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FORMULATION DEVELOPMENT AND EVALUATION OF GEMCITABINE HYDROCHLORIDE FOR INJECTION

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ABSTRACT

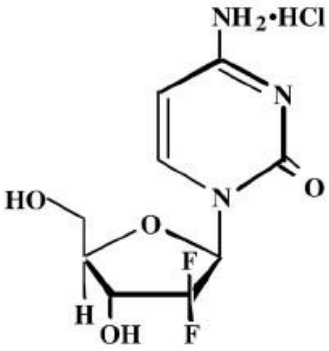
Products are manufactured in the lyophilized form due to their instability when in solution. There are many new parenteral products, including anti-infectives, biotechnology derived products, and in- vitro diagnostics which are manufactured as lyophilized products. In the present study, the anticancer drug was formulated as lyophilized dosage form. The description, identification, assay, solubility, melting point, solution stability, pH stability, compatibility of the drug with different excipients, glass transition temperature, rubber closure compatibility were determined. From the solubility studies, it was found that API was water soluble. pH-stability profile was generated over a pH range of 2.7- 3.3 and studies showed that the drug was stable in the range and pH of 2.9 was used for the study. FTIR was used to assess the compatibility of drug with selected excipients. Based on the results of FTIR, Mannitol PFG, Lactose Monohydrate and Dextrose Monohydrate were found to exhibit no interaction with the drug. Rubber closures compatibility studies reveal the absence of loss of drug with Stelmi and West Pharma rubber closures. The drug in solution form was subjected to stability studies and suggested that the drug in solution form was stable upto 48 hours. Finally lyophilized parenteral dosage form was developed with selected excipients. Short term accelerated stability studies were also conducted and from the results, it was concluded that the optimized formulation was found to be the stable lyophilized parenteral dosage form.

KEYWORDS: Anti-cancer drug, Freeze drying/Lyophilization, Stability, Freezing, Parenteral dosage form, Primary Drying, Secondary Drying.

1. INTRODUCTION

1.1 Drug and Excipient Profile

1.1.1 Gemcitabine Hydrochloride USP: Structure



Empirical Formula: C H F N O.HCl,

Melting Point: 286°C to 292°C 911234

Chemical Name: 4-amino-1-[(2R, 4R, 5R)-3, 3-difluoro-4-hydroxy-5- hydroxymethyl) oxolan-2-yl] pyrimidin-2- one

Molecular Weight: 299 Da Experimental Water Solubility: Soluble LogP: 0.14

1.1.1.1 Pharmacology

Gemcitabine is an antineoplastic anti-metabolite. Anti-metabolites masquerade as purine or pyrimidine - which become the building blocks of DNA. They prevent these substances becoming incorporated in to DNA during the "S" phase (or DNA synthesis phase of the cell cycle), stopping normal development and division. Gemcitabine blocks an enzyme which converts the cytosine nucleotide into the deoxy derivative. In addition, DNA synthesis is further inhibited because Gemcitabine blocks the incorporation of the thymidine nucleotide into the DNA strand.

1.1.1.2 Mechanism of Action

Gemcitabine inhibits thymidylate synthetase, leading to inhibition of DNA synthesis and cell death. Gemcitabine is a prodrug so activity occurs as a result of intracellular conversion to two active metabolites, gemcitabine diphosphate and gemcitabine triphosphate by deoxycitidine kinase. Gemcitabine diphosphate inhibits ribonucleotide reductase, the enzyme responsible for catalyzing synthesis of deoxynucleoside triphosphates required for DNA synthesis. Gemcitabine triphosphate (diflurorodeoxycytidine triphosphate) competes with endogenous deoxynucleoside triphosphates for incorporation into DNA.

1.1.2 Excipients used

1.1.2.1 mannitol

1.1.2.2 lactose monohydrate

1.1.2.3 Dextrose monohydrate

1.2 Lyophilization/ Freeze Drying

Freeze-drying (lyophilization) is a processing method commonly used in the pharmaceuticals and biologicals industry to improve the stability of thermally labile molecules mostly proteins and biomolecules. Some active ingredients are able to sustain 1only in weeks of storage as a liquid formulation, but they can be stored for years when freeze-dried using suitable excipients.

