# Chapter 6

# In vitro 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 (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 liposomes arrive at the target) to the requirements of the therapy.

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

a) Physicochemical characterizations of the systems, including liposomes processed into aerosols or reconstituted from freeze dried powders.

b) Various aspects of system optimisation such as the selection of liposome type, lipid composition and parameters of shelf life.

c) Criteria for quality assurance.

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 (each, separately) dilutions that the system is anticipated to undergo.

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 based 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 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 APPARATUS

Magnetic stirrer (Remi Scientific Equipments, Mumbai); Teflon coated bar magnet 25 x 7mm (S.D. Fine Chem Ltd., Boisar); water bath with 0-110°C display thermometer; open-ended dialysis tubing made of cellulose (retains proteins with molecular weight greater than 12000 daltons) having flat width 35mm and inflated diameter 21mm (Sigma Diagnostics, U.S.A.)

### 6.2.4 STUDIES OF KINETICS OF DRUG RELEASE FROM LIPOSOMES

#### 6.2.4.1 Preparation of dialysis sac

A 10cm long portion of the dialysis tubing was made into a dialysis sac by folding and tying up one end of the tubing with thread, taking care to ensure that there would be no leakage of the contents from inside the sac. The sac was then soaked overnight in PBS.

#### 6.2.4.2 Dialysis set up

The wet sac was gently opened and washed copiously with PBS. Then, it was filled up with PBS and examined for leaks. The sac was then emptied and 1ml of the liposomal suspension 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 a glass beaker containing 200ml of PBS, which acted as the receptor compartment. The temperature of the contents of the beaker was maintained at the appropriate level required using the thermostatically controlled heater of the magnetic stirrer. The contents of the beaker were stirred using a Teflon coated bar magnet (25 x 7mm) and the beaker was closed with aluminium foil to prevent any evaporative losses during the experimental run. The temperature of the system was monitored at regular intervals of time.

#### 6.2.4.3 Sampling

At predetermined intervals of time, 5ml aliquots were withdrawn from the receptor compartment and subjected to analysis using the methods elaborated in Chapter 3, Analytical methods. Fresh buffer was used to replenish the receptor compartment. Analysis was carried out immediately

after withdrawal. Studies were continued till about 70% of the entrapped drug was released.

**6.2.4.4 Data analysis**

Release kinetics studies were performed for all liposomal batches in triplicate. Table 6.1 - 6.12 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

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

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

$f = f_1 - f_1 e^{-k_1 t} + f_f - f_f e^{-k_f t}$

or $f = (f_1 + f_f) - f_1 e^{-k_1 t} - f_f e^{-k_f t}$

Thus,

$f = 1 - (f_1 e^{-k_1 t} + f_f e^{-k_f t})$ [using equation (3)]

or $1-f = f_1 e^{-k_1 t} + f_f e^{-k_f t}$ .....(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 Tables 6.1 –6.12. Figures 6.1 –6.12 depict the log of percent drug retained vs time curves (using regressed data). Kinetic parameters obtained from the above analysis are tabulated in Table 6.13.

**Table 6.1 *In vitro* drug release studies of conventional liposomes containing methotrexate**

| Time in Hrs (t) | Mean fraction drug retained (1-f) | | |
|---|---|---|---|
| | Actual (S.E) | Calculated | % Deviation |
| 0.5 | 0.982 (0.011) | 0.972 | 1.035 |
| 1 | 0.915 (0.009) | 0.926 | -1.132 |
| 2 | 0.841 (0.013) | 0.850 | -1.068 |
| 4 | 0.766 (0.019) | 0.748 | 2.419 |
| 6 | 0.684 (0.025) | 0.684 | 0.003 |
| 8 | 0.629 (0.014) | 0.640 | -1.742 |
| 24 | 0.466 (0.022) | 0.459 | 1.414 |
| 48 | 0.291 (0.031) | 0.294 | -1.050 |
| 72 | 0.189 (0.016) | 0.188 | 0.392 |

**Table 6.2** ***In vitro*** **drug release studies of liposomes containing methotrexate sterically stabilized with 5mol% mPEG5000-CC-PE**

| Time in Hrs (t) | Mean fraction drug retained (1-f) | | |
|---|---|---|---|
| | Actual (S.E) | Calculated | % Deviation |
| 0.5 | 1.000 (0.000) | 1.012 | -1.141 |
| 1 | 0.987 (0.011) | 0.972 | 1.503 |
| 2 | 0.912 (0.020) | 0.914 | -0.209 |
| 4 | 0.856 (0.021) | 0.844 | 1.408 |
| 6 | 0.795 (0.016) | 0.804 | -1.111 |
| 8 | 0.749 (0.011) | 0.776 | -3.416 |
| 24 | 0.621 (0.016) | 0.619 | 0.316 |
| 48 | 0.487 (0.024) | 0.445 | 9.376 |
| 72 | 0.306 (0.027) | 0.320 | -4.450 |

