CHAPTER 6

INVITRO DRUG RELEASE KINETICS FROM LIPOSOMES

6.1 INTRODUCTION

Studies of drug release / diffusion from liposomal systems are directed towards issues that are relevant to the *in vivo* as well as to the non *in vivo* arenas. In any dosage form, only the portion of the drug that is released has a therapeutic effect. This suggests that the extent of release of a liposome entrapped drug at a target tissue determines the ultimate bioavailability of the drug. Thus the rate and extent of drug release from liposomes can influence not only the onset, but also the degree and duration of the therapeutic activity of the drug. In order to derive relevant data from such studies, the experimental conditions should be set to fit the specific objectives, especially with respect to the extent of liposomes and drug dilutions that the system is anticipated to undergo (Margalit and Yerushalmi, 1996).

For liposomes in the *in vivo* arena, the drug release studies are expected to yield data and understanding that will lead to:

a) Minimizing the loss of encapsulated drug on route from the site of administration to the site of drug action.
b) The ability to match the rate of release (once the liposome reaches the target) to the requirements of the therapy.

The objectives of drug release studies that concern the non *in vivo* arena are

a) Physicochemical characterization of the systems, including liposomes processed into aerosols or reconstituted from freeze dried powders.
b) Various aspects of system optimization such as the selection of liposome type, lipid composition and parameters of shelf life.
c) Criteria for quality assurance.

Sustaining a unidirectional flux of the drug from the liposomal system into the bulk medium during the entire experiment is a key element in such kinetic studies. Data from experiments conducted under such conditions represent the worst-case scenario for drug loss from the liposomes. This knowledge can then be used to design shelf life conditions under which drug loss is minimized. e.g. such studies have revealed that, if the selected dosage form is liposome suspensions, storage should be at high liposome concentrations. Furthermore, the kinetic parameters determined for the system of interest become product specifications (or 'fingerprints') that will

constitute critical input into the data base on which the decision of whether to store with or without unencapsulated drug will rest. If the selected dosage form is a freeze dried powder of drug encapsulating liposomes, these fingerprints can be useful in defining the optimal conditions for reconstitution and in verifying that the reconstituted system has retained its original properties. Regardless of the dosage form selected for storage, retention of the same magnitudes of the dosage form selected for storage, retention of the same magnitudes of the kinetic parameters can be included within the battery of quality assurance tests. When it comes to surface modified liposomes, the processes of drug release add some concerns that are of interest both academic and industrial. A particular concern is the risk of drug (encapsulated) loss that can occur in the course of the modification itself, as well as in subsequent procedures of separation and purification. Kinetic studies can be used to determine whether such losses are significant at all and to evaluate the extent. Whether the modification is done on preformed drug encapsulating liposomes or on a single lipid component prior to liposome formation, such studies can also address the extent to which (if at all) the modification interferes with drug release and the optimal conditions for minimizing that interference. Thus such studies are an essential part of the product development process in case of liposomes. These studies are needed anew for each drug-liposome system, conducted with the specific drug of interest rather than with other models.

6.2 EXPERIMENTAL

6.2.1 REAGENTS

Disodium hydrogen phosphate, potassium dihydrogen phosphate and sodium chloride of analytical reagent (AR) grade (S.D. Fine Chem Ltd., Boisar).

6.2.2 SOLUTIONS

Phosphate buffered saline, pH 7.4 (PBS) was prepared as per the procedure given in the Indian Pharmacopoeia (1985).

6.2.3 STUDIES OF KINETICS OF DRUG RELEASE FROM LIPOSOMES

Drug release kinetic studies were performed by suspending the liposomes in suitable medium at controlled conditions of temperature (37°C) and agitation. At a predetermined time point aliquot of suspension was removed, replaced with the same amount of medium, centrifuged and the drug present in the supernatant and in the liposomal pellet was determined. Samples were taken for analysis according to the planned schedule, and liposomal pellets were then obtained by centrifugation in a centrifuge at 16,000rpm for 4 min after gentle shaking. The amount of drug present in supernatant and in liposomal pellet was estimated by diluting by the methods as described in earlier section.

6.2.4 DATA ANALYSIS

Release kinetics studies were performed on all liposomal batches in triplicate and duplicate runs were made for each batch. Table 6.1 - 6.10 shows the results of these studies. The data was analysed using the theoretical approach developed by Eyring (Margalit et. al., 1991). The mathematical expression for this approach is given by

$$f = \sum_{j=1}^{n} f_j (1 - e^{-kjt}) \qquad ..(1)$$

where f represents the response dependent experimental parameter which is the cumulative release, normalized to the total drug in the system, at time = 0

t represents time, the experimental independent parameter

n gives the total number of independent drug pools

f_j is the fraction of the total drug occupying the j^{th} pool at time = 0

k_j is the rate constant of the diffusion of the drug from the j^{th} pool

For a system containing encapsulated and unencapsulated drug the equation becomes

$f = f_l (1-e^{-klt}) + f_f (1-e^{-kft})$.(2)

where f = cumulative amount of drug released at time t normalized to the total amount in the preparation at time = 0

f_l = fraction of drug encapsulated in liposomes at time = 0

f_f = fraction of drug unencapsulated in liposomes at time = 0

k_l = rate constant for the diffusion of the encapsulated drug

k_f = rate constant for the diffusion of the unencapsulated drug

t = time

and $f_l + f_f = 1$.(3)

Expanding equation (2) gives

$$f = f_l - f_l e^{-k_l t} + f_f - f_f e^{-k_f t}$$

or $$f = (f_l + f_f) - f_l e^{-k_l t} - f_f e^{-k_f t}$$

Thus,

$$f = 1 - (f_l e^{-k_l t} + f_f e^{-k_f t})$$ [using equation (3)]

or $$1 - f = f_l e^{-k_l t} + f_f e^{-k_f t}$$.(4)

Therefore, mean values of the fraction of drug retained were obtained with respect to time and subjected to non-linear regression analysis using available software (Quickcalc, Plexus Supporting Systems, Ahmedabad). The regressed values for the various liposomal systems are detailed in Table 6.1 –6.10. Figure 6.1 –6.10 depict the log of percent drug retained vs. time curves (using regressed data). Kinetic parameters obtained from the above analysis are tabulated in Table 6.11.

