Identification of didanosine | results

DISCUSSION

The procured sample of didanosine was tested for its identification. The drug sample showed compliance with the data given in B. P. and Clarkes which reflects its quality and purity. Quality and purity of sample was also confirmed by the manufacturer. The lipids such as soyalecithin and cholesterol and all other excipients provided by the supplier confirmed by their identification test official in USP 24, IP and EP. All the excipients showed results in compliance with standard specifications.

STANDARD CALIBRATION CURVE OF DIDANOSINE

From the scanning of drug in 7. 4 pH phosphate buffer was concluded that the drug had λ_{max} of 249 nm. From the standard calibration curve of drug, it was concluded that drug obeys Beer-Lamberts law in concentration range of 0-20mcg/mL.

$R^2 = 0.9995$

Correlation coefficient values indicated the linear correlation between concentration and absorbance.

PREPARATION AND CHARACTERIZATION OF LIPOSOMES

Among the various methods thin film hydration method is widely used on a laboratory scale. In this method the lipids are casted as stacks of film from their organic solution using flash rotary evaporator under reduced pressure and then the film is dispersed in an aqueous medium. This method yields the liposomes with a heterogeneous size distribution. Also the liposomes that are formed are multilamellar in nature with some unilamellar vesicles. (Vyas and Khar, 2002).

Venkataram et al., 1990 have used PC to prepare liposomes in a drug to lipid ratio of 1: 10. The temperature during preparation has been kept 40ï,°C as the glass transition temperature of PC is very low. The drug entrapment into the liposomes depends mainly on Drug: Lipid ratio. In this study, the cholesterol and PC are selected as lipids in combination on basis of percent drug entrapped and rigidity (stability). S. Vemuri et al are stated that, cholesterol improves the fluidity of the bilayer membrane, reduces the permeability of water soluble molecules throught the membrane, and improve the stability of bilayer membrane in the presence of biological fluids such as blood/plasma. The hydration characteristic of lipid film was studied for different batches of liposomes and then liposomes were evaluated for % drug entrapment. From results of % drug entrapped of different batches of liposomes that were prepared and stability of liposomes under different temperature condition it was observed that as the percentage of cholesterol was increased there was subsequent increase in the stability and rigidity of liposomes but at the same time percentage drug entrapment reduced, Due to reduction in phosphatydilcholine. Formulation F4 containing 70% of PC and 30% cholesterol showed maximum drug entrapment (29. 41%) with satisfactory stability and rigidity i. e. showed spherical shape with only 1. 86% of drug leaching at 25 after 30 days. However, when PC was further increased to 80% keeping cholesterol to 20% there was increase in % drug entrapment but simultaneous decrease in stability and rigidity. The shape was lost and drug leaching was more i. e. 10. 25%.

The data was also treated statistically by using one way analysis (ANOVA) and found to be satisfactory significant difference (P < 0. 05), at 95% confidence interval.

PREPARATION AND CHARACTERIZATION OF DIDANOSINE PROLIPOSOMES

The proliposomes of Didanosine were prepared by powder thin film hydration (New, 1990). Here modified rotary evaporator unit was used as described by Lee et al., 1999. Different carriers like lactose, sodium chloride and lactose beads were used for preparation of proliposomes. Were hydrated with distilled water and the liposomes was analysed for % drug entrapped for different batches of proliposomes derived liposomes were as shown in the table 5. 3. The Lipid: Carrier ratio was kept 1: 10 as reported by Song et al., 2002.

The proliposomes of lactose were quite free flowing compared to the lactose that was used to prepare them and lactose proliposomes showed highest % drug entrapment (29. 17). They were less sticky. Also as the amount of lipid i. e. lecithin was increased the proliposomes powder was found to be very sticky. This is because the lipid is sticky at room temperature.

In case of sodium chloride the carrier was very free flowing but the proliposomes powder was very sticky compared to that made with either lactose or sorbitol. The particle size of the liposomes formed in sodium chloride and lactose beads was also greater than that formed with lactose. Here the carrier is non porous so majority of the lipid has to be deposited over the on the surface of the carrier, thereby maximizing the possibility of agglomeration and also because sodium chloride is hygroscopic. So it can be

said that such nonporous carriers are suitable only for high melting lipids. The results are in accordance with those observed by Payne et al.

As the amount of lipid was increased in case of sodium chloride the proliposomes were found to be extremely sticky because they tend to agglomerate (Payne et al., 1986a). The entrapment of the proliposomes made by using sodium chloride as a carrier was very low compared to that of either lactose or lactose beads because of the effect of the monovalent cation Na^{+}. Sodium ion has the effect of increasing the release of cyclosporine from the liposome hence it decreases the entrapment of drug in the liposomes (Al-Angary et al., 1995).

The proliposomes of lactose beads were found to be very free flowing just like the lactose beads from which the proliposomes were prepared but the surface area available for coating less compared to lactose powder and sodium chloride so film formed is thick so it yields multilammelar liposomes.