**Table 6.3** ***In vitro*** **drug release studies of liposomes containing methotrexate sterically stabilized with 7mol% mPEG2000-CC-PE**

| Time in Hrs (t) | Mean fraction drug retained (1-f) | | |
|---|---|---|---|
| | Actual (S.E) | Calculated | % Deviation |
| 0.5 | 0.982 (0.009) | 0.986 | -0.385 |
| 1 | 0.954 (0.016) | 0.943 | 1.169 |
| 2 | 0.884 (0.010) | 0.883 | 0.105 |
| 4 | 0.812 (0.028) | 0.819 | -0.853 |
| 6 | 0.782 (0.014) | 0.785 | -0.436 |
| 8 | 0.743 (0.019) | 0.762 | -2.492 |
| 24 | 0.631 (0.021) | 0.625 | 1.030 |
| 48 | 0.498 (0.019) | 0.466 | 6.991 |
| 72 | 0.334 (0.022) | 0.347 | -3.715 |

**Table 6.4** ***In vitro*** **drug release studies of liposomes containing methotrexate sterically stabilized with 5 mol% mPEG5000-IC-PE**

| Time in Hrs (t) | Mean fraction drug retained (1-f) | | |
|---|---|---|---|
| | Actual (S.E) | Calculated | % Deviation |
| 0.5 | 1.000 (0.000) | 0.990 | 1.011 |
| 1 | 1.000 (0.000) | 0.984 | 1.650 |
| 2 | 0.988 (0.014) | 0.971 | 1.704 |
| 4 | 0.954 (0.023) | 0.947 | 0.698 |
| 6 | 0.912 (0.028) | 0.924 | -1.306 |
| 8 | 0.874 (0.016) | 0.902 | -3.048 |
| 24 | 0.726 (0.018) | 0.745 | -2.514 |
| 48 | 0.597 (0.032) | 0.577 | 3.550 |
| 72 | 0.466 (0.019) | 0.470 | -0.905 |

**Table 6.5** ***In vitro*** **drug release studies of conventional liposomes containing tamoxifen citrate**

| Time in Hrs (t) | Mean fraction drug retained (1-f) | | |
|---|---|---|---|
| | Actual (S.E) | Calculated | % Deviation |
| 0.5 | 1.000 (0.000) | 1.015 | -1.442 |
| 1 | 0.994 (0.002) | 0.992 | 0.240 |
| 2 | 0.964 (0.025) | 0.952 | 1.318 |
| 4 | 0.907 (0.013) | 0.889 | 2.041 |
| 6 | 0.847 (0.019) | 0.842 | 0.588 |
| 8 | 0.775 (0.011) | 0.805 | -3.722 |
| 24 | 0.611 (0.027) | 0.619 | -1.361 |
| 48 | 0.465 (0.033) | 0.434 | 7.209 |
| 72 | 0.294 (0.018) | 0.304 | -3.247 |

**Table 6.6** ***In vitro*** **drug release studies of liposomes containing tamoxifen citrate sterically stabilized with 5mol% mPEG5000-CC-PE**

| Time in Hrs (t) | Mean fraction drug retained (1-f) | | |
|---|---|---|---|
| | Actual (S.E) | Calculated | % Deviation |
| 0.5 | 1.000 (0.000) | 1.012 | -1.173 |
| 1 | 0.997 (0.015) | 0.990 | 0.698 |
| 2 | 0.955 (0.016) | 0.953 | 0.241 |
| 4 | 0.912 (0.021) | 0.896 | 1.758 |
| 6 | 0.865 (0.027) | 0.856 | 1.082 |
| 8 | 0.795 (0.014) | 0.825 | -3.607 |
| 24 | 0.674 (0.019) | 0.676 | -0.231 |
| 48 | 0.539 (0.026) | 0.517 | 4.165 |
| 72 | 0.388 (0.022) | 0.397 | -2.141 |

**Table 6.7** ***In vitro*** **drug release studies of liposomes containing tamoxifen citrate sterically stabilized with 7mol% mPEG2000-CC-PE**

| Time in Hrs (t) | Mean fraction drug retained (1-f) | | |
|---|---|---|---|
| | Actual (S.E) | Calculated | % Deviation |
| 0.5 | 1.000 (0.000) | 1.004 | -0.414 |
| 1 | 1.000 (0.000) | 0.994 | 0.624 |
| 2 | 0.972 (0.012) | 0.974 | 0.190 |
| 4 | 0.883 (0.016) | 0.937 | -5.742 |
| 6 | 0.816 (0.024) | 0.903 | -9.640 |
| 8 | 0.731 (0.021) | 0.872 | -16.189 |
| 24 | 0.634 (0.013) | 0.693 | -8.4895 |
| 48 | 0.566 (0.026) | 0.527 | 7.3303 |
| 72 | 0.398 (0.013) | 0.410 | -3.0163 |