Table 6.1 ***In vitro*** **drug release studies of neutral liposomes containing cyclosporine**

Time in hrs (t)	Mean fraction drug retained (1-f)		
	Actual	Calculated	% Deviation
1	1.000	1.017	-1.67
2	0.982	0.970	1.19
4	0.948	0.905	4.79
6	0.862	0.859	0.2556
8	0.764	0.825	-7.47
24	0.684	0.644	6.138
48	0.456	0.452	0.872
72	0.312	0.317	-1.616

Table 6.2 ***In vitro*** **drug release studies of positive charged liposomes containing cyclosporine**

Time in hrs (t)	Mean fraction drug retained (1-f)		
	Actual	Calculated	% Deviation
1	1.000	1.017	-1.68
2	0.975	0.967	0.763
4	0.946	0.897	5.45
6	0.854	0.849	0.593
8	0.752	0.813	-7.47
24	0.695	0.629	10.47
48	0.414	0.438	-5.57
72	0.312	0.306	2.11

Table 6.3 ***In vitro*** **drug release studies of negative charged liposomes containing cyclosporine**

Time in hrs (t)	Mean fraction drug retained (1-f)		
	Actual	Calculated	% Deviation
1	1.000	1.000	-1.24
2	0.963	0.9579	0.1497
4	0.926	0.8901	3.88
6	0.892	0.846	5.54
8	0.723	0.812	-10.86
24	0.708	0.637	11.37
48	0.436	0.449	-3.00
72	0.318	0.318	0.057

Table 6.4 ***In vitro*** **drug release studies of conventional liposomes containing leuprolide acetate**

Time in hrs (t)	Mean fraction drug retained (1-f)		
	Actual	Calculated	% Deviation
1	0.905	0.906	3.09
2	0.806	0.837	-3.52
4	0.77	0.730	1.09
6	0.654	0.654	-6.53
8	0.589	0.597	-8.96
24	0.351	0.376	-10.49
48	0.209	0.214	0.59
72	0.114	0.122	2.19

Table 6.5 ***In vitro*** **drug release studies of sterically stabilized liposomes (mPEG-5000-CCPE) containing leuprolide acetate**

Time in hrs (t)	Mean fraction drug retained (1-f)		
	Actual	Calculated	% Deviation
1	0.956	0.973	-1.935
2	0.902	0.915	1.175
4	0.856	0.790	4.67
6	0.728	0.708	0.741
8	0.650	0.651	-6.59
24	0.472	0.406	4.15
48	0.366	0.212	1.03
72	0.201	0.111	-1.34

Table 6.6 ***In vitro*** **drug release studies of sterically stabilized liposomes (mPEG-2000-CCPE) containing leuprolide acetate**

Time in hrs (t)	Mean fraction drug retained (1-f)		
	Actual	Calculated	% Deviation
1	0.968	0.988	-2.104
2	0.912	0.918	-0.6923
4	0.879	0.812	8.244
6	0.748	0.738	1.36
8	0.642	0.684	-6.24
24	0.495	0.503	-1.57
48	0.395	0.365	8.28
72	0.256	0.266	-3.61

Table 6.7 ***In vitro*** **drug release studies of conventional liposomes containing DNA**

Time in hrs (t)	Mean fraction drug retained (1-f)		
	Actual	Calculated	% Deviation
1	0.922	0.934	0.8233
2	0.850	0.851	0.3769
4	0.768	0.753	1.01
6	0.692	0.702	-1.77
8	0.604	0.661	-8.68
24	0.490	0.425	15.22
48	0.229	0.219	4.27
72	0.108	0.113	-4.85

Table 6.8 ***In vitro*** **drug release studies of sterically stabilized liposomes (mPEG5000-CCPE) containing DNA**

Time in hrs (t)	Mean fraction drug retained (1-f)		
	Actual	Calculated	% Deviation
1	0.963	0.967	-5.7
2	0.886	0.884	0.267
4	0.790	0.774	2.10
6	0.702	0.709	-0.957
8	0.652	0.666	-2.09
24	0.520	0.491	5.91
48	0.305	0.324	-5.97
72	0.221	0.214	3.12

Table 6.9 ***In vitro*** **drug release studies of sterically stabilized liposomes (mPEG2000-CCPE) containing DNA**

Time in hrs (t)	Mean fraction drug retained (1-f)		
	Actual	Calculated	% Deviation
1	0.970	0.975	-0.525
2	0.902	0.899	-0.2895
4	0.814	0.801	1.62
6	0.742	0.742	0.034
8	0.682	0.702	-2.79
24	0.546	0.523	4.33
48	0.336	0.348	-3.49
72	0.235	0.232	1.45

Table 6.10 ***In vitro*** **drug release studies of cationic sterically stabilized liposomes (mPEG2000-CCPE) containing DNA**

Time in hrs (t)	Mean fraction drug retained (1-f)		
	Actual	Calculated	% Deviation
1	0.980	0.989	-0.926
2	0.922	0.909	1.47
4	0.810	0.809	0.127
6	0.756	0.752	0.575
8	0.692	0.713	-2.99
24	0.560	0.534	4.82
48	0.342	0.354	-3.46
72	0.238	0.234	1.318

Table 6.11 Parameters of release kinetics for conventional and sterically stabilized liposomes containing cyclosporine, leuprolide acetate and DNA

Batch	f_f	f_l	$k_f(h^{-1})$	$k_l(h^{-1})$	$t_{1/2}$ (h)
CL	0.1594	0.9186	0.3535	0.0148	46.91
CPL	0.1785	0.9026	0.3328	0.015	46.06
CNL	0.1814	0.8991	0.3613	0.0145	47.98
LL	0.2055	0.7192	0.1491	0.0259	26.77
SLL5000	0.3221	0.7366	0.2585	0.0173	40.04
SLL2000	0.3865	0.6881	0.2215	0.0132	52.71
DL	0.1933	0.8192	0.496	0.0275	25.24
SDL5000	0.3424	0.743	0.3624	0.0173	40.13
SDL2000	0.2928	0.7865	0.372	0.017	40.8
CSDL2000	0.2988	0.8057	0.416	0.0171	40.5

Figure 6.1 ***In vitro*** **drug release studies of neutral liposomes containing cyclosporine**

