EFFECTS OF PH, SALT, TEMPERATURE ON CONVENTIONAL LIPOSOMES SIZE ENLARGEMENT ANALYZED BY OPTICAL MICROSCOPE

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RESEARCH ARTICLE

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ABSTRACT:

The objective of the present study was to investigate the effects of experimental conditions like pH, salt, temperature on the interactions of conventional liposomes. The model drug Stavudine was entrapped in the liposomes composed of Phosphotidyl Choline and cholesterol in the ratio of 200mg: 80mg and prepared by thin film hydration method. Liposomes preparation was characterized and compared for particle size, mean dispersion, entrapment efficiency, Zeta potential and the size enlargement studies were carried out in optical microscope by using Magnus.

Key words: Liposomes, Stavudine, Temperature, Size Enlargement, pH, Salt.

Introduction:

Liposomes are becoming increasingly important as carriers of biologically important molecules in living system [1-4]. Liposomes have a variety of routes of administrations including oral, dermal, intravenous, ophthalmic.[5] Liposomes mimic cells and are used for encapsulation and sustained release of drugs in modern therapies. They are aggregates containing a continuous bilayer of phospholipids around in aqueous space. There has been much interest in liposomes as drug delivery carriers, not only because of wide range of biologically active substances can be encapsulated, but that can be administered to man or animals without any adverse effect. Incorporation in to liposomes can reduce the toxicity and produce sustained release.[6] Liposomes, after three decades of research, are still gaining increasing interest with special emphasis more recently on their use as drug carrier systems. For therapeutic purpose they must be loaded with active substances. This is more achieved with lipophilic and hydrophilic molecules as they have tendency to be incorporated in the liposomal membrane. This is more essential achieved with amphiphile molecules like phospholipids and cholesterol as they have a tendency to be incorporated in the liposomal membrane. In contrast, hydrophilic molecules must be encapsulated in the aqueous interior, which in general, cannot easily be performed in an efficient manner[7]. A variety of liposomes preparations were introduced and they have to fulfill the basic requirements (a) Produced liposomes should be homogeneous (b) Efficient of encapsulation of hydrophilic drugs. Whereas thin film hydration technique achieves high efficiency and easy method to produce in the laboratories.[8] The concept of this project was fairly to entrap hydrophilic drugs like zidovudine. The ratio of volume inside the liposomes compared to the total aqueous volume of the preparation is encapsulation efficiency. The increasing lipid concentration more liposomes per unit volume of the preparation are formed. When phospholipid dispersed in aqueous medium at certain concentrations results in highly viscous dispersions up to semisolid consistency. So, the phospholipid, cholesterol and hydration times were changed to achieve maximum entrapment efficiency. The prepared liposomes were

investigated at different temperature, pH and salt concentration for their stability.

Zidovudine, the first anti HIV compound approved for clinical use is widely used for the treatment of AIDS either alone or combination with other antiviral agents. However, the main limitation to therapeutic effectiveness of zidovudine its dose dependant hematological toxicity, low therapeutic index, short biological half life and poor bioavailability.[9] Zidovudine available in the market as conventional tablet form. After oral administration, it is rapidly absorbed from GIT exhibiting a peak plasma concentration of 1.2µg/ml at0.8 hours.[10] In the systemic circulation, it is first converted to Azidothimidine triphosphate, which is pharmacologically active and prevents the replication of the HIV virus. The biological half-life of zidovudine is 4 hours.

Materials and methods:

Materials:

The materials used in the study were Zidovudine a gifted sample of Alkem Laboratories Ltd, Raigad. Phosphotidyl choline, Qualigens, Mumbai. Cholesterol, Qualigens, Mumbai. Chloroform, Merck, Mumbai. Methanols, Qualigens, Mumbai.were Purchased. All in the study, other ingredients used were of analytical grade.

