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Formulation Design and Development of Stable Lyophilized Dosage Form of an Alkylating Agent: Carmustine

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ABSTRACT

Carmustine belongs to the category of alkylating agent meant for chemotherapy to treat certain types of brain tumors. The main objective of this work is to develop a novel stable lyophilized dosage form for carmustine with respect to that of the available brand product. The stabilization of the drug product is critical due to the storage condition of the drug substance (-20°C) and the storage condition of drug product (2-8°C). Carmustine for injection was prepared by using lyophilization technique with suitable solvent system. As per the literature review, none of the functional excipient present in the finished product as the drug substance is directly lyophilized with the help of suitable solvent system.

The selection of solvent system depends upon the solubility and stability of drug substance and also feasibility during manufacturing. Dehydrated alcohol, acetone and dimethyl sulfoxide were evaluated for the solublization and stabilization of the drug substance. During the formulation design, the formulated bulk solution was evaluated for the impact of process temperature, process time and compatibility with the contact parts. Due to the usage of organic solvents, the formulated bulk solution was freezed at -50°C and the primary drying was done at -15°C (less than critical product temperature) followed by secondary drying at 15°C. The lyophilization process was optimized with respect to the critical quality attributes of assay, related substances, pH of the reconstituted solution, reconstitution time and residual solvents. There was no significant change in description, pH, assay and related substances of bulk solution up to 24 hrs at refrigerated condition (2-8°C). The bulk solution was found to be compatible with SS 316 vessel, glass, silicon tubing, PVDF filters and nylon filters. The total time for optimized lyophilization process was about 61 hours. The optimized freeze dried product meets the predefined specifications. Based on the

observations and conclusions, it can be concluded that the carmustine for injection can be stabilized by lyophilization process with the proposed organic solvents i.e dehydrated alcohol, acetone and dimethyl sulfoxide.

Key words: Alkylating Agent, Critical Product Temperature, Critical Quality Attributes and Optimized Freeze Dried Product.

INTRODUCTION

Freeze drying, also known as lyophilization, is widely used for pharmaceuticals to improve the stability and long term storage stability of labile drugs, especially protein drugs. Freeze drying is a dehydration process in which aqueous or non-aqueous solution is first frozen and subsequently dried by sublimation process under the presence of vacuum (primary drying). The remaining solid cake undergoes additional drying at elevated temperatures and forms a porous cake with high internal surface area.

Carmustine is one of the nitrosoureas which acts by causing cross-links in DNA and RNA, leading to the inhibition of DNA synthesis, RNA production and RNA translation (protein synthesis) [1,2]. It also binds to and modifies (carbamoylates) glutathione reductase and leads to cell death. Carmustine is chemically known as 1, 3-bis (2-chloroethyl)-1-nitrosoureas. The molecular formula for carmustine is $C_5H_9Cl_2N_3O_2$ and its molecular weight is 214.06. The molecular structure for carmustine was depicted in Figure 1. Literature revealed that Carmustine is highly soluble in alcohol and lipids but poorly soluble in water.

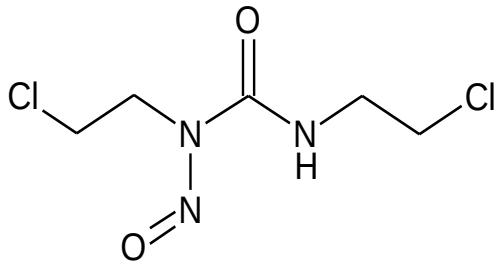

Figure 1: The chemical structure of Carmustine[1]

Carmustine is having low melting point (30.5°C -32.0°C) [3,4]. Exposure of the drug substance or drug product to this temperature or above will cause drug substance to liquefy which appears as an oil film and this will be the sign of decomposition. The drug substance is also a strong vesicant which requires very careful handling. As per the literature of the brand product, the drug substance is directly lyophilized in amber colored vials with organic solvents. The total study was divided in to two parts i.e first one is the stabilization of the drug substance in suitable organic solvent system and second one is optimization of lyophilization process.

MATERIALS AND METHODS

Chemicals and Reagents

Carmustine drug substance gift sample was provided by SP Accure Labs private limited. Dehydrated alcohol, acetone and dimethyl sulfoxide were procured from Merck specialties private limited.

Instruments and Equipments Details

Freezedrier, (Model: Super Modulyo, Make: Edwards), HPLC with PDA detector (Make: Waters, Model No.: 2998 PDA 2695 pump), Electronic Balance (Make: Mettler-Toledo, Model No.: XS-205 dual range), pH meter (Make: Mettler-Toledo, Model No.: FEP20 FIVE Easy Plus PH), Flexible Poly Urethane Isolator (Make: PFI systems Limited, Model No.: IB821), Powered air purifying respirator (Make: BLS Italy, Model No.: JS 3025 PAPR SGE2600 CE), Vacuum oven (Make: Osworld India, Model No.: OVOR-G-11), Chiller (Make: Julabo) and Hot plate (Make: IKA).