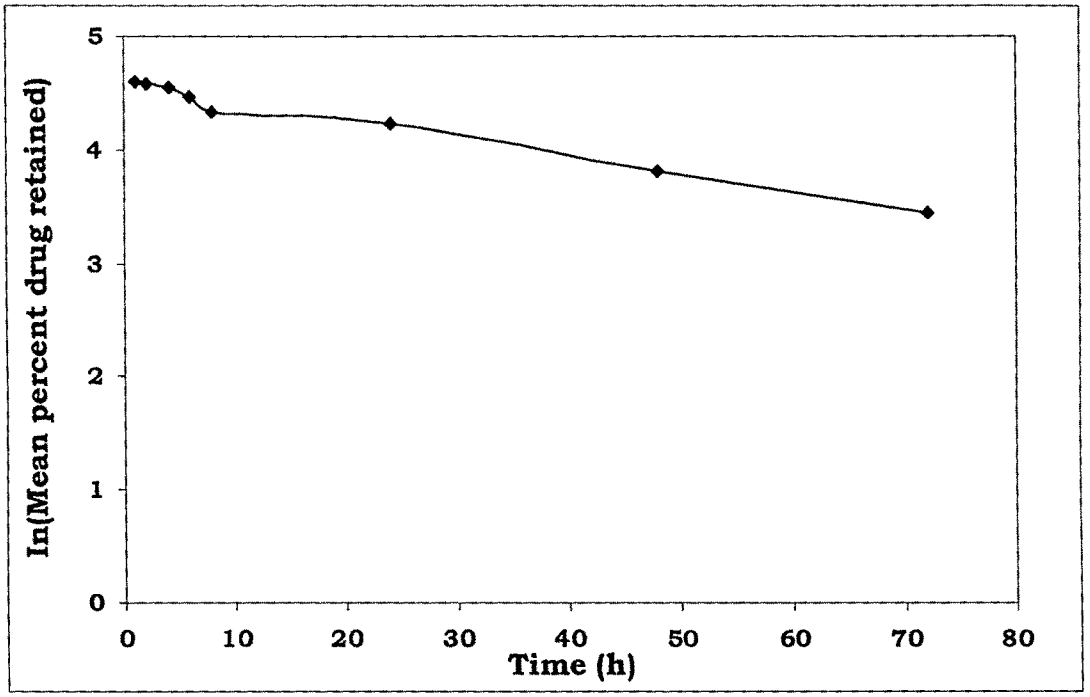


Figure 6.2 ***In vitro*** **drug release studies of positive charged liposomes containing cyclosporine**

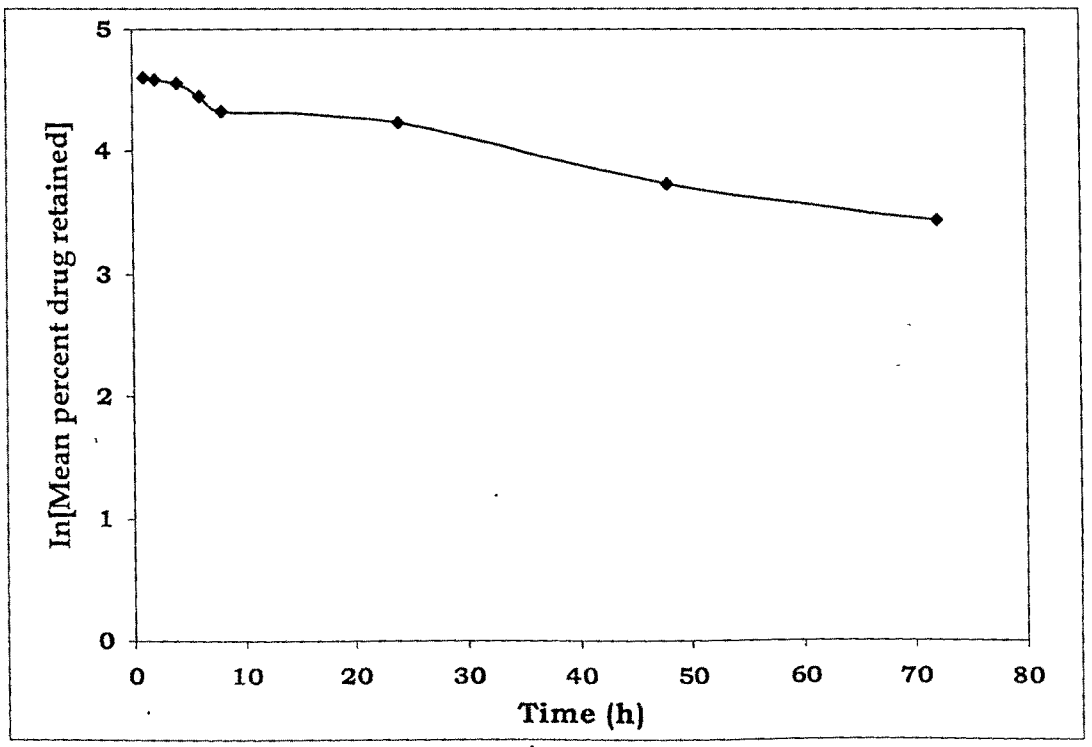


Figure 6.3 ***In vitro*** **drug release studies of negative charged liposomes containing cyclosporine**