Methods:

Preparation of liposomes: [11, 12]

Multilammellar liposomes were prepared by using thin film hydration method Accurately weighed quantity of Drug, Phosphotidyl Choline, Cholesterol was taken. Phosphotidyl Choline, Cholesterol in the ratio of 200mg: 80mg were dissolved in two parts of chloroform and one part of methanol mixture in 250 ml spear shaped flask. The chloroform and methanol mixture was evaporated at 45^{o}C under reduced pressure (600psi) at 150rpm using Rota evaporator. After the chloroform and methanol was completely evaporated. The flask was kept overnight under vacuum pressure to remove residual solvent. The thin film was hydrated using drug-containing 0.9%w/v Saline solution for 8 hours until vesiculation was completed.

Estimation of Entrapped Drug in Liposomes [13]

Zidovudine entrapped within the liposomes was estimated after removing the unentrapped drug. The unentrapped drug was separated from the liposomes by subjecting the dispersion to configuration in a cooling centrifuge (Remi Equipments, Mumbai, India) at 15 000 rpm at a temperature of –4°C for 30 minutes, whereupon the pellets of liposomes and the supernatant containing free drug were obtained. The liposome pellets were washed again with distilled water to remove any unentrapped drug by centrifugation. The supernatant was analyzed for the drug content after suitable dilution with saline solution by measuring absorbance at 267 nm using Shimadzu U-V 1700(U-V Spectrophotometer). The percentage drug entrapment in the liposomes was calculated from the difference between the initial drug added and the drug detected in the supernatant.

Transmission electron microscopy:

Zidovudine liposomal dispersion was examined by Transmission electron microscope to find out the presence of spherical vesicles and type of vesicles.

Determination of zeta potential:[14]

The zeta potential is the overall charge a particle acquires in a particular medium. Both mean particle size and charge of the particles can be measured on a Malvern Zetasizer. The zeta potential of a liposome preparation can help to predict the fate of the liposomes in vivo and invitro. Any subsequent modification of the liposome surface can also be monitored by measurement of the zeta potential.

Results and discussion:

Liposomes have been used as a carrier system to deliver medications in to the skin in order to achieve the therapeutic effect with lower systemic absorption. Several novel carrier systems were suggested to be appropriate for

topical drug delivery of liposomes. To achieve the desirable therapeutic affect of liposomes as drug carriers, they must be loaded with sufficient amount of active compounds. Therefore liposomes with Zidovudine were prepared by thin film hydration method with the effects of the formulation variables, lipid phase composition (Phosphotidyl Choline and cholesterol) and hydration time on the drug entrapment efficiency, vesicle size and mean distribution of liposomes. Transmission electron microscope revealed the presence of spherical vesicles (Liposomes). Regardless of the preparation procedure used, liposomes were of a mean diameter around 1110.509nm in diameter with the low cholesterol level. In the optical microscope it was found that the average diameter of the liposome vesicle was 2.467µm. The mean particle size and charge of the liposome were increased with the increased concentration of cholesterol. However, liposome prepared by the thin film hydration method was of a more homogenous size distribution. This was probably a consequence of the preparation procedure, in the better case.

Table-1: Composition, Hydration time, Mean particle size

S. No	PC: CH (mg)	Drug (mg)	Hydration time	EE
1	200:200	10	02	35±0.8
2	200:100	10	04	42±1.2
3	200:80	10	06	53±1.0
4	200:80	10	08	59±1.6
5	200:60	10	08	51±1.8

PC- Phosphotidyl Choline
CH-Cholesterol
EE-Entrapment Efficiency
(n=3).

