Research Article
CODEN (USA): IJPLCP

[Dave et al., 3(6): June, 2012]
ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Effect of cryoprotectant on lyophlisation of doxorubicin –HCl loaded chitosan nanoparticles

Riddhi Dave *[1], Rakesh Patel[2], Jayvadan Patel[3]and Hetangi Chauhan[1]
1, Saraswati Institute of Pharmaceutical Sciences, Dhanap. Gandhinagar, (Gujrat) - India
2, S.K.Patel College of Pharmaceutical Education & Research, Kherva, (Gujrat) - India
3, Nootan College of Pharmacy, Visnagar, (Gujrat) - India

Abstract
Nanoparticles stability of formulation is very poor when it is in form of aqueous suspension. So, Freeze-drying becomes a good technique to improve the stability of colloidal nanoparticles. Shelf life of colloidal particles can be enhanced by lyophilisation by several folds. Cryoprotectants are added during lyophilisation to protect the intactness of particles in injectable products. The present study is aimed at studying the role and influence of type of cryoprotectant and its concentration in the lyophilization of Doxorubicin- HCl Chitosan Nanoparticles.

Key-Words: Cryoprotectants, stability, colloidal particles, dispersibility

Introduction
A formulation can be defined as a medium in which one or more active components (chemical or biological) remain in a stable environment and maintain specified potency limits for specific period of time. The use of nanotechnology as drug delivery spreads rapidly due to advantage like drug targeting to various organs [1]. In Nanoparticles, stability of formulation is very poor when it is in form of aqueous suspension due to either physical instability problems like agglomerate formation or chemical incompatibility like hydrolysis of drug [2]. Lyophilisation (Freeze drying) is widely used process for pharmaceuticals to improve the stability of product. Stability of solutions and suspension can be improved by converting into solids by lyophilisation[3] in which water is removed from sample after freeze drying by vacuum desorption or sublimation.

Lyophilization stabilizes the formulation by slowing the kinetic clock of the degradation process. It alters the clock by removing the solvent component or components to levels that no longer support chemical reactions or biological growth. This removal is accomplished, first, by freezing the formulation, that is, separating the solutes from the solvent or solvents and immobilizing any solvent in the interstitial region between the solvent crystals.

*** Corresponding Author**
E.mail: riddhi_dave2002@yahoo.com
Mob. +91- 9898907056

Shelf life of colloidal particles can be enhanced by lyophilisation by several folds and during freeze drying cryoprotectants such as sucrose, dextrose are added to protect the sample from stresses. [4]

Freezing and Drying the Formulation[5,6]

Freezing

Formation of ice during freezing results in dramatic changes in concentrations of the active ingredient and the excipient or excipients of the formulation. Freezing produces a complex, glassy system that may be homogeneous or heterogeneous. This complex system, at this time, can only be produced in the interstitial region of ice crystals as a result of the freezing process. Lyophilization is considered to be an important process in the stability of injectable products. Cryoprotectants are substance which protects the intactness of particles in injectable products. Hence optimization of the type and concentration of cryoprotectant necessary to obtain stable injectable product is critical. In the frozen state, the mobility of the water in the glassy interstitial region approaches, and the formulation is considered completely frozen. As the temperature of the matrix increases, the glassy interstitial region softens the electrical resistivity of the interstitial region decreases, or the conductivity of the system increases. Such a change in the electrical nature of the matrix is associated with the onset of mobile water within its interstitial region. As temperature further increases, the interstitial region slowly takes on liquid like characteristics, while surrounding ice crystals remain frozen[7].

Drying

For lyophilization to occur, the solvent is first removed by sublimation while the temperature of the frozen matrix is maintained below the eutectic or collapse temperature of the formulation. This is the primary drying process. The chamber pressure and product and shelf temperatures, during primary drying, are based on the formulation's eutectic or collapse temperature. The resulting cake volume approaches the original fill-volume.

Primary drying at temperatures greater than that of the collapse or eutectic temperature of the formulation (sometimes referred to as vacuum drying or cryodrying) can lead to some collapse of the cake resulting from the presence of mobile water in the matrix interstitial region during primary drying and formation of meltback, a result of liquid states in the interstitial region.

After primary drying, the residual moisture on the resulting cake surface is reduced to levels that no longer support biological growth and chemical reaction. This process is secondary drying[8]. The reduction of moisture in the cake during secondary drying is accomplished by increasing the shelf temperature and reducing the partial pressure of water vapor in the container. The required partial pressure of water vapor and shelf temperature are ascertained from stability studies of lyophilized or vacuum-dried products having varied amounts of residual moisture.