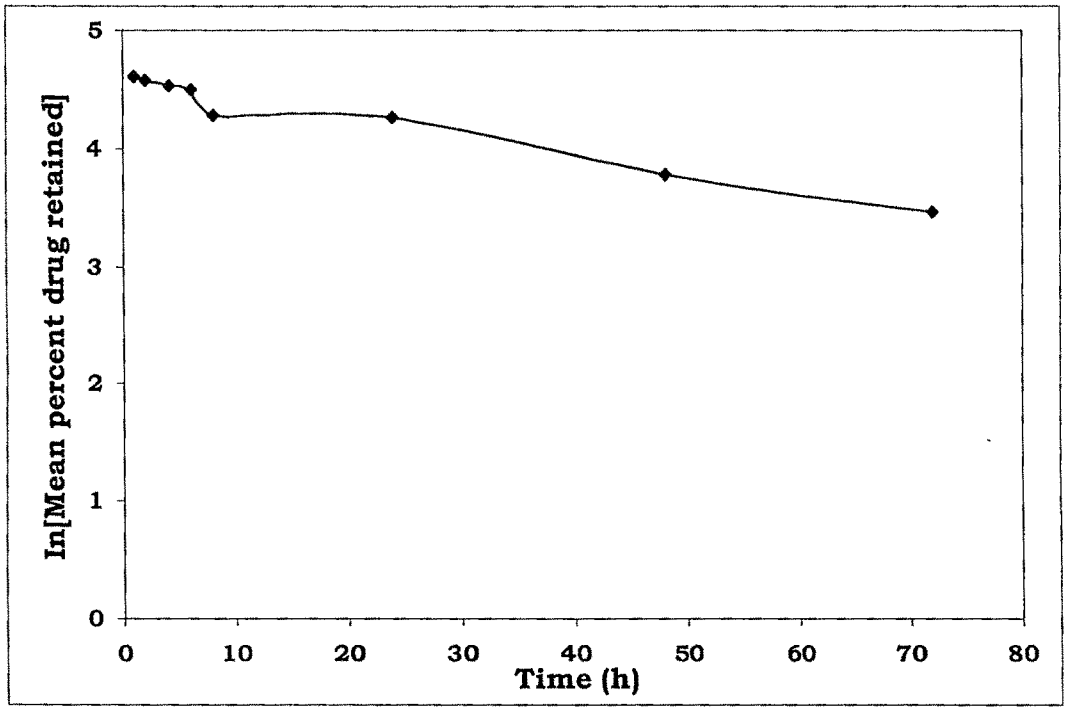


Figure 6.4 ***In vitro*** **drug release studies of conventional liposomes containing leuprolide acetate**

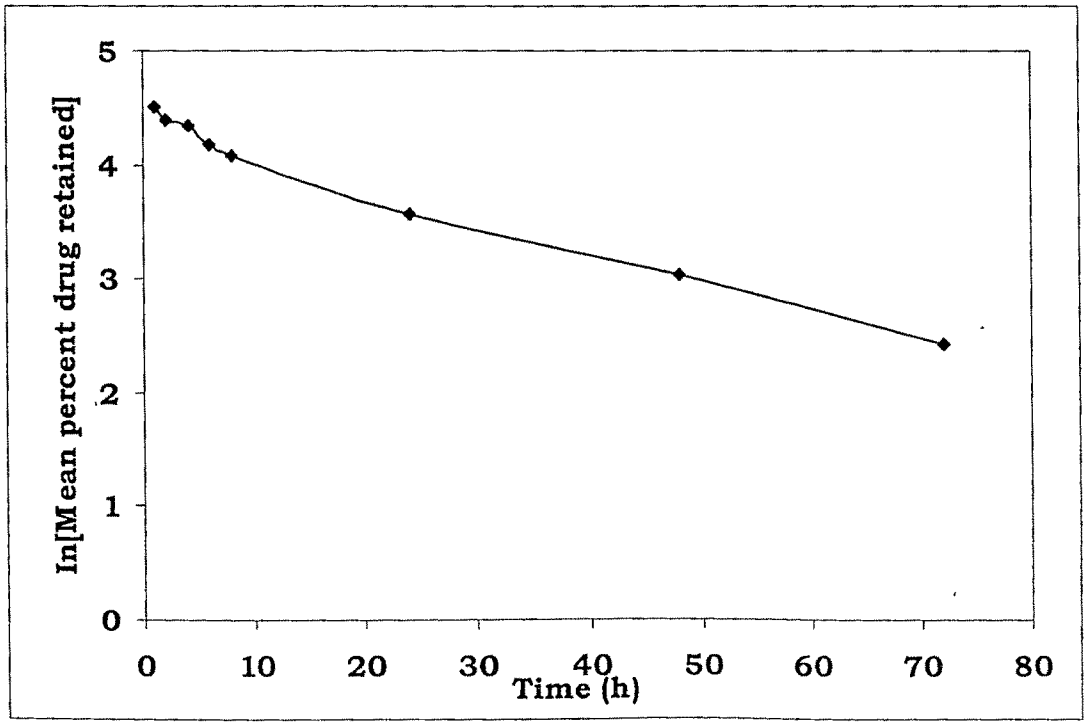


Figure 6.5 *In vitro* drug release studies of sterically stabilized liposomes (SLL5000) containing leuprolide acetate

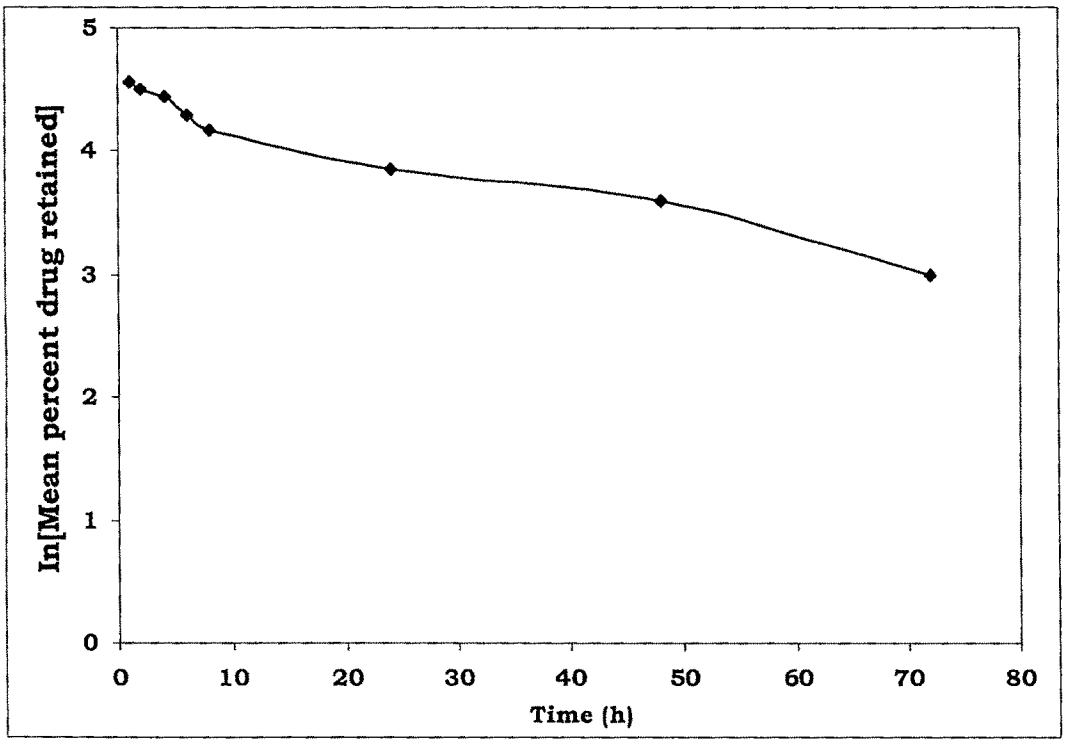


Figure 6.6 *In vitro* drug release studies of sterically stabilized liposomes (SLL2000) containing leuprolide acetate

