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Nonaqueous Formulations and Evaluation of Daptomycin Injection

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ABSTRACT

Daptomycin is an antibacterial drug and is a lipopeptide antibiotic used in the treatment of systemic and life-threatening infections caused by Gram-positive organisms. It is a naturally occurring compound found in the soil saprotroph Streptomyces roseosporus. Daptomycin is commercially available in the market as a lyophilized dosage form in various geographies. Approved strengths are 350 mg and 500 mg. Commercially, there is no solution form availability of Daptomycin. The reconstitution time is very long which is for about 15 minutes. The lengthier reconstitution time looks difficult during the emergency time and also pack insert mentions on the foam formation during the reconstitution time. Literature suggested that the drug candidate is very unstable in the liquid dosage form. It undergoes degradation in the presence of water upon long storage. Earlier attempts of aqueous-based daptomycin formulations were not fruitful due to the presence of a significant amount of known and unknown impurities. However, attempts with nonaqueous formulations were found satisfactory with respect to the control of impurities formation.

INTRODUCTION:

The increase in infections caused by Gram-positive pathogens and the rise in antibiotic-resistant bacterial strains have prompted the need for novel antibiotics.[1,2] Recent reports indicate that more than 25% of *Staphylococcus aureus* infections in Europe are caused by methicillin-resistant *S. aureus* (MRSA), and the majority of these isolates are resistant to additional antibiotics.[3]

Daptomycin, a fermentation product produced by *Streptomyces roseosporus*, is a cyclic lipopeptide antibiotic with potent bactericidal activity against most Gram-positive organisms including multiple antibiotic-resistant and -susceptible strains[4-12].

Daptomycin is an intravenously administered cyclic lipopeptide antibacterial agent with potent bactericidal activity against a broad range of Gram-positive organisms. In 2003, daptomycin for injection received approval from the US Food and Drug Administration (FDA) for the treatment of patients with complicated skin and skin structure infections (cSSSIs); in 2006, it was approved for the treatment of patients with *Staphylococcus aureus* bacteremia, including those with right-sided infective endocarditis caused by methicillin-susceptible and methicillin-resistant isolates. Daptomycin has been used to treat patients with bacterial infections of the skin and underlying tissues as well as infections that have entered the bloodstream. Daptomycin is provided by the manufacturer as a powder that requires mixing with a liquid before injection.

Daptomycin, originally designated as LY 146032, was discovered by researchers at Eli Lilly and Company in the late 1980s. LY 146032 showed promise in phase I/II clinical trials for the treatment of infection caused by Gram-positive organisms. Lilly ceased development because high-dose therapy was associated with adverse effects on skeletal muscle, including myalgia and potential myositis.

The rights to LY 146032 were acquired by Cubist Pharmaceuticals in 1997, which following U.S. Food and Drug Administration (FDA) approval in September 2003, for use in people older than 18 years, began marketing the drug under the trade name Cubicin. Cubicin is marketed in the EU and several other countries by Novartis following its purchase of Chiron Corporation, the previous licensee[13,14].

Daptomycin is supplied in single-use vials containing 500 mg daptomycin as a sterile, lyophilized powder. In some regions of the world, single-use vials containing 350 mg

daptomycin as a sterile, lyophilized powder are also available. There are no Daptomycin formulations available in India. In the US, the product is approved on September 12, 2003, for 500 mg strength which is a reference product. According to the reference product [CUBICIN] pack insert[16], the reconstitution time is very long which is for about 15 minutes. The lengthier reconstitution time looks difficult during the emergency time and also pack insert mentions on the foam formation during the reconstitution time. There is a need to overcome both problems.

Daptomycin is approved by the DCGI for use as an anti-emetic drug. In India, the drug product is approved as the Lyophilized powder Injection of 350 mg/ vial. Lyophilization is time consuming, tedious, and involves cumbersome procedures. Further, it involves expensive technology to develop a lyophilized product. Hence, an attempt to develop a non-lyophilized drug product such as liquid formulation would offer convenience for practitioners by avoiding the reconstitution step when preparing the drug for administration.

Daptomycin is a cyclic lipopeptide antibacterial agent derived from the fermentation of *Streptomyces roseosporus*. The chemical name is N-decanoyl-Ltryptophyl-D-asparaginyl-L-aspartyl-L-threonylglycyl-L-ornithyl-L-aspartyl-D-alanyl-L-aspartylglycyl-Dseryl-threo-3-methyl-L-glutamyl-3-anthraniloyl-L-alanine ε1-lactone. The chemical structure is:

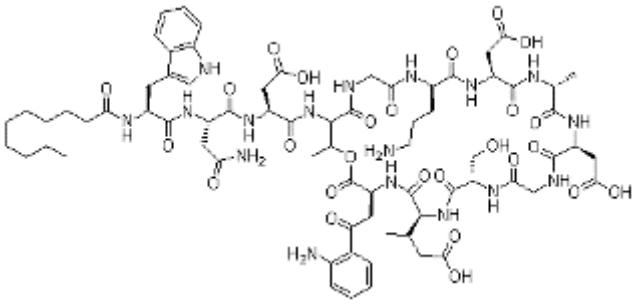

Figure No. 1: Structure of Daptomycin

The empirical formula is $C_{72}H_{101}N_{17}O_{26}$; the molecular weight is 1620.67.