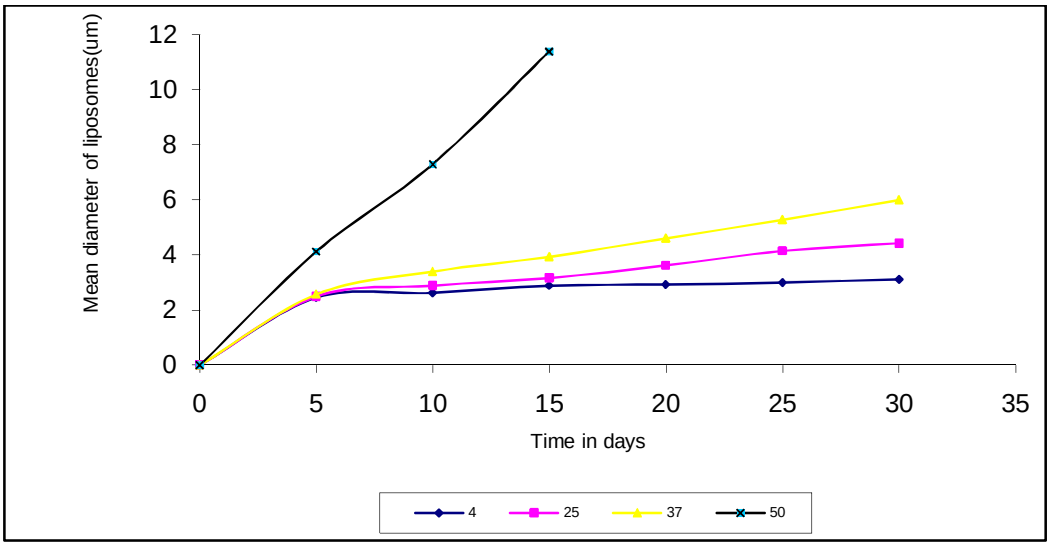
Effect of temperature of liposomes size enlargement: [15]

The conventional liposomes containing zidovudine were taken and stored at different temperatures like 4°C, 25°C, 37°C, and 50°C for 30 days. The influences of temperature on liposomes diameter as a function of time have been investigated and are shown in the fig-1 and values of the size enlargement of liposomes shown in table–2. In the higher temperatures, the liposomes may aggregates and form clusters. This investigation is to find out the effects on size enlargement of liposomes and its suitable storage temperature. The liposomal coalescence is not reversible and it is sensitive to temperature. The aggregation of liposomes due to Brownian motion while increasing the temperature, there will be increasing in motion of the liposomes vesicles. The reaction limited clusters aggregation process, the average cluster size grows exponentially with the time. After aggregation liposomes tends to form large vesicles via coalescence. In this case that the decreasing the temperature of zidovudine liposomes increased the viscosity of liposomal suspension and the liposomal coalescence rate decreased. So it is shown in this study that a low temperature i.e. 4°C increases the stability of liposomes in relation to their size.

Table-2 Effect of temperature on liposome size enlargement

S.No	Time in days	4°C	25°C	37°C	50°C
0	0	0	0	0	0
1	5	2.458	2.498	2.575	4.13
2	10	2.635	2.891	3.4	7.3
3	15	2.896	3.17	3.94	11.4
4	20	2.941	3.621	4.61	-
5	25	3.01	4.15	5.28	-
6	50	3.126	4.426	6	-

Figure-1 : Effect of temperature on liposome size enlargement



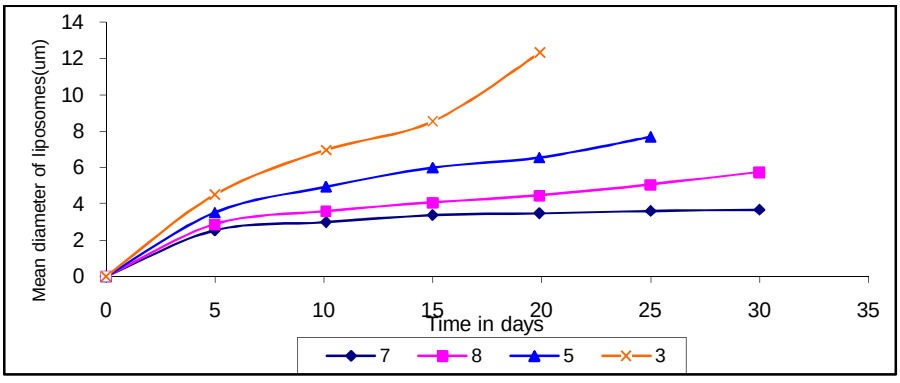
Effect of pH on liposomes size enlargement: [16]

The conventional liposomes containing zidovudine were stored at different values like 3,5,7,8. The influences of pH on liposomes diameter as a function of time have been investigated and are shown in fig-2 and values of the size enlargement of liposomes shown in table-3. It was found that the effect of pH on liposome stability is significant. The pH range above5-8, there is no significant change in the size enlargement. But the decreased pH (pH-3), the rate of membrane fusion may occur in the neutral liposomes.

