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DEVELOPMENT AND CHARACTERIZATION OF CYCLOPHOSPHAMIDEMONOHYDRATENOVELLYOPHILIZED COMPOSITION FOR INJECTION

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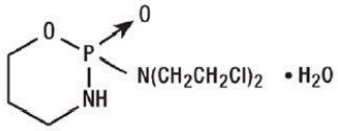
ABSTRACT

Cyclophosphamide is a synthetic antineoplastic drug substance chemically related to the nitrogen mustards. Cyclophosphamide is used to treat various types of cancers and few autoimmune disorders. The current investigation was designed to prepare a stable cyclophosphamidemonohydrate lyophilized composition for injection prepared by a process comprising lyophilization of solution comprising cyclophosphamide monohydrate, dehydrated alcohol and water for injection. In another aspect, there is provided a lyophilized composition comprising cyclophosphamide monohydrate, wherein the composition retains cyclophosphamide monohydrate after lyophilization process. In another aspect, there is provided a lyophilized composition comprising cyclophosphamide monohydrate, wherein the composition retains cyclophosphamide monohydrate after lyophilization and during storage i.e. shelf-life.

INTRODUCTION

Cyclophosphamide is a synthetic antineoplastic drug chemically related to the nitrogen mustards and has the following structure



Cyclophosphamide is used to treat various types of cancers and few autoimmune disorders**.** Cyclophosphamide exists at least in monohydrate and anhydrous forms. The chemical name for Cyclophosphamide monohydrate is 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate, 2H-1,3,2-Oxazaphosphorin-2-amine, N,N bis (2-chloroethyl) tetrahydro-, 2-oxide. The U.S patent number US 4,537,883 discloses a stable rapidly dissolving lyophilized and hydrated composition of cyclophosphamide with sodium bicarbonate. The disadvantages associated with the product described in this patent are the large size of the vials required for lyophilization and time taken to solubilize the product. The U.S patent number US 4,659,699 discloses the process for freeze drying of cyclophosphamide. The two stage process described in the patent involves freeze drying of an aqueous solution of cyclophosphamide to yield a hydrate of cyclophosphamide. In the first stage, cyclophosphamide is freeze dried with an excipient until the moisture content is less than 2% by weight. In the second stage, the freeze dried material is rehydrated until the moisture content of the product is in the critical range i.e. 2-7% by weight. The process

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described in this patent requires the use of high quantity of excipients for maintaining the stability of the product. The PCT publication number WO 2014068585 discloses the process for producing lyophilized compositions of cyclophosphamide monohydrate, wherein the process does not need rehydration step. The lyophilization is carried out in presence of solvent or mixture of solvents. The Canadian patent application number CA 2063058 discloses a process for hydrating a lyophilized composition of cyclophosphamide having a hydrated form as its most stable form and a bulking agent. The process comprises contacting the lyophilized composition with atomized water in a sealed chamber maintained at a reduced pressure, maintaining the relative humidity in the chamber at greater than 90% and, preferably, greater than 95% for a period of time sufficient to convert the composition to its most stable hydrated form, and recovering the hydrated composition. The Indian patent application number IN 212/MUM/2013 discloses a lyophilized cyclophosphamide injection in a unit dosage form with appropriate lyo-protection using the direct lyophilization process with short lyo-cycle to overcome the cumbersome lyophilization processes employed in prior art. In the past, a pharmaceutical composition has been marketed, containing the cyclophosphamide monohydrate in the form of a coarse powder, mixed with common salt for the purpose of making it flowable through hopper. Currently available formulation of cyclophosphamide monohydrate for injection is sterile powder filling which includes sodium chloride as an aid to increase flow property. Such powder filling may not be readily reconstituted in water and hence external heating of the glass vials may be required with long reconstitution time. However, in the manufacturing practice, powder filling tends to be difficult. In addition, during the processing and storage of dry powder premix formulation, a glassiness and or stickiness could be acquired by the premix composition giving unattractive material with inferior solubility characteristics and decreased potency. Therefore, the above compositions have been replaced by freeze-dried cyclophosphamide monohydrate compositions. The technique known as lyophilization is often employed for freeze-dried injectable pharmaceuticals which exhibit poor stability in aqueous solution. This process involves freeze drying the frozen solutions leaving only solid dried components of the original liquid. Cyclophosphamide monohydrate as such is a stable form, but it loses water at high temperatures. Hence, maintaining proper vacuum and temperature during manufacturing is important. Therefore, there is a need to develop a composition comprising cyclophosphamide monohydrate that overcomes the disadvantages of compositions and processes known in the art.