As per the literature available, the daptomycin substance undergoes severe degradation in the aqueous environment. Hence, an attempt to develop a composition focusing on adequate stability while enhancing the solubility necessary for the required therapeutic dose. Hence, an attempt is made to evaluate the simple aqueous based formulations of Daptomycin.

MATERIALS AND METHODS:

Daptomycin was procured from Manus Biopharma, Gujarat. Propylene Glycol, PEG 400, Polysorbate 80, Glycerine, Soyabean oil, Ethanol, and DMSO were purchased from the commercial sources. All required chemicals used were of standard grade.

Preparation of Daptomycin Formulations

A total of 3 formulations were prepared. The concentration chosen of Daptomycin is 25 mg/mL based on the solubility. Initially, the drug substance was dissolved in the solvents and added one by one excipient as per the composition table given below. Finally, the volume is made to solvent as per the composition table. The pH of the formulation was adjusted to 6.5 ± 0.2.

Table No. 1: Formulation of Daptomycin Injection.

Sr. No.	Ingredients	NADF1	NADF2	NADF3
1	Daptomycin	25 mg/mL	25 mg/mL	25 mg/mL
2	Ethanol	10 mg/mL	10 mg/mL	10 mg/mL
3	Dimethyl Sulfoxide	10 mg/mL	10 mg/mL	10 mg/mL
4	Propylene Glycol	350 mg/mL	--	--
5	PEG 400	--	350 mg/mL	--
6	Glycerine	--	--	350 mg/mL
7	Polysorbate 80	3 mg/mL	3 mg/mL	3 mg/mL
8	Citric Acid	QS to pH	QS to pH	QS to pH
9	Sodium Hydroxide	QS to pH	QS to pH	QS to pH
10	Soyabean oil	QS to 1 mL	QS to 1 mL	QS to 1 mL

NADF Stands for Nonaqueous Daptomycin Formulations.

Evaluation of Daptomycin Formulations

Physical evaluation

***Description*:** This is a physical observation made by an individual.

pH: pH was measured using a pH meter at about 25°C temperature by diluting the one part of formulations with 10 mL of water.

Water Content: water content of all the formulations was measured using Karl-Fischer USP 921 <Method1a>.

Light Transmission: All the formulations were tested for light transmission at 650 nm using a UV spectrophotometer. The formulations were diluted in a 1:10 ratio with water and then measured.

Chemical Evaluation:

Assay: HPLC method was adopted to measure the active drug content from the 3 formulations. The active obtained is expressed as a percent of labelled amount of Daptomycin content. The obtained value of drug content is expected to be within limits of 90.0% to 110.0% (General compendia like USP & BP requirement).

Related Substances: % content of known and unknown impurities were determined by the HPLC method.

RESULTS AND DISCUSSION:

The results are compiled in Table No. 2. A clear yellow color solution was observed in all three formulations. The pH of all 3 formulations was adjusted to 6.5 ± 0.2. Light transmission measured for the three formulations found close to 100 % indicating the clear transmission of the liquid formulation when each of the formulations was transmitted through a UV spectrophotometer at 650 nm. The water content of all three formulations was less than 1 %. Concerning the chemical analysis of all the three formulations, it was observed that all three formulations have shown satisfactory assay levels indicating the correct input of % content of daptomycin vs label claim. It also indicates that the analytical method employed for estimating the % content of Daptomycin is correct. From the analysis of the related substances, it was observed that all the 3 known formulations have a higher amount of known and unknown impurities.

Table No. 2: Physical and Chemical Evaluation of Aqueous Daptomycin Formulations.

Sr. No.	Formulation Codes	Description	pH	LT (in%)	Water Content	Assay (in %)	Related Substances
1	NADF1	@	6.34	98.6	0.56%	98.29%	Anhydro Daptomcyin: 0.52% Beta-isomer: 1.74% Lactone Hydrolysis: 0.38% Single Highest UNK Imp: 0.32% Total Imp: 2.88%
2	NADF2	@	6.14	98.5	0.48%	97.8%	Anhydro Daptomcyin: 0.68% Beta-isomer: 2.03% Lactone Hydrolysis: 0.45% Single Highest UNK Imp: 0.38% Total Imp: 3.58%
3	NADF3	@	6.24	99.4	0.52%	97.2%	Anhydro Daptomcyin: 0.65% Beta-isomer: 1.93% Lactone Hydrolysis: 0.55% Single Highest UNK Imp: 0.28% Total Imp: 3.72%

@: Description: A clear colorless solution. LT is Light Transmission.

CONCLUSION:

The overall characterization of all three formulations concluded that no physical description complication was observed. Analytical results of pH and light transmission test parameters were found satisfactory. The pH of the formulations is adjusted towards the neutral side. Chemical evaluation such as assay test parameter result was observed satisfactory wherein the level of assay in all the three formulations is around 98%.

However, concerning impurities formation, all the known impurities such as Anydro-Daptomycin, beta-Isomer, and Lactone Hydrolysis beta-isomer impurity is on a higher side in all the three formulations. However, better control of Anhydro daptomycin and Lactone hydrolysis impurities were observed in non-aqueous formulations. Whereas beta-isomer impurity level in all the three formulations was on the higher side. However, better control on the unknown impurity formation was also seen in non-aqueous formulations. From the above experiment, it can be concluded that daptomycin degradation in non-aqueous formulations was lesser when compared to aqueous formulations.

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