Lyophilization is considered to be an important process in the stability of injectable products. Cryoprotectants are substance which protects the intactness of particles in injectable products. Hence optimization of the type and concentration of cryoprotectant necessary to obtain stable injectable product is critical. The present study is aimed at studying the role and influence of type of cryoprotectant and its concentration in the lyophilization of Chitosan Nanoparticles.

Material and Methods

Doxorubicin- HCl is obtained as a gift sample from astron pharmaceuticals Ahmedabad, All other reagents like Sucrose, Mannitol, Dextrose were obtained from commercial source. Cellophane membrane (Cutt off 12000 Hi Media), Heto-Lab equipment, Malvern Particle Size Analyzer (Malvern Master Sizer 2000 SM UK), Remi Magnetic Stirrer (Remi Scientific Equipment, Bombay)

Lyophilization process

For the present study, Sucrose[9], Dextrose[10] and Mannitol[10] were selected as cryoprotectant in ratio of 1:1, 1:2 and 1:3 (Nanoparticles:Cryoprotectant). Two ml aqueous solution of cryoprotectant (Mannitol, Dextrose, and Sucrose) was added to 8 ml of doxorubicin- HCl Nanoparticles solution. The above solutions were mixed well and then each set Nanoparticle suspension was freezed in a deep freezer at -20^0 C. The samples were then vacuum dried in Heto lab equipment below 27º C for 24 hrs.

Evaluation of lyophilized Nanoparticles

After lyophilization the lyophilized Nanoparticles were subjected to Characterization of Nanoparticles, like Particle size analysis, physical properties like flowability and % release study.

Studies of Kinetics of drug release from Nanoparticles[11]

Cellulose dialysis bag (Cutt off 12000 Hi Media) soaked overnight in PBS (Phosphate buffer solution). The wet sacs were gently open and wash copiously with PBS then it was filled with PBS and examined for leaks. The sac was then emptied and 1 ml of Nanosuspension to be investigated was accurately transferred into the sac, which thus became the donor compartment. The sac was once again examined for any leaks and then was suspended in glass beaked containing 20 ml of PBS, which acted as receptor compartment. The content of the beaker was stirred using Teflon coated bar magnet and the beaker was closed with aluminium foil to prevent any evaporative losses during the experimental work. At predetermined interval of time 1 ml aliquots were withdrawn from the receptor compartment and analyzed at λmax 480 nm. Cumulative drug release is calculated. Fresh buffer was used to replenish the receptor compartment. All experiments were repeated thrice and the average values were taken.

The % drug release was determined by the formula

$$\% \text{ Drug diffused} = \frac{CrVr}{CdVd} \times 100$$

Cr = Conc. of drug in the receptor compartment

Vr = Volume of the receptor compartment

Cd = Conc. of drug in the donor compartment

Vd = Volume of donor compartment

Results and Discussion

Particle size analysis was carried out by Malvern Particle size analyzer before lyophilization and it was observed 346 nm (Ionic gelation method) and 215 nm (w/o emulsion method). After lyophilization with different cryoprotectant with different ratios the particles size was carried out and mentioned in table 1 and it is also in nano range. The graph of particles size after lyophilisation is shown in (Figure 1 and Figure 2).

Table 1: Particle size analysis of reconstituted nanoparticles after lyophilization

Cryoprotectant	Ratio	Particle size (nm) NPs (Ionic Gelation Method)	Particle size (nm) NPs (W/O Method)
Mannitol	1:1	934	678
	1:2	876	621
	1:3	843	584
Dextrose	1:1	897	656
	1:2	842	603
	1:3	809	567
Sucrose	1:1	377	266
	1:2	365	258
	1:3	353	234

Fig.1: Particle size analysis of NPs after lyophilization (Ionic gelation method)