During the freeze drying cycle, the protein formulation is first frozen to separate the solvent (water) from the solutes (protein and excipients) after which the ice is removed under vacuum conditions by a process known as sublimation. Finally, any residual water in the solid matrix may be removed by a process known as desorption. Overall, the freeze drying cycle can be viewed mainly as a three step process: freezing, primary drying and secondary drying.

1.2.1 Principles f Lyophilization

The lyophilization cycle can be broken down into three main steps: Freezing, Primary Drying and Secondary Drying.

1.2.1.1. Freezing transforms the water into ice crystals and the solids into two or more phases; usually a crystalline ice phase and an amorphous freeze concentrate phase containing the active pharmaceutical ingredient (API) and excipients.

1.2.1.2. Primary Drying is the step where the solvent is removed by a process known as sublimation where ice is converted to water vapour. Both a vacuum and an increase in the shelf temperature are required to promote sublimation.The primary drying rate is dependent on the vacuum and temperature setpoints along with an efficient freezing step. Primary drying is completed when all the ice crystals have been removed from the formulation, and the volume occupied by the resulting cake is equivalent to that of the frozen matrix.

1.2.1.3. Secondary drying involves the removal of bound water by a process known as desorption. The remaining water to be removed is usually around 15 – 20% w/w of the solute.[26] The step involves increasing the temperature parameters to higher setpoints than

that of primary drying in order to achieve desorption and achieve minimum moisture content within the Lyophilised cake without reducing the volume of the cake .

2.0 MATERIALS AND METHODOLOGY

2.1 Materials

Table 01: List of Material.

S No:	Materials
1	Gemcitabine HCl
2	Ethanol
3	Mannitol (PFG)
4	Lactose Monohydrate
5	Dextrose Monohydrate
6	Sodium chloride
7	Sodium hydroxide
8	Hydrochloric acid
9	Water for injection

2.2 Formulation

Prior to formulation pre formulation studies are done and results are given below Mannitol (PFG), Lactose Monohydrate and Dextrose Monohydrate were selected as bulking agents in the following ratio,

F1: Gemcitabine 40mg/mL+ Mannitol (PFG) 40mg/mL,

F2: Gemcitabine 40mg/mL+ Lactose Monohydrate 40mg/mL and F3: Gemcitabine 40mg/mL+ Dextrose Monohydrate 40mg/mL.

Sodium acetate anhydrous was used as buffering agent, Further lyo cycle was developed.

Table 02: Comparison of ingredients of formulations.

S.No	Ingredients	F1 (mg/ml)	F2 (mg/ml)	F3 (mg/ml)
1	Gemcitabine	40	40	40
2	Sodium acetate anhydrous	2.5	2.5	2.5
3	Mannitol (PFG)	40 (1:1)	---	---
4	Lactose Monohydrate	---	40 (1:1)	---
5	Dextrose Monohydrate	---	---	40 (1:1)
6	0.1N HCl	q.s	q.s	q.s
7	0.1N NaOH	q.s	q.s	q.s
8	WFI	Upto 1ml	Upto 1ml	Upto 1ml

2.2.1 Method of formulation

A batch of 15 vials of 200mg dose were prepared for all formulations as per below given method and evaluated the cake characteristics, water content, pH, Reconstitution time.

2.2.1.1 Collected about 70% (of total batch size) of WFI in all the 3 separate containers labeled as F1, F2, F3 and purged with filtered nitrogen gas and stirred at a rate of 300 rpm for more than 10 min, after that the pH of WFI was found to be 5.91 at a temperature of 27.5^{o}C. Required amount of sodium acetate anhydrous was added to all formulations and stirred for ~ 5min at 300 rpm to form clear solution and pH was found to be 8.0.

