches revealed that the lyophilized cakes in all batches were white, as shown in Table 2 . The moisture content, a critical factor, was found to be within the acceptable range of 4%-6% for batches I (6.10%), II (5.56%), III (5.86%), IV (6.23%), and V (4.75%), indicating that all products had moisture content within the limits. The cake formation varied: batch I exhibited bulging, batch II showed melt back, and batch III had layers of cake, whereas batches IV and V showed complete cake formation without issues such as sticking or melting back. The reconstitution times for batches I, II, and III were 21-24 seconds, whereas those for batches IV and V were shorter at 19 seconds. The pH of the reconstituted products in all batches was within the USP limits of 11-11.5, indicating no stability problems, such as hydrolysis and esterification. The assay results, determined by HPLC, were within the USP specification range of 97.50%-100.50%. Additionally, the colour value and % light transmittance, assessed using the UV method, were within the USP limits for all batches. Based on these results, batches IV and V were identified as good formulations. Given that cake formation plays a crucial role in the final selection of the formulation,

Table 2: Preformulation study data of Acyclovir sodium and analysis of Acyclovir sodium formulation

Preformulation studies data of Acyclovir sodium

Sr. No.	Tests	Standards	Results
1	Description	A White or almost white crystalline powder	White crystalline powder
2	Solubility	Freely soluble in water Slightly soluble in methanol Practically insoluble in Chloroform and Acetone	Passes
3	Melting Point	257°C	Passes
4	Identification by I R	Sample spectrum must be Concordant with the Standard spectrum	Passes
5	Foreign matter	Sample should be free from black particles and foreign matter	Passes
6	pH	Not less than 11	11.3
7	Water content (by KFT)	Between 4 and 6%	4.78%
8	Assay	Between 98.0 and 101.0%	99.50%
9	Related compounds by HPLC Guanine Unknown impurity Total impurity	Not more than 0.5% Not more than 0.1% Not more than 1.0 %	0.14% 0.02% 0.16%

Analysis of Acyclovir sodium formulation

Evaluation parameters	Batch-I	Batch-II	Batch-III	Batch-IV	Batch-V
Appearance	White in colour	White in colour	White in colour	White in colour	White in colour
Moisture content %	6.10%	5.56%	5.86%	6.23%	4.75%
Cake formation	Bulging of cake	Melt back	Cracking of cake	Good cake formation	Good cake Formation
Reconstitution time(sec)	24 sec	21 sec	23 sec	19 sec	16 sec
pH(initial)	11.03	11.12	10.99	11.30	11.31
pH (After 24 hours)	11.01	11.09	10.97	11.29	11.34
Assay	97.50%	98.32%	98.12%	99.90%	100.50%
Color value (At 430nm)	0.0213	0.0298	0.0193	0.0198	0.0195
Light transmittance (At 630nm)	98.21%	98.61%	96.96%	98.95%	99.56%
Particulate matter	3665	4021	4121	4900	4201
$\geq 10\mu$m: Not more than 6000/vial $\geq 25\mu$m: Not more than 600/vial	348	398	438	468	235

complete and stable cakes in batches IV and V were significant. Furthermore, moisture content is important for preventing chemical reactions and microbial growth, with batch V having the lowest moisture content compared to batch IV. The reconstitution time was also shorter in batch V. Therefore, batch V was selected as the optimized formulation and subjected to further studies including particulate matter determination and differential scanning calorimetry.

Particulate matter analysis was performed for the batch-V formulation, and the particulate matter was found to be 4201 (10 μm) and 235 (25μm). The results showed that the values were within the limits of the USP.

Differential scanning calorimetry (DSC) was performed on the lyophilized drug product at a ramp rate of 5°C/min from -60°C to 60 °C. The DSC thermogram of the acyclovir sodium liquid formulation before lyophilization exhibited an endothermic peak at -32.03°C, indicating its eutectic point. The optimized lyophilized product, reconstituted with 10 mL of WFI, showed an endothermic peak at -33.37 °C, demonstrating no change in the eutectic point. This result confirmed that the crystalline nature of the drug was preserved after lyophilization.

Stability studies were performed in accordance with ICH guidelines at 40 °C ± 2 °C and 75% RH ± 5% RH. The optimised lyophilised formulation was analysis at one-month intervals over a period of three months. The evaluated parameters included the colour of the product, reconstitution time, pH, assay, particulate matter, colour value, and percentage light transmittance, as summarised in Table 2. After the first month, the colour of the product remained consistent with the initial formulation, with no observable colour changes. The reconstitution time was 17 seconds, and the pH was 11.35, showing no significant deviations from the initial values. HPLC assay results

indicated that the formulation remained within the USP limits. The colour value and percentage light transmittance, determined via UV methods, were 0.0196 and 99.58%, respectively, and were also within the USP limits. Particulate matter counts were recorded as 4224 for particles $\geq$10 μm and 239 for particles $\geq$25 μm, indicating that the product was stable after one month. During the second and third months, the appearance of the product remained unchanged and no colour variations were observed. All the evaluation parameters continued to meet the specified limits, as shown in Table 2 .