Based on the above results the carrier was finalized. In subsequent experiments Lactose was used as a carrier and the formulation was optimized by a 3^{2} factorial design. The effect of the two independent variables viz. Drug: Lipid ratio and Lipid: Carrier ratio was studied on dependant variables like entrapment and mean particle size. All other processing factors like vacuum applied, speed of rotation of round bottom flask; temperature, amount of surfactant etc were kept constant.

OPTIMIZATION OF PROLIPOSOMES

A prior knowledge and understanding of the process and the process variables under investigation are necessary for achieving a more realistic

model. Based on the results of the preliminary experiments, drug: lipid ratio was found to be a major variable in determining PDE and Lipid: carrier ratio in determining the PMD. Hence, these variables were selected to find the optimized condition for higher PDE and PMD using 3^2 factorial design. By using 3^2 factorial designs, 9 batches of didanosine liposomes were prepared varying the two independent variables at three levels as recorded in thetable. The prepared batches were evaluated for % drug entrapment (PDE) and mean particle size, which were taken as dependent variables and the results were recorded in thetableA substantial high drug entrapment was achieved in liposomes of variable X_1 (drug: Lipid = 1: 15) and X2 (Lipid: Carrier = 1: 15).

EVALUATION STUDIES OF DIDANOSINE PROLIPOSOMES

A) Microscopy of proliposome

The microscopy of proliposomes revealed that the surface was smooth due to the coating of the lipid and some of the particles were agglomerated. The pictures of proliposomes are as shown in Figure. After hydration with deionized water a series of time-lapse photographs of proliposome hydration are as shown below in Figure. Here the formation of liposomes form proliposomes is shown. The results indicate that the process of dissolution/disintegration may occur by a progressive hydration of the lipid surface of the proliposome, taking the form of liposomes ‘ budding off’ from the central core of the proliposome until both hydration of the lipid and dissolution of the carrier is complete. Although only an imitation of the process of proliposome hydration (due to absence of hand shaking to aid

proliposome dispersion), this approach was thought to offer a reasonable indication of the process (Payne et al., 1986b).

Finally the liposomes are formed that are multilamellar with a heterogeneous size distribution. The photographs reveal the multilamellar nature (Figure). Also the Maltice crosses shown in Figure indicate that the vesicles are multilamellar in nature. Also there are many unilamellar vesicles.

B) Scanning electron micrographs.

The scanning electron micrographs of carriers and optimized batch of proliposomes made with different carriers were taken and are as shown in the Figure. From the SEM pictures it is evident that after coating the surface becomes somewhat smooth and the surface defects are no more visible and a thick coating is also seen in proliposomes compared to the carrier alone. After coating of lipid on the surface the particles looks quite opaque and smooth compared to the carrier.

EVALUATION STUDIES OF PROLIPOSOME DERIVED LIPOSOMES

A) % Drug entrapped

By using 3^2 factorial designs, 9 batches of didanosine liposomes were prepared varying the two independent variables. Various methods have been reported for determination of drug content in liposomes that involve separation of free drug from liposomes either by centrifugation or by dialysis or by sephadex column. Here the separation was affected by sephadex G25 column as reported by Guo et al., 2001. Here the PDE was calculated from the difference between the initial drug added and the drug detected after

separation of the free drug. The results of various batches are as shown in the table.

It was found that the % drug entrapped was highest when the Drug: Lipid ratio was highest. Also the Lipid: Carrier ratio was found to affect the entrapment of the drug into the liposomes. It was found that the highest % entrapment for all three levels of X_2 was obtained at +1 level of X_1 that is 21. 37% at –1 level, 26. 73% at 0 level and 30. 89% at +1 level of X_2. Graphical presentation figure shows the effect of the independent variable (drug: lipid ratio) on % drug entrapped. The results indicate that as the drug to lipid ratio increases the entrapment of the drug in the liposomes increases, as didanosine is a hydrophilic drug, which finds place within the core.

B) Particle size analysis

Particle size analysis results of various batches of proliposome derived liposomes are as shown in the table. The results are expressed as particle mean diameter. The particle size of the liposomes decreases as the amount of the carrier increases because there is a greater surface area available for thin lipid film formation which gives rise to a small particle size compared to a thick film that is formed when the amount of carrier is decreased (Hwang et al., 1997).

The graph (figure) represents the relationship between lipid: carrier ratio on mean particle size. Mean particle size decreases as the lipid: carrier ratio increases because as the surface area increases thin film formation occurs that gives rise to smaller particle size.

C) Stability Studies

The optimized formulation was subjected to stability studies at 4 ^{0} C, 25 ^{0} C, 45 ^{0} C for 60 days. They were evaluated for physical appearance, entrapment efficiency, drug content etc. All the results obtained are within the limits and no major changes were identified physically.