The inventors of the present subject matter have found that it is possible to prepare a stable cyclophosphamide monohydrate lyophilized composition for injection prepared by a process comprising lyophilization of a solution comprising cyclophosphamide monohydrate, dehydrated alcohol and water for injection.

MATERIALS AND METHODS

Materials

Cyclophosphamide APIis the gift sample of MSN Labs, Hyderabad, and Telangana, India.

Dehydrated Alcohol is the gift sample of Alembic Pharmaceuticals Ltd. Vadodara, Gujarat, India.

FORMULATION SCREENING STUDIES

The pharmaceutical composition of the subject matter comprises cyclophosphamide monohydrate prepared by a process comprising lyophilization of a solution comprising cyclophosphamide monohydrate and dehydrated alcohol or a mixture of dehydrated alcohol and water for injection in suitable proportions. The solvent is later removed during the freeze drying process.

Selection of solvent system

Based on the fact that cyclophosphamide monohydrate is susceptible to hydrolytic degradation, the solubility study of cyclophosphamide monohydrate in various solvents was carried out at two different temperature ranges and the result is presented in Table 1.

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Table 1: Solubility Studies of cyclophosphamide monohydrate in individual solvents

S. No	Solvent	Solubility Temperature	Solubility
1	Dehydrated alcohol	RT (20-30°C)	666.6 mg/mL
2	Dehydrated alcohol	2-8°C	362.0 mg/mL
3	Acetone	RT (20-30°C)	758.9 mg/mL
4	Acetone	2-8°C	397.8 mg/mL
5	Tertiary butyl alcohol	RT (20-30°C)	375.7 mg/mL

Table 2: Batch manufacturing detailsformulation 1 and formulation 2

Formulation No	Batch Size	Solvent mixture Composition (%V/V)		Fill Volume
		Acetone	Tertiary butyl alcohol	
Formulation 1	100 mL	70	30	1 mL
Formulation 2	100 mL	30	70	2 mL

Table 3: Results after lyophilization for formulation 1 and formulation 2

Formulation No	Residual solvent (ppm)		Water content % (w/w)
	Acetone	Tertiary butyl alcohol	
Formulation 1	3899	2456	4.58
Formulation 2	50	5839	3.89

Table 4: Batch manufacturing details formulation 3 and formulation 4

Formulation No	Batch Size	Solvent mixture Composition (%V/V)		Fill Volume
		Acetone	Water	
Formulation 3	100 mL	80	30	1.5 mL
Formulation 4	100 mL	90	70	1 mL

Table 5: Results after lyophilization for formulation 3 and formulation 4

Formulation No	Residual solvent (ppm)	Water content % (w/w)
	Acetone	
Formulation 3	8599	7.99
Formulation 4	5085	5.89

Table 6: Batch manufacturing details formulation 5 and formulation 6

Formulation No	Batch Size	Solvent mixture Composition (%V/V)		Fill Volume
		Dehydrated alcohol	Water	
Formulation 5	100 mL	90	10	2mL
Formulation 6	100 mL	95	5	1 mL

Table 7: Results after lyophilization for formulation 5 and formulation 6

Formulation No	Residual solvent (ppm)	Water content % (w/w)
	Dehydrated alcohol	
Formulation 5	6599	6.58
Formulation 6	5123	5.89

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Table 8: Batch manufacturing details formulation 7

Formulation No	Batch Size	Solvent mixture Composition (%V/V)		Fill Volume
		Dehydrated alcohol	Water	
Formulation 7	100 mL	90	10	1mL

Table 9: Results after lyophilization for formulation 7

Formulation No	Residual solvent (ppm)	Water content % (w/w)
	Dehydrated alcohol	
Formulation 7	3599	6.12

STABILITY DATA OF FORMULATION 7 (500MG/VIAL):