2.2.1.2 Required amount of Mannitol PFG, 3 gm of Lactose monohydrate and Dextrose Monohydrate were added to F1, F2 and F3 respectively and stirred for ~ 10 min

2.2.1.3 Required amount of Gemcitabine HCl was added to all formulations and stirred for ~15 min

2.2.1.4 The pH of all solutions was adjusted to 2.9 (limit – 2.7 to 3.3) using 0.1 NaOH and 0.1N HCl

2.2.1.5 The final volume was made with WFI using volumetric flask and again pH of solution was performed and found to be 2.93

2.2.1.7 All the formulations were sterilized using 0.22µ PVDF filter under aseptic conditions

2.2.1.8 5 ml of bulk solution was filled into each vial under laminar air flow, partially stoppered and transported to lyophilizer

2.2.1.9 Each formulation was lyophilized separately not to interact the results of each other

2.2.1.10 Different trials were done to optimize the lyo cycle with changing freezing, drying temperatures and drying rates and times.

After completion of lyophilization, vacuum was released, vials were stoppered and sealed. Vials were analyzed for evaluation parameters.

2.2.2 Development of lyophilization cycle

Lyophilization or freeze drying is a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor without passing through a liquid phase. The process consists of three separate, unique and interdependent processes; freezing, primary drying and secondary drying.

Different lyophilization cycle were tried to optimize the cycle which gives stable and uniform cake.

Results obtained from final set cycle was given in the subsequent chapters.

Lyo Cycle

Table: 03: Lyophilization cycle (trial 04) total time = 54.8 hr.

Step	Temp(^{o}C) °C	Ramp duration (min) (min)	Soak duration (min) (min)	Pressure (mbar) (mbar)
Freezing				
1	-25	30	50	-
2	-5	20	30	-
3	-45	40	120	-
Primary Drying				
4	-20	180	720	0.400
5	-10	20	600	0.400
6	0	20	740	0.300
7	10	20	180	0.300
8	25	30	220	0.200
Secondary Drying				
9	40	30	240	0.100

Finally above Lyophilization cycle gave satisfactory results and all formulations showed good cake structure and it was taken as optimized cycle. So for the purpose of further evaluation tests and stability studies, a batch of 40 vials of 200mg dose were prepared ,5 ml of bulk solution was filled into each vial under laminar air flow, partially stoppered and transported to lyophilizer. After completion of lyophilization, vacuum was released, vials were stoppered and sealed. Vials were initially analyzed for evaluation parameters and charged for stability studies.

3. RESULTS

3.1 Preformulation Study Results

Table 04: Preformulation Study Results.

Sl.No	Tests	Results
1	Description	A white crystalline solid
2	Identification by IR	The sample spectrum exhibited maxima only at the same wave length as that of standard spectrum.
3	Assay (HPLC)	99 % (The retention time of the major peak in the chromatogram of the assay preparation corresponds to that in the chromatogram of the standard preparation)
4	Solubility	Soluble in water, slightly soluble in methanol, practically insoluble in alcohol and polar organic solvents.
5	Melting point	0289 C
6	pH	2.83 (range – 2.7 to 3.3)

Identification by IR

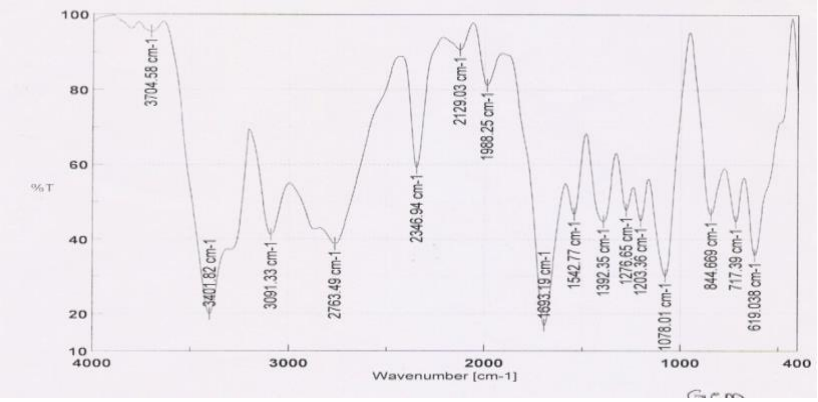


Figure 01: IR spectrum of standard Gemcitabine.