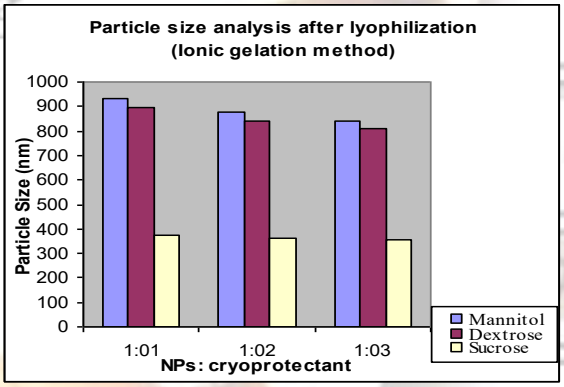
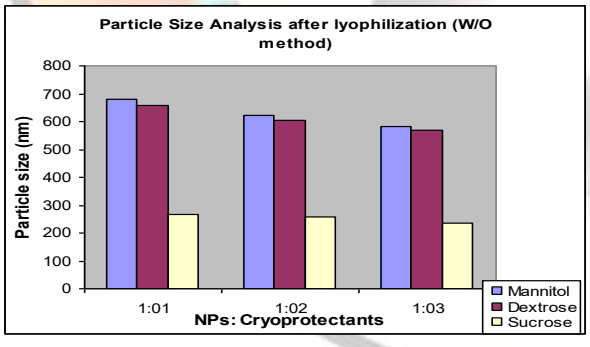


Fig. 2 Particle size analysis of NPs after lyophilization (W/O method)



Flow Property and Redispersibility

The lyophilized powder was checked for physical properties like flowability[12] and redispersibility[13]. For Redispersibility, the lyophilized Nanoparticulate powder was dissolved in 10 ml Deionised distilled water and examined that the samples were converted in clear solution or not.

Table 2: Flowability and redispersibility of reconstituted nanoparticles after lyophilization

Cryoprotectant	Ratio	NPs (Ionic Gelation Method)		NPs (W/O Method)	
		Flowability	Redispersibiliy	Flowability	Redispersibiliy
Mannitol	1:1	-	--	-	--
	1:2	-	--	-	--
	1:3	+	+	+	+
Dextrose	1:1	--	-	--	-
	1:2	-	--	-	--
	1:3	+	--	+	--
Sucrose	1:1	++	++	++	++
	1:2	+	+	+	+
	1:3	+	+/-	+	+/-

--- Lump formation, -- Very poor,
- Poor, -/+ Moderately Good, + Good, ++ Very Good

Drug Release Study

After lyophilization with different cryoprotectant, there is least increase in particle size with sucrose in all proportion. There is minimum increase in particle size with sucrose 1:3 proportion. But better results were obtained with sucrose 1:1 proportion in case of Redispersibility and flowability. Redispersibility and Flowability was very good in case of sucrose 1:1 proportion. Based on that observation the Sucrose 1:1 was selected as the best cryoprotectant for this system. The release study of the Nanoparticles was also carried out before and after lyophilization. There is no change in release profile before and after lyophilization. It means the sucrose (Nanoparticles:Cryoprotectant) would not affect particle size, flowability, Redispersibility and release pattern of the Nanoparticles.

Acknowledgement

The authors are thankful to astron pharmaceuticals for the gift sample of Doxorubicin- HCl.

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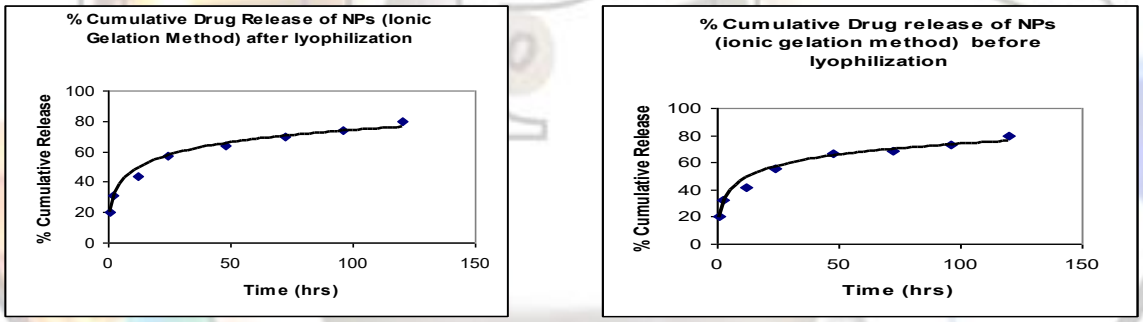
Fig. 3: % Cumulative Release → Time (hrs) of Lyophilized Nanoparticles (Ionic Gelation Method) Before and After Lyophilization



Graph 4: % Cumulative Release → Time (hrs) of Lyophilized Nanoparticles (W/O emulsion Method) Before and After Lyophilization.