The results confirm that the product maintains stability under accelerated conditions, supporting an expected shelf-life of two years for batch-V. Based on these findings, it is clear that batch-V was effectively optimised as the target lyophilised product of acyclovir sodium IV injection (Table 3).

Table 3: Stability data of optimized formulation for Batch-V

Duration	First month	Second month	Third month
Stability condition	40°C $\pm$2°C / 75% RH	40°C $\pm$2°C / 75% RH	40°C $\pm$2°C / 75% RH
Appearance	White in color	White color	White in color
pH	11.35	11.39	11.40
Light transmittance (at 630nm)	99.58%	99.61%	99.62%
Color valve (at 430nm)	0.0196	0.0198	0.0199
Reconstitution time	17 sec	16 sec	17 sec
Assay	100.50%	100.10%	99.91%
Particulate matter $\geq$10μm: Not more than 6000/vial $\geq$25μm: Not more than 600/vial	4224 239	4265 241	4296 246

We observed superior stability compared to existing marketed formulations, thereby achieving the goal of developing a stable and long-lasting lyophilised acyclovir sodium IV injection.

CONCLUSION

Batch-V, with a duration of 51.75 hours, was chosen as the best formulation due to its good cake formation, pH, particulate matter, water content, colour value, and light transmission being within USP limits. Stability studies confirmed the stability of the formulation, indicating that lyophilisation is advantageous for developing a stable injectable dosage form of acyclovir sodium.

REFERENCES

1. Kudra T, Mujumdar AS. Advanced drying technologies;vol. 438. 2nd ed. and others, editor;CRC Press. 2018. Available from: https://www.researchgate.net/publication/329319389_Advanced_drying_technologies_second_edition.
2. Ratti C. Hot air and freeze-drying of high-value foods: a review. *Journal of food engineering*. 2001;49(4):311–319. Available from: https://doi.org/10.1016/S0260-8774(00)00228-4.
3. Bubnovich V, Reyes A, Quijada E, Mahn A. Numerical simulation of lyophilization of carrot slices at atmospheric pressure in a fluidized bed. *Journal of food engineering*. 2012;109(4):659–667. Available from: https://doi.org/10.1016/j.jfoodeng.2011.11.030.
4. Vivet-Boudou V, Didierjean J, Isel C, Marquet R. Nucleoside and nucleotide inhibitors of HIV-1 replication. *Cellular and Molecular Life Sciences*. 2006;63(2):163–186. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11136153/pdf/18_2005_Article_5367.pdf.
5. De Clercq E, Neyts J. Therapeutic potential of nucleoside/nucleotide analogs against poxvirus infections. *Journal of Medical Virology*. 2004;14(5):289–300. Available from: https://doi.org/10.1002/rmv.439.
6. Cm G, Jordheim L, Dumontet C. Pyrimidine nucleoside analogs in cancer treatment. *Expert review of anticancer therapy*. 2003;3:717–728. Available from: https://doi.org/10.1586/14737140.3.5.717.
7. Parker WB, Secrist JA, Waud WR. Purine nucleoside antimetabolites in development for the treatment of cancer. *Current opinion in investigational drugs*. 2004;5(6):592–596. Available from: https://pubmed.ncbi.nlm.nih.gov/15242246/#:~:text=Five%20purine%20analogs%20have%20been,nelarabine%20and%208%2Dchloroadenosine).
8. Chen C, Han D, Cai C, Tang X. An overview of liposome lyophilization and its future potential. *Journal of controlled release*. 2010;142(3):299–311. Available from: https://doi.org/10.1016/j.jconrel.2009.10.024.
9. Kasper J, Winter G, W F. Recent advances and further challenges in lyophilization. *European Journal of Pharmaceutics and Biopharmaceutics*. 2013;85:162–169. Available from: https://doi.org/10.1016/j.ejpb.2013.05.019.
10. Jeffs LB, Palmer LR, Ambegia EG, Giesbrecht C, Ewanick S, Maclachlan I. A scalable, extrusion-free method for efficient liposomal encapsulation of plasmid DNA. *Pharmaceutical research*. 2005;22:362–372. Available from: https://link.springer.com/article/10.1007/s11095-004-1873-z.
11. Nath N, Mcneal E, Obenhuber D, Pillari B, Shelton L, Stevens-Riley M, et al. Particulate contaminants of intravenous medication and the limits set by USP General Chapter h788i. *Pharmacopeial Forum*. 2004;30(6):2272–2280. Available from: https://www.researchgate.net/publication/237675806_Particulate_Contaminants_of_Intravenous_Medication_and_the_Limits_Set_by_USP_General_Chapter_h788i.
12. DR I. Stability testing of new drug substances and products Q1A (R2). In: In Proceedings of the International Conference on Harmonization. 2003. Available from: https://database.ich.org/sites/default/files/Q1A%28R2%29%20Guideline.pdf.