Selection of Excipients

Carmustine for injection is commercially available as a sterile lyophilized powder for injection and typically contains no preservative which is not meant for multiple dose vial. None of the excipients are listed in the finished product, hence the carmustine drug substance stability was evaluated in acetone, dehydrated alcohol and dimethyl sulfoxide (co-solvent mixture). Carmustine active requires special storage condition i.e deep freezer (-20°), due to this it was difficult to conduct the drug-excipient binary mixture compatibility studies because the exposure temperatures are too high than the storage condition of API (usually 55°C and 40°C for two weeks and four weeks). Hence the excipient compatibility and also the stability of the drug substance were evaluated in the solvent system selection stage by holding it for several hours.

Selection of Suitable Solvent System

The drug substance is highly soluble in alcohol and lipids and poorly water soluble drug. The drug substance solubility was evaluated in dimethyl sulfoxide, dehydrated alcohol and acetone (individually and co-solvent system). The detailed work plan was depicted in (Table 1). Drug substance stability was evaluated in co-solvent system of dehydrated alcohol, acetone and dimethyl sulfoxide. The prepared bulk solution was evaluated for stability at different processing temperatures of 2-8°C and at room temperature (25°C) with respect to the holding time of 24 hours. The bulk solution was also subjected for the compatibility studies with the contact parts like SS316 vessel, glass vessel, silicon tubing, nylon and PVDF filters. The preparation of bulk solution involves the addition of drug substance to the organic solvent (processed at required temperature) and followed by volume makeup. The sample of bulk solution was periodically withdrawn for evaluating the physic-chemical parameters at different processing temperatures.

Table 1: Solvent selection studies for Cramustine**.**

S. No.	Name of the solvent	Trial Vs (%)				
		A	B	C	D	E
1.	Water for Injection	100	0	0	0	0
2.	Dehydrated alcohol	0	100	0	0	60
3.	Acetone	0	0	100	0	30
4.	Dimethyl sulfoxide	0	0	0	100	10

Critical Product Temperature Determination

Based on the outcomes of the solvent selection studies, the optimized bulk solution was subjected for freeze drying microscopic studies for determination of critical product temperature. The prepared formulation was subjected for Lyostat freeze drying microscope, equipped with Linksys32 image and data capture software [5,6].

Development and Optimization of Lyophilization Cycles [7-11]

Freeze drying microscopic studies revealed that the critical product temperature was found to be -10°C. Complete freezing of the co-solvent bulk solution was observed as -50°C. Hence -50°C temperature was considered for freezing during freezing stage. After completion of freezing, the frozen structure was sublimated below the critical product temperature which was set as -15°C. For desorption of the solid cake, the drying temperature selected was 15°C based on the nature of the drug substance. Various lyophilization cycles were evaluated for optimizing the desired cycle to get the consistent product by modifying the different ramp, hold rates of temperature and vacuum. Finally, the product developed with optimized lyophilization cycle was subjected stability studies. Several lyophilization cycles were evaluated but only four typical lyophilization cycles were presented in (Table 2, 3, 4 & 5).

Table 2: Trial Lyophilization cycle for Carmustine for Injection 100 mg

Step Name	Temperature (°C)	Time (Minutes)		Vacuum (mTorr)
		Ramp	Hold	
Freezing	-45	120	240	-
	-25	60	120	-
	-45	60	420	-
Primary Drying	-40	60	660	250

	-15	120	600	250
	0	180	420	200
	5	60	-	150
Secondary Drying	5	-	240	150

Table 3: Trial Lyophilization cycle for Carmustine for Injection 100 mg

Step Name	Temperature (°C)	Time (Minutes)		Vacuum (mTorr)
		Ramp	Hold	
Freezing	-10	30	10	-
	-20	30	10	-
	-30	30	10	-
	-55	180	300	-
Primary Drying	-12	225	500	150
	5	300	720	150
	15	40	900	150
	20	60	-	75
Secondary Drying	20	-	300	75

Table 4: Trial Lyophilization cycle for Carmustine for Injection 100 mg

Step Name	Temperature (°C)	Time (Minutes)		Vacuum (mTorr)
		Ramp	Hold	
Freezing	-10	30	10	-
	-20	30	10	-
	-30	30	10	-
	-50	180	300	-
	-15	225	500	150

Primary Drying	5	300	900	150
	15	40	960	150
	20	60	-	75
Secondary Drying	20	-	480	75