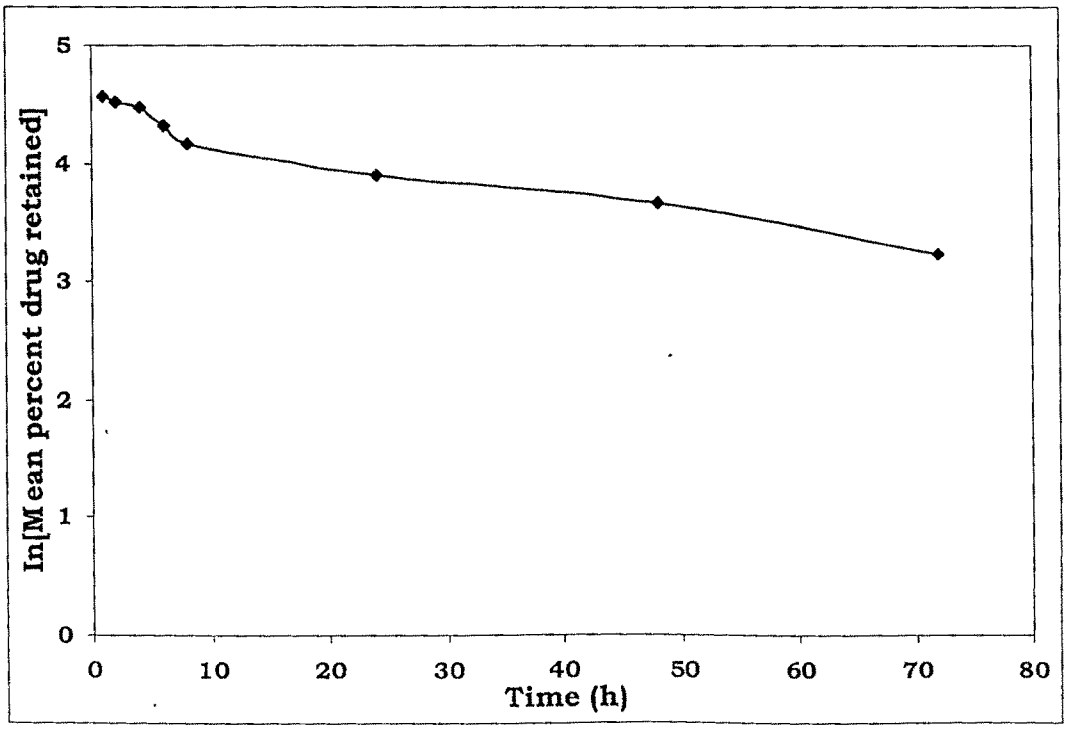


Figure 6.7 *In vitro* drug release studies of conventional liposomes containing DNA

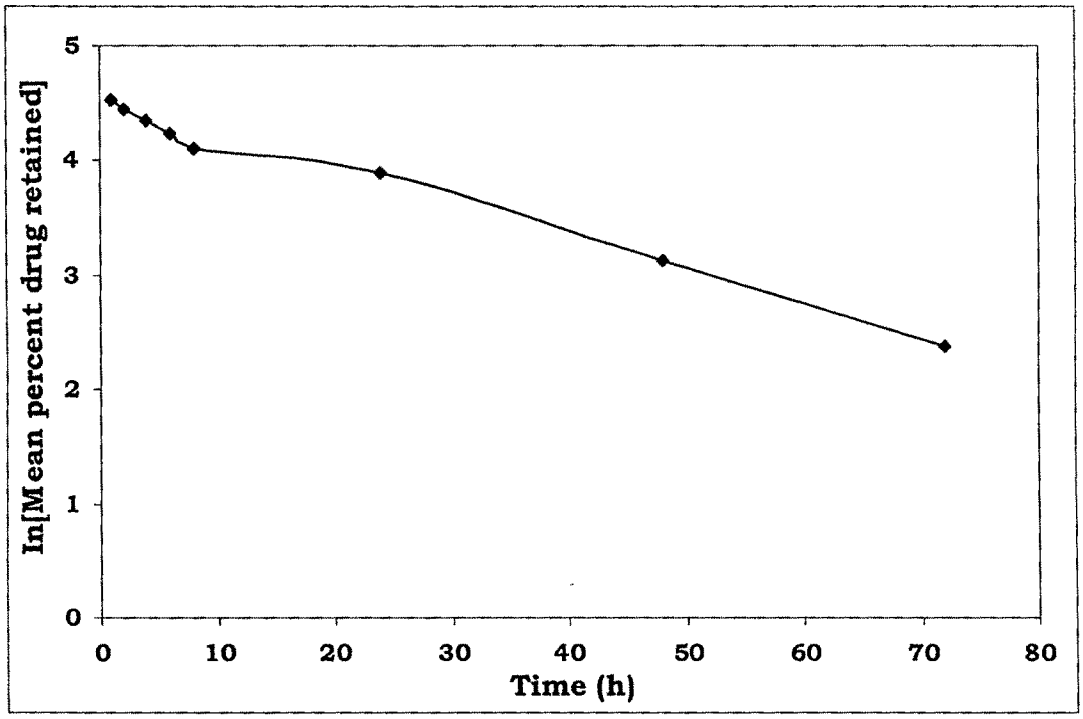


Figure 6.8 *In vitro* drug release studies of sterically stabilized liposomes (SDL5000) containing DNA