Assay by HPLC

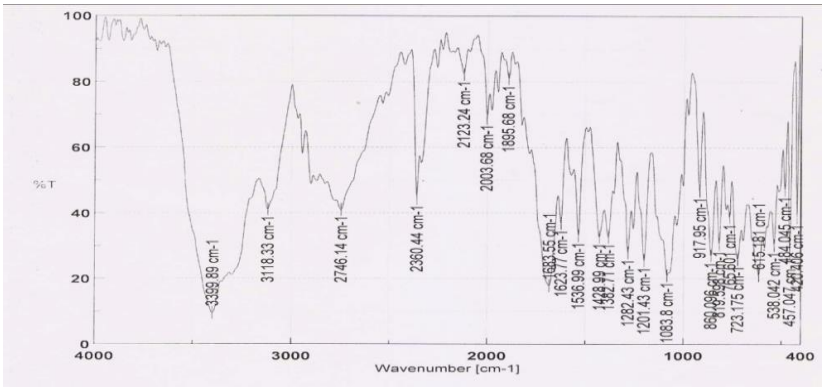


Figure 02: Chromatogram of Gemcitabine HCl.

3.1.1 Solution Stability

At USP controlled room temperature (20-25^{0}C).

Table 05: Solution stability.

Sl. No	Time period	Asssay (%)	Appearance
1	Initial	99.0	Clear colorless
2	After 24 hr	98.3	Clear colorless
3	After 48 hr	94.6	Straw colored

3.1.2 pH-stability profile

Table 06: pH stability.

Sl. No	pH	Assay in %		
		Initial	First week	Second week
1	2.7	101.3	99.2	97.57
2	3.3	101.1	98.76	96.45

3.1.3 Compatibility studies with excipients

Table 07: Compatibility with excipients.

Sl.No	Combination	Results
1	Gemcitabine HCl	Compatible
2	Gemcitabine HCl+Mannitol PFG	Compatible
3	Gemcitabine HCl +Lactose Monohydrate	Compatible
4	Gemcitabine HCl +Dextrose Monohydrate	Compatible

3.1.4 Rubber closure compatibility

Table 08: Rubber closure compatibility.

Sl. no	Rubber stopper	Assay(%)		
		Initial	24^{th} hr	48^{th} hr
1	Control	100.1	99.8	99.6
2	Stelmi Pharma	100.0	99.8	99.5
3	West Pharma	100.1	99.7	99.5

3.2 FORMULATION

Lyo Cycle

Table 09: Evaluation of lyo cycle 04.

S.No	Formulation	Evaluation parameters			
		Description	Water content (%w/w)	Reconstitution Time (sec)	pH
1	F1	White lyophilized cake	1.0	40	2.98
2	F2	White lyophilized cake	1.2	35	2.93
3	F3	White lyophilized cake	1.1	45	3.13

Evaluation of stability study samples of optimized lyophilized formulations

Condition

- $40^{o}C \pm 2^{o}C/75\% \pm 5\%$ RH
- Inverted position

Table 10: Evaluation of Stability study samples of F1.

S.No		Gemzar®	Limits	Initial	1st month	2nd month	3rd month
1	**Description**	**White crystalline powder**	**White to off white crystalline powder**	**White crystalline powder**	**White crystalline powder**	**White crystalline powder**	**White crystalline powder**
2	Water content (%w/w)	1.0	0.8– 2.0	1.1	1.0	1.0	0.9
3	Reconstitution time (sec)	40	30-90	40	40	40	43
4	pH of reconstituted solution	2.93	2.70- 3.30	2.98	3.02	3.03	3.05
5	Assay (%)	98.76	97.50–101.50	100.32	99.92	99.87	99.46
6	Related substances (%)						
	i. Cytosine	0.005	NMT 0.1	0.002	0.002	0.006	0.014
	ii. Gem α anomer	0.004	NMT 0.1	0.003	0.003	0.003	0.003
	iii. Any other impurity	0.003	NMT 0.2	0.002	0.004	0.004	0.005
	iv. Total impurity content	0.012	NMT 0.3	0.007	0.009	0.013	0.025

Table 11: Evaluation of Stability study samples of F2.