Table 5: Optimized Lyophilization cycle for Carmustine for Injection 100 mg

Step Name	Temperature (°C)	Time (Minutes)		Vacuum (mTorr)
		Ramp	Hold	
Freezing	-10	30	10	-
	-25	20	60	-
	-50	120	360	-
Primary Drying	-15	200	525	200
	5	300	720	200
	10	40	900	200
	15	60	-	100
Secondary Drying	15	-	300	75

RESULTS AND DISCUSSION

The drug substance was precipitated out when the drug substance was added to the cooled water for injection. The drug substance was dissolved completely in dehydrated alcohol within four minutes without leaving any solid residues. The drug substance was dissolved completely in acetone within eight minutes without leaving any solid residues. The drug substance was dissolved completely in dimethyl sulfoxide within twelve minutes without leaving any solid residues. The drug substance was dissolved completely in co-solvent mixture (60%:30%:10%) within nine minutes without leaving any solid residues. The appearance of solution was found to be clear pale yellow colored solution [12,13].

The order of addition of solvents and the drug substance is critical as they differ each other with respect to their solubility and stability profiles. Among these three solvents, dimethyl sulfoxide is used to help the freezing phenomena during freezing step in

lyophilization process hence it will be used during the batch volume makeup step. Dimethyl sulfoxide is not adding to the co-solvency mixture during compounding because the DMSO gets freeze due to the compounding temperature of 2-8°C. Hence DMSO at room temperature shall be added to the drug solution after compounding and due to its low volume DMSO will be intact in the formulation after batch volume makeup.

The typical analytical results for the stability of the drug substance at different processing temperatures (2-8°C & 25°C) in co-solvent system was depicted in (Table 6). Drug substance was found to be more stable at a processing temperature of 2-8°C. There was no significant change in the description, pH of the bulk solution, assay and carmustine related compound-A at a processing temperature of 2-8°C with a hold time of 24 hours. Hence bulk solution compounding temperature was selected as 2-8°C. At the same temperature, the compatibility studies were conducted with all contact parts. There was no significant change in the description, pH of the bulk solution, assay and carmustine related compound-A. Carmustine bulk solution was found to be compatible with all contact parts i.e SS316 vessel, glass vessel, platinum cured silicon tubing, nylon and PVDF filters. The analytical results for compatibility studies were depicted in (Table 7 & 8) [14-16].

Table 6: Analytical Results for Stability of Carmustine in Co-solvent Mixture (60:30:10) at a Processing temperatures of 2-8°C and 25°C

Processing temperature	Time points (hrs)	Description	pH of bulk solution	Assay (%)	Carmustine related compound-A (%)
2-8°C	Initial	Clear pale yellow colored solution	5.28	99.8	Not detected
	3	Clear pale yellow colored solution	5.31	99.7	Not detected
	6	Clear pale yellow colored solution	5.32	100.1	Not detected
	12	Clear pale yellow colored solution	5.30	99.2	Not detected
	18	Clear pale yellow colored solution	5.29	99.5	Not detected
	24	Clear pale yellow colored solution	5.28	99.6	Not detected
25°C	Initial	Clear pale yellow colored solution	5.32	98.5	0.28
	3	Clear pale yellow colored solution	5.31	97.2	0.39
	6	Clear pale yellow colored solution	5.28	96.7	0.46
	12	Clear pale yellow colored solution	5.20	95.4	1.25

	18	Hazy solution	5.19	90.1	2.38
	24	Hazy solution	5.12	86.6	3.51

Table 7: Compatibility Study Results of Carmustine bulk solution with SS316 vessel and Glass at 2-8°C

Name of the contact part	**Time points (hrs)**	**Description**	**pH of bulk solution**	**Assay (%)**	**Carmustine related compound-A (%)**
SS316 Vessel	Initial	Clear pale yellow colored solution	5.25	99.5	Not detected
	3	Clear pale yellow colored solution	5.27	99.9	Not detected
	6	Clear pale yellow colored solution	5.30	99.4	Not detected
	12	Clear pale yellow colored solution	5.28	99.6	Not detected
	18	Clear pale yellow colored solution	5.22	99.2	Not detected
	24	Clear pale yellow colored solution	5.24	99.0	Not detected
Glass	Initial	Clear pale yellow colored solution	5.30	100.4	Not detected
	3	Clear pale yellow colored solution	5.31	99.9	Not detected
	6	Clear pale yellow colored solution	5.28	100.2	Not detected
	12	Clear pale yellow colored solution	5.27	100.0	Not detected
	18	Clear pale yellow colored solution	5.30	99.7	Not detected
	24	Clear pale yellow colored solution	5.29	99.8	Not detected

Table 8: Compatibility Study Results of Carmustine bulk solution with Silicon tubing, Nylon and PVDF filters at 2-8°C