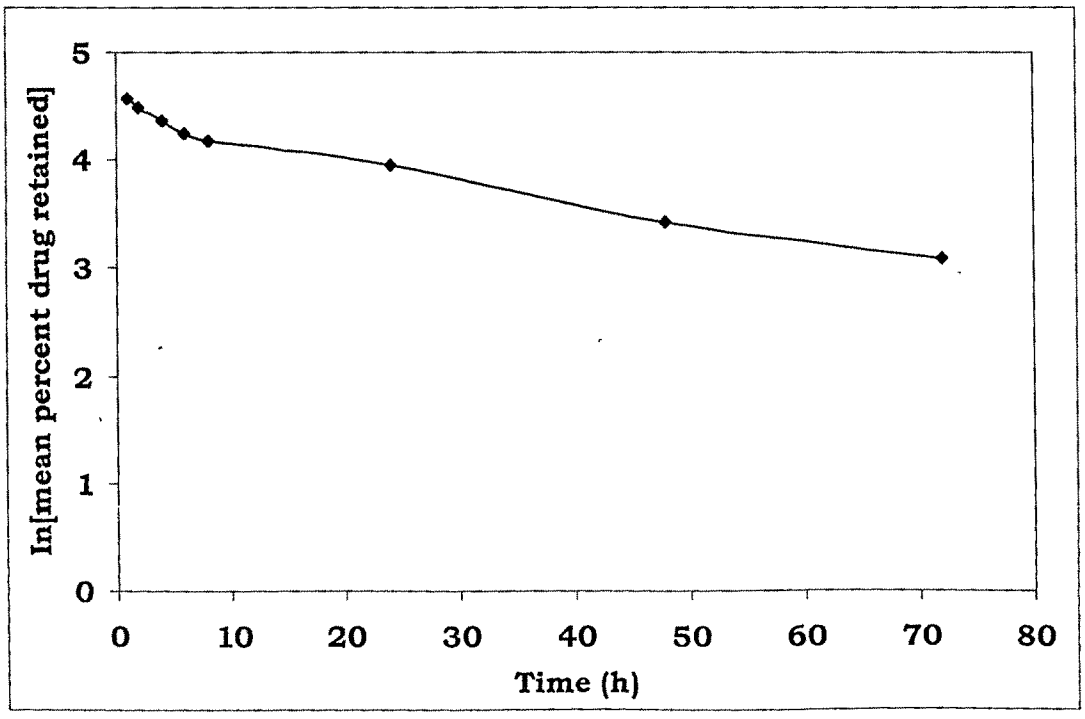


Figure 6.9 *In vitro* drug release studies of sterically stabilized liposomes (SDL2000) containing DNA

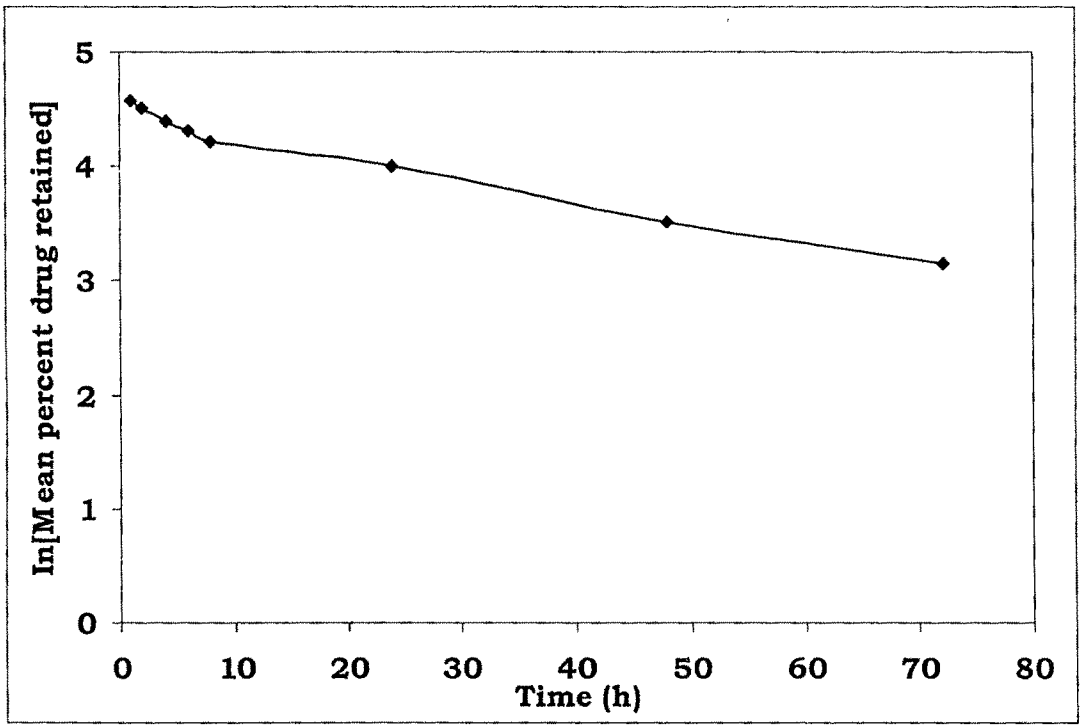
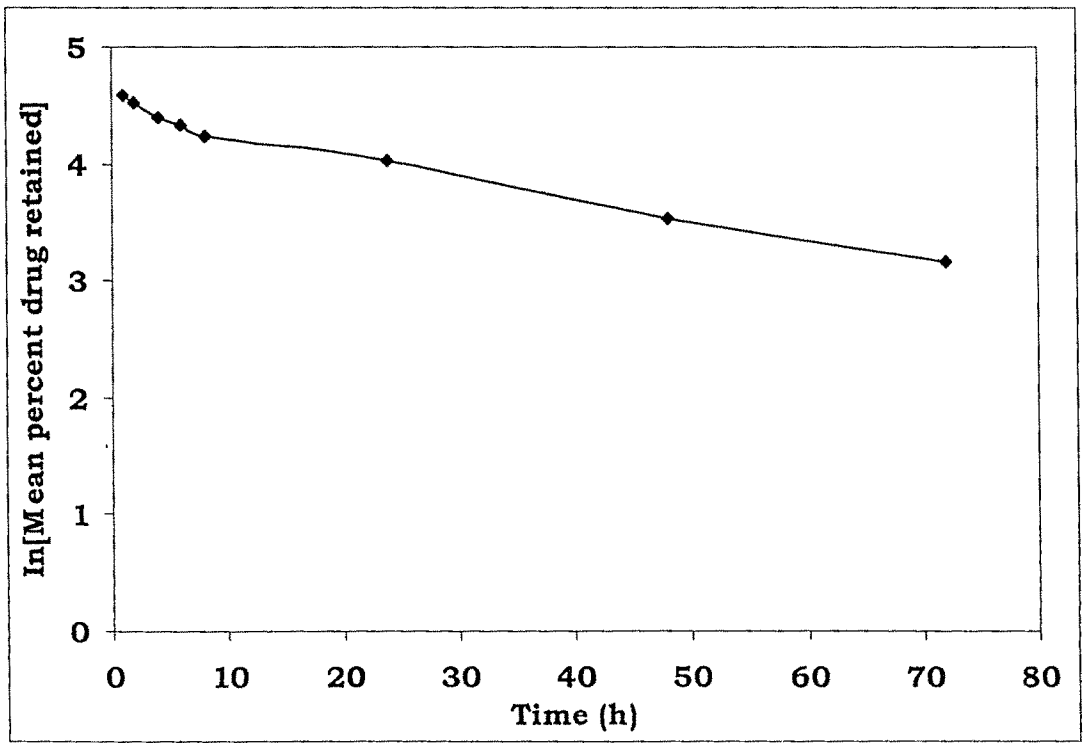