Table 10: Physicochemicalstability data with Batch manufacturing formulation 7

Stability condition		2-8 ° C	25°C/60 %RH data
Time point	Initial	6M	6M
Stability Orientation		Inverted	Inverted
Tests	Results		
Alcohol Content	2600 ppm	2135	1900ppm
Water content % (w/w)	6.49 %	6.15%	5.9 %
Assay (%)	99.8		99.1
Related Substances (%)			
Related compound A	ND	0.116	0.343
Related compound B	0.128	0.09	0.078
Related compound C	ND	0.015	0.035
Related compound D	0.086	0.12	1.238
Any individual unspecified degradant	0.011	0.127	0.685
Total Impurities	0.258	1.395	3.547

Table 11: Comparative Physicochemical testing of formulation 7 and Reference standard (Mfg. by Baxter) Results

Product	500mg/Vial	
	Reference Product (Mfg. by Baxter)	Formulation 7
Batch #Expiry date	J7082F- Oct 2020	Not Applicable
Description	white to off white powder	white to off white powder
Assay (%)	100.7	99.8
Water content (%)	5.39	6.49
Related Substances (%)		
Cyclophosphamide related compound A	Not detected	ND
Cyclophosphamide related compound B	0.083	0.128
Cyclophosphamide related compound D	ND	ND
Cyclophosphamide related compound C	ND	0.086
Any individual unspecified degradant	0.493 at RRT 1.17	0.011
Total degradation products	1.629	0.258

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Figure 1: Overlay of IR Spectra of cyclophosphamide monohydrate USPAPI and formulation prepared according to formulation 7.

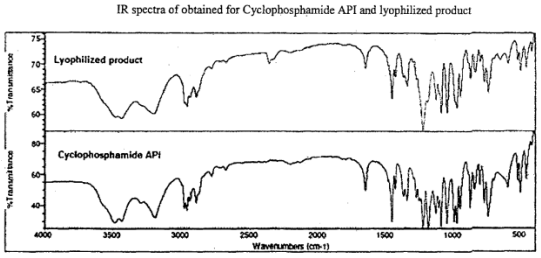
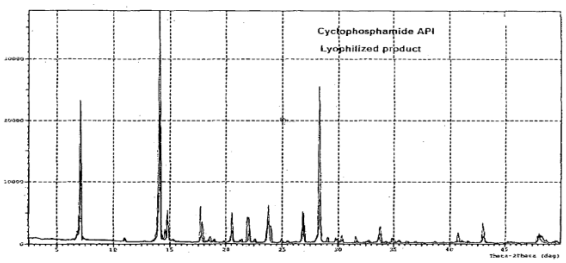


Figure-1

Figure 2: XRD was performed with vials prepared using manufacturing formula 7 and compared with for Cyclophosphamide monohydrate USP API for hydrate form confirmation, overlay of the same is reproduced as figure-2, as below:

Figure 2



Observation and Conclusion:
Cyclophosphamide monohydrate has higher solubility in dehydrated alcohol and Acetone compare to Tertiary butyl alcohol.

Formulation 1 and 2:

Objective: To manufacture the bulk solution of Cyclophosphamide monohydrate using Acetone, Tertiary butyl alcohol and Lyophilization.The batch manufacturing details and results for formulation 1 and formulation 2 are provided in table 2 and table 3 respectively.

Observation and Conclusion: From the above data it was observed that the water content was found lower side of acceptable range for Cyclophosphamide monohydrate (5.8 to 7.8 % w/w). Whereas solvent levels are elevated. Hence Formulation 1 and 2 was not evaluated further.

Formulation 3 and 4:

Objective: To manufacture the bulk solution of Cyclophosphamide monohydrate using Acetone, water and lyophilization.The batch manufacturing details and results for formulation 3 and formulation 4 are provided in Table 4 and Table 5respectively.

Observation and Conclusion: From the above data it was observed that the water content was found within acceptable range for Cyclophosphmide monohydrate (5.8 to 7.8 % w/w) for formulation 4. Whereas solvent levels are elevated. Hence Formulation 3 and 4 was not evaluated further.

Formulation 5 and 6:

Objective: To manufacture the bulk solution of Cyclophosphamide monohydrate using Dehydrated Alcohol, water and lyophilization.
The batch manufacturing details and results for formulation 5 and formulation 6 are provided in Table 6 and Table 7 respectively.