Table-3: Effect of different pH on liposomes size enlargement

S.No	Time in days	Effect of size in pH-7	Effect of size in pH-8	Effect of size in pH-5	Effect of size in pH-3
0	0	0	0	0	0
1	5	2.54	2.88	3.53	4.52
2	10	2.98	3.6	4.95	6.98
3	15	3.37	4.07	6	8.56
4	20	3.47	4.46	6.56	12.34
5	25	3.6	5.06	7.7	--
6	30	3.67	5.75	--	--

Figure-3 : Effect of different pH on liposomes size enlargement

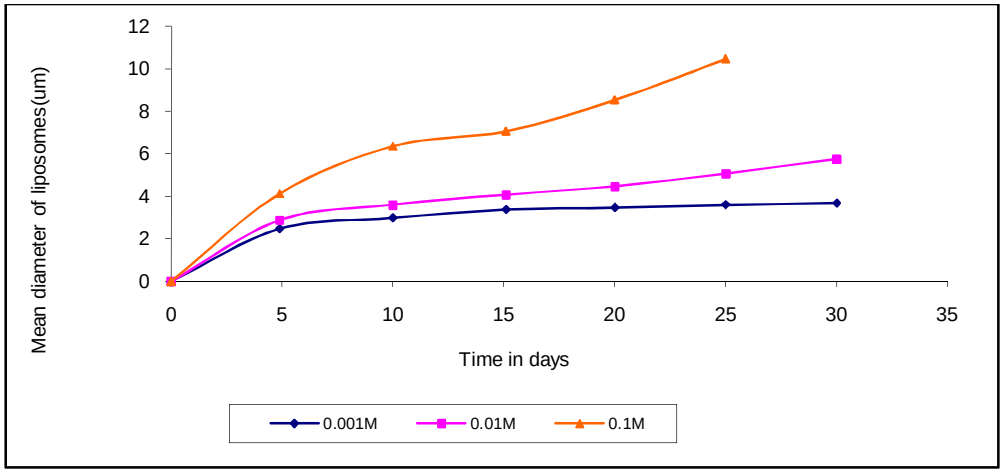


Effect of calcium chloride on liposomes size enlargement: [17]

Table-4 Effect of 0.001M, 0.01M, 0.1M $CaCl_2$ on liposomes size enlargement

S.No	Time in days	0.001M CaCl2	0.01M $CaCl_2$	0.1M $CaCl_2$
0	0	0	0	0
1	5	2.48	2.88	4.13
2	10	2.98	3.6	6.35
3	15	3.37	4.07	7.05
4	20	3.47	4.46	8.52
5	25	3.6	5.06	10.45
6	30	3.67	5.75	--

Figure-4: Effect of 0.001M, 0.01M, 0.1M $CaCl_2$ on liposomes size enlargement



The conventional liposomes containing zidovudine were stored at different molar ratio of calcium chloride concentration. I.e. 0.001M, 0.01M, 0.1M solution at 25°C at pH7 reported. The influences of pH on liposomes diameter as a function of time have been investigated and are shown in fig-2 and values of the size enlargement of liposomes shown in table-4.in the range of 0.001M to 0.01M there is no significant change in the size enlargement. Above 0.01M i.e. 0.1M calcium chloride salt may induce aggregation behavior of conventional liposomes occurs only at a high concentration salt level.

Conclusion

The stability of liposomes with respect to aggregation was evaluated by using different salt concentrations, different pH and different temperatures. With the increasing concentration of the electrolytes were more effective for liposome aggregation. The highest level of stability of liposomes was observed at pH-7. The temperature dramatically influenced the aggregation. Low temperature was suitable for the storage of liposomes.

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