		Gemzar®	Limits	Initial	1st month	2nd month	3rd month
1	Description	White crystalline powder	White to off white crystalline powder	White crystalline powder	White crystalline powder	White crystalline powder	White crystalline powder
2	Water content (%w/w)	1.0	0.8– 2.0	1.3	1.2	1.0	1.0
3	Reconstitution time (sec)	40	30-90	40	40	35	35
4	pH of reconstituted solution	2.93	2.70- 3.30	2.93	3.05	3.16	3.27
5	Assay (%)	98.76	97.50 – 101.50	99.89	98.64	97.45	94.5
6	Related substances (%)						
	i. Cytosine	0.005	NMT 0.1	0.002	0.002	0.002	0.04
	ii. Gem α anomer	0.004	NMT 0.1	0.003	0.008	0.09	0.24
	iii. Any other impurity	0.003	NMT 0.2	0.03	0.04	0.04	0.04
	iv. Total impurity content	0.012	NMT 0.3	0.035	0.05	0.132	0.32

Table 12: Evaluation of Stability study samples of F3.

S.No		Gemzar®	Limits	Initial	1st month	2nd month	3rd month
1	Description	White crystalline powder	White to off white crystalline powder	White crystalline powder	White crystalline powder	White crystalline powder	White crystalline powder
2	Water content (%w/w)	1.0	0.8– 2.0	1.2	1.1	0.9	0.9
3	Reconstitution time (sec)	40	30-90	45	45	40	40
4	pH of reconstituted solution	2.93	2.70- 3.30	2.95	3.03	3.09	3.14
5	Assay (%)	98.76	97.50– 101.50	100.43	99.62	98.56	95.82
6	Related substances (%)						
	i. Cytosine	0.005	NMT 0.1	0.009	0.02	0.02	0.07
	ii. Gem α anomer	0.004	NMT 0.1	0.003	0.006	0.03	0.09
	iii. Any other impurity	0.003	NMT 0.2	0.03	0.09	0.15	0.25
	iv. Total impurity content	0.012	NMT 0.3	0.042	0.116	0.200	0.41

4.0 DISCUSSION

The objective of this research work is to develop a stable injectable formulation of the Gemcitabine by lyophilization technique, which overcomes the limitation with respect to shelf life.

As a primary step, the Gemcitabine was subjected to preformulation studies. The drug was identified by IR spectrum and which complies with standard drug spectrum. The assay was carried out by HPLC assay method which was found to be 99 %. The Gemcitabine exhibit Solubility in water and melting point was found to be 28^{o}C Solution stability shows decrease in drug content as time progresses, initial drug content was 99.0 mg and after 48 hours 94.6 mg. it clearly shows that drug degradation proceeds as time proceeds. The drug was stable in the pH range of 2.7-3.3, so further formulation was carried out in this pH range. From compatibility study it was found that Gemcitabine compatible with Mannitol PFG, Lactose Monohydrate and Dextrose Monohydrate. Glass transition temperature was found to be -42.49^{O}C to - 40.98^{O}C and was helpful in designing freeze drying cycle. Two different manufacturers (Stelmi Pharma and West Pharma) of rubber closures were tested for suitability and West Pharma stoppers were recommended for the optimized batches based on

analytical data of rubber stopper compatibility and ease of availability.

FORMULATION

Lyophilization technique was adopted to formulate parenteral dosage form of Gemcitabine. The lyophilization was carried out in different cycles and formulation by varying the total cycle time, freezing, primary drying and secondary drying time, ramp time and holding time and keeping the quantities of all the active pharmaceutical ingredient and excipients as constant for all the trials.

Final approach lyo cycle was carried for 54.8 hour in which annealing process was tried because annealing gives larger crystals which are very easy to dry. In all F1, F2 and F3 cake structure was intact, crystalline and elegant in appearance, pH of the reconstituted solutions were in a range of USP limits, reconstitution time was about 35-45 second, water content was in-between 1.0 – 1.2 %. Since final lyo cycle gave best results compared to initial trials of lyo cycle. As the desired characteristics of lyophilized product should be uniform, distinct, intact with sufficiently dry and porous, this was obtained from final lyo cycle with all formulations, hence the product was replicated with final lyo cycle and charged for accelerated stability studies.