Figure 6.10 *In vitro* drug release studies of cationic sterically stabilized liposomes (CSDL2000) containing DNA



6.3 RESULTS AND DISCUSSION

Studies of drug release from charged liposomal systems containing cyclosporine, conventional and sterically stabilized liposomal systems containing leuprolide acetate and DNA were performed. It was expected that such studies would throw light regarding the effect of charge and steric stabilizing agents on the release of entrapped drugs from the liposomes. Kinetic parameters obtained from these studies can also help to evaluate the possible utility of these systems in actual clinical use.

Tables 6.1 to 6.10 contain the experimental data along with the results of non-linear regression of the data of the release of cyclosporine, leuprolide acetate and DNA from charged and sterically stabilized liposomes. The log of percent drug retained against time plots using the results of non-linear regression are shown in figures 6.1 to 6.10. Calculated kinetic parameters for various systems are tabulated in Table 6.11.

Examination of the above data overall reveals that the release of cyclosporine, leuprolide acetate and DNA from conventional and sterically stabilized liposomes follows a biphasic pattern. In all the cases, an initial, rapid release of the drug occurs (the rate of release being described by the value of the parameter k_f) followed by a subsequent, slow and sustained release, the rate being given by the value of k_l. While the unencapsulated fraction of the drug (described by the kinetic parameter f_f) is responsible for the initial phase, the later part of the release profile can be attributed to the release of the encapsulated fraction of the drug (given as f_l).

6.3.1 RELEASE STUDIES OF CHARGED LIPOSOMES CONTAINING CYCLOSPORINE

Table 6.1 (and figure 6.1) presents the release of cyclosporine from neutral liposomes (CL). From the values of the kinetic parameters (table 6.11), it can be seen that around 16% of the total drug associated with the system is in the unencapsulated form. As part of the preparation procedure, liposomes are repeatedly centrifuged before storage, to remove any unentrapped drug. This 'cleansing' procedure sets up a moderate electrochemical gradient during storage. Drug from the interior of the liposomes diffuses to the external environment in response to this gradient.

Thus, the unencapsulated fraction is partly the result of this movement of drug. However, the drug present in the outermost bilayer of the liposomes at the time of the study contributes in a major way to the unencapsulated drug fraction since this portion of the drug entrapped does not traverse any membrane before encountering the external medium and this behaves in a manner similar to free drug. The unencapsulated cyclosporine is released at a rate of $0.3535h^{-1}$. 91.86% of the drug present in the encapsulated form is released at a rate of $0.0148h^{-1}$, the slower rate (as compared to that of unencapsulated drug) signifying that the drug has to traverse membrane barriers before diffusing into the external medium. The half-life of the CL is around 47h. This, therefore, indicates that cyclosporine containing liposomes have the potential for use as sustained drug delivery systems.

Table 6.2 (figure 6.2) depicts the results of the release studies conducted for positive charged liposomes (CPL) containing cyclosporine. Around 17.8% of the total drug associated with the system is in the unencapsulated form (Table 6.11). The unencapsulated cyclosporine is released at a rate of $0.3328h^{-1}$. 90% of the drug present in the encapsulated form is released at a rate of $0.015h^{-1}$, the slower rate (as compared to that of unencapsulated drug) signifying that the drug has to traverse membrane barriers before diffusing into the external medium. The half-life of the CPL is around 46h. This, therefore, indicates CPL has the potential for use as sustained drug delivery systems.

Table 6.3 (figure 6.3) depicts the results of the release studies conducted for negative charged liposomes containing cyclosporine. Around 18% of the total drug associated with the system is in the unencapsulated form (Table 6.11). The unencapsulated cyclosporine is released at a rate of $0.3613h^{-1}$. 90% of the drug present in the encapsulated form is released at a rate of $0.0145h^{-1}$, the slower rate (as compared to that of unencapsulated drug) signifying that the drug has to traverse membrane barriers before diffusing into the external medium. The half-life of the CNL is around 47.9h. This, therefore, indicates that CNL can be used as sustained drug delivery systems.

6.3.2 RELEASE STUDIES OF CONVENTIONAL LIPOSOMES CONTAINING LEUPROLIDE ACETATE AND DNA

Table 6.4 (and figure 6.4) represents the results of release studies conducted for conventional liposomes containing leuprolide acetate. Around 33%, of the total drug associated with the system, is in the unencapsulated form as shown in Table 6.11. Partly, the presence of this drug fraction can be explained in terms of the electrochemical gradient being in operation during storage in a similar manner to that already discussed above for conventional liposomes containing cyclosporine. The high aqueous solubility of leuprolide acetate makes this gradient more pronounced than in the case of cyclosporine. A majority of the unencapsulated fraction of the drug is due to the presence of the drug in the outermost bilayer of the liposomes (value of f_1 = 0.6582 h^{-1}), a portion of the drug is associated with the bilayer due to its intrinsic hydrophobicity and due to the presence of phosphatidyl choline in the bilayer which enhances the solubility of leuprolide acetate in this lipidic region. The high rate of release of this unencapsulated fraction (0.2326 h^{-1}) points out the hydrophilic nature of leuprolide acetate. The rate of release of the encapsulated fraction is 0.0235h^{-1}, the greater rate compared to that for conventional liposomes containing cyclosporine, drawing attention to the greater fluidity of the bilayer in case of conventional liposomes containing leuprolide acetate and to the importance of the hydrophilicity of leuprolide acetate. The half-life for drug release from conventional liposomes containing leuprolide acetate is around 29.6h indicating that these liposomes can be used as sustained drug delivery systems.