Name of the contact part	Time points (hrs)	Description	pH of bulk solution	Assay (%)	Carmustine related compound-A (%)
Platinum cured silicon tubing	Initial	Clear pale yellow colored solution	5.32	101.5	Not detected
	3	Clear pale yellow colored solution	5.31	100.9	Not detected
	6	Clear pale yellow colored solution	5.30	101.0	Not detected
	12	Clear pale yellow colored solution	5.31	101.2	Not detected
	18	Clear pale yellow colored solution	5.29	100.8	Not detected
	24	Clear pale yellow colored solution	5.30	101.3	Not detected
Nylon filter	Initial	Clear pale yellow colored solution	5.28	99.6	Not detected
	3	Clear pale yellow colored solution	5.26	99.9	Not detected
	6	Clear pale yellow colored solution	5.27	99.4	Not detected
	12	Clear pale yellow colored solution	5.29	99.5	Not detected
	18	Clear pale yellow colored solution	5.26	100.1	Not detected
	24	Clear pale yellow colored solution	5.30	99.5	Not detected
PVDF filter	Initial	Clear pale yellow colored solution	5.24	100.4	Not detected
	3	Clear pale yellow colored solution	5.27	100.2	Not detected
	6	Clear pale yellow colored solution	5.28	99.8	Not detected
	12	Clear pale yellow colored solution	5.23	100.5	Not detected
	18	Clear pale yellow colored solution	5.29	100.1	Not detected
	24	Clear pale yellow colored solution	5.22	99.6	Not detected

LYOSTAT images are depicted in Figure 2 which clearly shows the phenomena of freezing, sublimation with and without collapse structure.

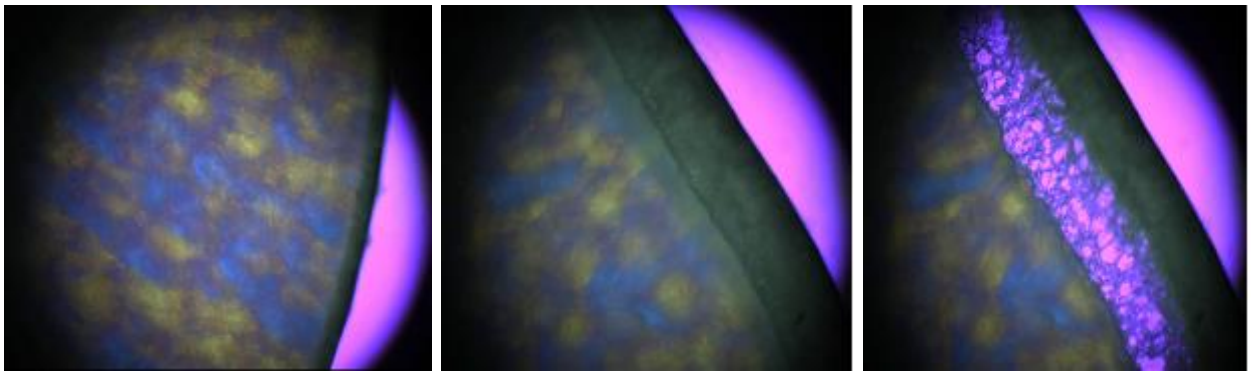

Figure 2: LYOSTAT Images for Critical Product Temperature of Carmustine

Several lyophilized cycles were evaluated. Among the different trials, cake was liquefied and disappeared in some of the vials. The cake appearance was found to be good with the optimized lyophilization cycle. The total drying time for the optimized lyophilization cycle was 3645 minutes (60.75 hours) for effective removal of organic solvents and consistent quality of drug product. The analytical results of the formulation were depicted in Table 9.

Table 9: Analytical Results of Carmustine for injection with optimized lyophilization cycle

S. No.	Name of the CQA	Analytical Results
1.	Description	Pale yellow colored lyophilized powder at the bottom of the vial
2.	Assay	100.2%
3.	Water content	0.12%
4.	Reconstitution time	25 seconds
5.	pH of the reconstituted solution	5.30
6.	Carmustine related compound-A	Not detected
7.	Residual solvents a. Ethanol b. Acetone c. Dimethyl sulfoxide	 3157 ppm 428 ppm 196 ppm

CONCLUSIONS

From the solvent selection and compatibility studies, it can be concluded that the drug substance is stable with proposed co-solvent mixture at a processing temperature of 2-8°C and also found to be compatible with the all contact parts. The optimized

lyophilization cycle can produce good consistent product with less water content and residual solvents. The prepared carmustine for injection meets all pre-defined specifications which were determined with quality target product profile analysis. Finally it can be concluded that the formula designed with proposed organic co-solvent mixture was comparable with that of the brand product.

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