The accelerated stability studies were carried out as per ICH guidelines

- 40°C ± 2°C/75 % ± 5 % RH, (accelerated studies)
- Inverted position

The lyophilized formulations of all F1, F2 and F3 under stability studies were analyzed at time interval of first month, second month and third month. Products were evaluated for parameters such as description, reconstitution time, pH, assay, water content and related substances. F1 showed better results in case of water content, pH, assay, related substances compared to F2 and F3. F1 exhibited all the evaluation parameters within the USP limits and comparable to marketed formulations (Gemzar®) where as F2 showed reduced assay content of 94.5 % after 3rd month which was beyond the limit and more impurity content like Gem α anomer (0.24 %) and total impurity content (0.32 %), F3 showed assay content of 95.82 % after 3rd month which is also low compared to USP limits and increased impurity content (0.41 %). Hence the F1 in which bulking agent was Mannitol PFG was found to be better compared to other formulations F2 and F3.

There fore, Formulation F1 and final approachlyo cycle was found to be suitable for developing lyophilized injectable product of the Gemcitabine that appears to have better stability and reconstitution properties. Hence, the objective of developing an optimized lyophilized dosage form of the Gemcitabine was achieved.

5. SUMMARY

The objective of this research work was to increase stability of Gemcitabine which is used for pancreatic adenocarcinoma, NSCLC (non small cell lung cancer), ovarian cancer and breast cancer. Since, the Gemcitabine is soluble in water and exhibits short term stability at room temperature, if dispensed as liquid injectable dosage form. Lyophilization is one of the techniques employed to increase the stability of the drug in the present research work. Gemcitabine was formulated by lyophilization technique for parenteral administration.

The present research work is to formulate injectable dosage form of Gemcitabine and optimize the lyophilization cycle.

The objective of the study was to perform pre-formulation studies of the drug, selecting suitable vehicle, formulation of injectable dosage form, lyophilization of the formulation, evaluation of the lyophilized product, and finally to perform short term stability studies.

The method adopted is lyophilization and formulations were prepared using water for injection. All the formulated lyophilized formulations were analyzed initially and subjected to short term accelerated stability studies and further evaluation parameters of lyophilized formulations were studied in the time interval of 1st month 2nd month and 3rd month.

The results after 2nd month stability studies concluded that formulation and final lyo cycle was found to be the best formulation passing all the evaluation criteria like identification, assay, percentage water content, reconstitution time, pH and stability, which were within the limits. Finally, it was concluded that the fourth cycle lyophilization protocol was the best process for the formulated Gemcitabine.

6.0 CONCLUSION

The present research work was designed to develop a lyophilized injectable dosage form of pyrimidine analogue, Gemcitabine which is an anticancer agent. As the Gemcitabine was found to be unstable after 48 hr at controlled room temperature, if dispensed as liquid dosage form, which is evident from solution stability studies. Hence the present goal of the project

work is to envisage and overcome the drawbacks associated with Gemcitabine and to formulate a stable formulation by lyophilization technique.

Based on the physicochemical properties of the drug, lyophilization technique was adopted to improve the cake characteristics of the lyophilized form of the Gemcitabine. Four different Lyo cycle were designed and investigated to optimize the product characteristics.

The cycle (optimized cycle) gave better results with respect to the parameters evaluated such as Description, Assay, pH, Water Content, Reconstitution Time. Hence it was decided to subject final approach lyo Cycle formulation for stability studies. From the three months accelerated stability studies data, the F1 showed better assay content and impurity levels were found to be within the USP limits compared to other formulations.

From the above results it was concluded that lyophilization technique proved to be an advantageous tool for the development of stable injectable dosage form for the Gemcitabine. Hence our objective to develop a stable lyophilized injectable of the Gemcitabine was achieved. Lyophilization cycle time can be reduced through optimization.

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