Observation and Conclusion: From the above data it was observed that the water content was found within acceptable range for Cyclophosphamide monohydrate (5.8 to 7.8 % w/w) for formulation 5 and formulation 6. Whereas solvent levels are elevated. Hence further worked on lyophilization cycle to get desired solvent content (i.e ≤ 5000 PPM).

Formulation 7:

Objective: To manufacture the bulk solution of Cyclophosphamide monohydrate using Dehydrated Alcohol, water and lyophilization.
The batch manufacturing details and results for formulation 7 provided in Table 8.

Worked on lyophilization cycle to get desired solvent content (i.e ≤ 5000 PPM). Freeze drying process involves removal of solvent from a frozen mass under reduced atmospheric pressure. In the context of this

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subject matter the term freeze drying, drying and lyophilization shall be used interchangeably. Lyophilization helps to stabilize pharmaceutical composition by reducing the solvent component(s) to levels that no longer support chemical reactions or biological growth. Since drying during lyophilization takes place at a low temperature, chemical decomposition is also reduced. The use of organic solvents requires more attention in the freeze-drying process. Lower temperatures are required to freeze and condense solvents and they can easily bypass the condenser and end up causing damage to the vacuum pump. However liquid nitrogen (LN2) traps may be required to catch/condense certain solvents with very low freezing temperatures or dry vacuum pumps are used. The freezing temperature of ethanol is -114.1 °C.It was observed that lyophilization employing traditional/usual freezing temperature (-45°C to -50°C) do not result in complete freezing. Most of the vials were not frozen and were observed to be liquefied i.e. collapsed vials observed at end of lyophilization cycle. This may be due to insufficient evaporation of alcohol from vials. Surprisingly, inventors have found that when freezing step was carried at about -55°C, powder in the vials was not collapsed.The lyophilized composition formulation 7,wherein the lyophilization process comprises:rapidly freezing to -55°C and applying chamber vacuum 150mTorr at -55°C, employing a chamber pressure of about 150 mTorr with shelf temperature from -55°C to -40°C, raising the shelf temperature to -25°C with 600mTorr chamber pressure

Observation and Conclusion: From the above data it was observed that the water content was found within acceptable range for Cyclophosphamide monohydrate (5.8 to 7.8 % w/w) for formulation 7 and solvent content was as desired (i.e ≤ 5000 PPM) with reorganized lyophilization cycle parameters. Hence further evaluated stability of formulation.

Observation and Conclusion: From the above data it was observed that the water content was found within acceptable range for Cyclophosphamide monohydrate (5.8 to 7.8 % w/w) for formulation 7upon stability. Hence solvent system comprises of dehydrated alcohol, water and lyophilized 500mg/Vial (formulation 7)was selected to proceed further evaluation of formulation of cyclophosphamidemonohydrate

Comparative physicochemical testing between formulation 7 and USA reference standard product:

To demonstrate the equivalence of formulation 7 product to the Reference Standard Product, pharmaceutical equivalence testing was conducted. Testing included the drug product key parameters Results are summarized in Table 18. Since RLD (CYTOXAN®) is discontinued from USA market, comparative pharma equivalence testing was performed for formulation 7 product with Reference standard (RS)Mfg.by Baxter.

Observation and Conclusion: Results of Formulation 7 Cyclophosphamide for Injection 500mg/Vial were comparable to that of respective Reference Product (Mfg.by Baxter). Hence Formulation7Cyclophosphamide Injection 500mg/Vial is pharma equivalent to that of Reference Product (Mfg.by Baxter).

BRIEF DESCRIPTION OF THE DRAWINGS- Figure 1 is an overlay of IR Spectra of cyclophosphamide monohydrate USPAPIand that of a formulation prepared according to formulation 7.

CONCLUSION:

The stability data of Cyclophosphamide monohydrate formulation 7 demonstrates formulationis stable, physicochemical results of Formulation 7 Cyclophosphamide Injection 500/Vial were comparable to that of respectiveReference Product (Mfg.by Baxter). Hence Formulation 7 Cyclophosphamide Injection 500mg/Vial is pharma equivalent to that of Reference Product (Mfg.by Baxter). Cyclophosphamide novel lyophilized formulation 7is stable monohydrate form based on Figure 2 XRD overlay and IR spectra.Furtherscalability of formulation studies are recommended in commercial scale level, wherein the formulation composition retains cyclophosphamide monohydrate after lyophilization and during storage i.e. shelf-life.

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