Table 6.7 (and figure 6.7) represents the results of release studies conducted for conventional liposomes containing DNA. Around 32.5%, of the total drug associated with the system, is in the unencapsulated form as shown in Table 6.11. Partly, the presence of this drug fraction can be explained in terms of the electrochemical gradient being in operation during storage in a similar manner to that already discussed above for conventional liposomes containing cyclosporine. A majority of the unencapsulated fraction of the drug is due to the presence of the drug in the outermost bilayer of the liposomes (value of f_1 = 0.8235h^{-1}), a portion of

the drug is associated with the bilayer due to its intrinsic hydrophobicity and due to the presence of phosphatidyl choline in the bilayer which enhances the solubility of DNA in the lipidic region. The high rate of release of this unencapsulated fraction (0.8235 h^{-1}) points out the hydrophilic nature of DNA when compared with the same parameter in case of conventional liposomes containing cyclosporine. The rate of release of the encapsulated fraction is 0.0275h^{-1}, the greater rate compared to that for conventional liposomes containing cyclosporine, drawing attention to the greater fluidity of the bilayer in case of conventional liposomes containing DNA and to the importance of the hydrophilicity of DNA. The half-life for drug release from conventional liposomes containing DNA is around 25.17h indicating that these liposomes can be used as sustained drug delivery systems.

6.3.3 RELEASE STUDIES OF STERICALLY STABILIZED LIPOSOMES CONTAINING LEUPROLIDE ACETATE AND DNA

Sterically stabilized liposomes of leuprolide acetate were prepared using methoxy polyethylene glycol derivates (mPEG5000-CC-PE and mPEG2000-CC-PE) and the results of release studies of these liposomes are shown in table 6.5 and table 6.6 (figure 6.5 and figure 6.6) respectively. The rate of release of free leuprolide acetate from sterically stabilized liposomes SLL5000-CC-PE and SLL2000-CC-PE (k_f = 0.2585h^{-1} and 0.2215h^{-1}) is increased as compared to that of the conventional liposomes [LL] (k_f = 0.1491h^{-1}) respectively as shown in table 6.11. Methoxy polyethylene glycol derivates, when included in the bilayer of the liposomes, increases the percentage of unencapsulated drug (f_f = 0.3221h^{-1} and 0.3865h^{-1} for SLL5000-CC-PE and SLL2000-CC-PE respectively) as compared to that of conventional liposomes (f_f = 0.2055h^{-1}). This increase in permeability, coupled with the enhanced electrochemical gradient, due to the presence of hydrophilic polyethylene glycol chains, on the liposomal surface, is responsible for the increased drug content at the surface. The conclusion is well supported by the lower rate of release of encapsulated leuprolide acetate (k_l = 0.0173h^{-1} and 0.0132h^{-1} for SLL5000-CC-PE and SLL2000-CC-PE respectively) as compared to that of conventional liposomes (k_l = 0.0259h^{-1}) which is possible only if a part of the drug is also present in

the aqueous compartment and has to traverse a hydrophobic barrier to reach the exterior. This phenomenon is also responsible for the increase in half-life of these liposomes ($t_{1/2}$ = 40.04h and 52.41h for SLL5000-CC-PE and SLL2000-CC-PE respectively) as compared to that of conventional liposomes ($t_{1/2}$ = 26.77h) indicates that leuprolide acetate containing sterically stabilized liposomes have the potential for use as sustained drug delivery systems.

Sterically stabilized liposomes of DNA were prepared using methoxy polyethylene glycol derivates (mPEG5000-CC-PE and mPEG2000-CC-PE) and the results of release studies of these liposomes are shown in tables 6.8-6.10 (and figures 6.8-6.10) respectively. The rate of release of unencapsulated DNA from sterically stabilized liposomes SDL5000-CC-PE, SDL2000-CC-PE and CSDL2000-CC-PE (k_f = 0.3624h^{-1}, 0.372h^{-1} and 0.416h^{-1}) is increased as compared to that of the conventional liposomes [DL] (k_f = 0.498h^{-1}).

Methoxy polyethylene glycol derivates, when included in the bilayer of the liposomes, increases the percentage of unencapsulated drug for SDL5000-CC-PE, SDL2000-CC-PE and CSDL2000-CC-PE (f_f = 0.3424h^{-1}, 0.2928h^{-1} and 0.299h^{-1}) respectively as compared to that of conventional liposomes (f_f = 0.1933h^{-1}). This increase in permeability, coupled with the enhanced electrochemical gradient, due to the presence of hydrophilic polyethylene glycol chains, on the liposomal surface, is responsible for the increased drug content at the surface. The conclusion is well supported by the lower rate of release of encapsulated DNA for SDL5000-CC-PE, SDL2000-CC-PE and CSDL2000-CC-PE (k_l = 0.0173h^{-1}, 0.017h^{-1} and 0.0171h^{-1}) respectively as compared to that of conventional liposomes (k_l = 0.0275h^{-1}) which is possible only if a part of the drug is also present in the aqueous compartment and has to traverse a hydrophobic barrier to reach the exterior. The presence of the hydrophilic steric stabilizing agents causes delay in the release of the drug from liposomes which is responsible for the increase in half life of these liposomes ($t_{1/2}$ = 40.13h, 40.8 and 40.5h for SDL5000-CC-PE, SDL2000-CC-PE and CSDL2000-CC-PE respectively) as compared to that of conventional liposomes ($t_{1/2}$ = 25.24h) indicates that

DNA containing sterically stabilized liposomes have the potential for use as sustained delivery systems.

6.3.5 CONCLUSIONS

Considering the above observations made with conventional liposomes and liposomes sterically stabilized with various agents, following conclusions can be drawn:

1. All the batches of liposomes (conventional and sterically stabilized) have the potential for sustained drug delivery.
2. Incorporation of charge into liposomes of cyclosporine does not alter the half life of drug release to a great extent as compared to neutral liposomes of the same drug. Sterically stabilizing agents into liposomes of leuprolide acetate and DNA causes a significant increase in the half life of the drug in comparison to conventional liposomes. Not much difference between the various agents in this regard is obtained, the most promising results being obtained with liposomes of sterically stabilized with mPEG2000-CC-PE.
3. The various kinetic parameters could be explained using the physicochemical properties of the steric stabilizing agents and the nature of the composition of the liposomal membrane. These observations also emphasised the importance of considering the hydrophobicity or hydrophilicity of the drug in determining membrane composition.
4. The low values of the standard deviation (Table 6.1-6.10) and the fulfillment of the criterion $f_f + f_l = 1$ in all the cases point to the good fit between the experimental and calculated data and to the applicability of Quickcalc software to these types of analyses.

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