**Table 6.8** ***In vitro*** **drug release studies of liposomes containing tamoxifen citrate sterically stabilized with 5mol% mPEG5000-IC-PE**

| Time in Hrs (t) | Mean fraction drug retained (1-f) | | |
|---|---|---|---|
| | Actual (S.E) | Calculated | % Deviation |
| 0.5 | 1.000 (0.000) | 1.026 | -2.485 |
| 1 | 1.000 (0.000) | 0.994 | 0.628 |
| 2 | 0.974 (0.011) | 0.943 | 3.320 |
| 4 | 0.902 (0.021) | 0.874 | 3.190 |
| 6 | 0.823 (0.016) | 0.831 | -0.964 |
| 8 | 0.742 (0.027) | 0.801 | -7.331 |
| 24 | 0.687 (0.019) | 0.657 | 4.522 |
| 48 | 0.531 (0.013) | 0.500 | 6.244 |
| 72 | 0.365 (0.029) | 0.380 | -3.964 |

**Table 6.9** ***In vitro*** **drug release studies of conventional liposomes containing cytarabine**

| Time in Hrs (t) | Mean fraction drug retained (1-f) | | |
|---|---|---|---|
| | Actual (S.E) | Calculated | % Deviation |
| 0.5 | 1.000 (0.000) | 1.001 | -0.064 |
| 1 | 0.974 (0.012) | 0.973 | 0.086 |
| 2 | 0.924 (0.022) | 0.924 | -0.023 |
| 4 | 0.827 (0.016) | 0.845 | -2.076 |
| 6 | 0.745 (0.011) | 0.781 | -4.648 |
| 8 | 0.661 (0.029) | 0.729 | -9.281 |
| 24 | 0.487 (0.013) | 0.454 | 7.228 |
| 48 | 0.229 (0.019) | 0.231 | -0.754 |
| 72 | 0.116 (0.022) | 0.117 | -1.091 |

**Table 6.10** ***In vitro*** **drug release studies of liposomes containing cytarabine sterically stabilized with 5mol% mPEG5000-CC-PE**

| Time in Hrs (t) | Mean fraction drug retained (1-f) | | |
|---|---|---|---|
| | Actual (S.E) | Calculated | % Deviation |
| 0.5 | 0.991 (0.008) | 0.987 | 0.386 |
| 1 | 0.932 (0.011) | 0.941 | -0.927 |
| 2 | 0.865 (0.015) | 0.867 | -0.245 |
| 4 | 0.794 (0.023) | 0.772 | 2.922 |
| 6 | 0.710 (0.015) | 0.714 | 0.566 |
| 8 | 0.655 (0.027) | 0.675 | -3.009 |
| 24 | 0.502 (0.018) | 0.507 | -0.897 |
| 48 | 0.365 (0.011) | 0.340 | 7.231 |
| 72 | 0.221 (0.024) | 0.229 | -3.388 |

**Table 6.11** ***In vitro*** **drug release studies of liposomes containing cytarabine sterically stabilized with 7mol% mPEG2000-CC-PE**

| Time in Hrs (t) | Mean fraction drug retained (1-f) | | |
|---|---|---|---|
| | Actual (S.E) | Calculated | % Deviation |
| 0.5 | 1.000 (0.000) | 1.004 | -0.361 |
| 1 | 0.963 (0.011) | 0.959 | 0.443 |
| 2 | 0.894 (0.016) | 0.895 | -0.090 |
| 4 | 0.858 (0.023) | 0.824 | 4.123 |
| 6 | 0.786 (0.018) | 0.786 | 0.048 |
| 8 | 0.694 (0.012) | 0.759 | -8.505 |
| 24 | 0.591 (0.022) | 0.603 | -1.902 |
| 48 | 0.445 (0.016) | 0.429 | 3.786 |
| 72 | 0.301 (0.027) | 0.305 | -1.361 |

**Table 6.12** ***In vitro*** **drug release studies of liposomes containing cytarabine sterically stabilized with 5mol% mPEG5000-IC-PE**

| Time in Hrs (t) | Mean fraction drug retained (1-f) | | |
|---|---|---|---|
| | Actual (S.E) | Calculated | % Deviation |
| 0.5 | 0.964 (0.012) | 0.961 | 0.299 |
| 1 | 0.902 (0.025) | 0.909 | -0.758 |
| 2 | 0.831 (0.011) | 0.832 | -0.060 |
| 4 | 0.764 (0.019) | 0.741 | 3.101 |
| 6 | 0.672 (0.026) | 0.691 | -2.748 |
| 8 | 0.646 (0.017) | 0.657 | -1.683 |
| 24 | 0.489 (0.020) | 0.485 | 0.842 |
| 48 | 0.326 (0.019) | 0.312 | 4.606 |
| 72 | 0.195 (0.014) | 0.200 | -2.641 |

**Table 6.13 Parameters of release kinetics for conventional and sterically stabilized liposomes containing methotrexate, tamoxifen citrate and cytarabine**

| Batch | $f_f$ | $f_l$ | $k_f(h^{-1})$ | $k_l(h^{-1})$ | $t_{1/2}$ (h) |
|---|---|---|---|---|---|
| ML | 0.3080 | 0.7176 | 0.3318 | 0.0186 | 37.2883 |
| SML5000-CC-PE | 0.1991 | 0.8607 | 0.4778 | 0.0137 | 50.4713 |
| SML2000-CC-PE | 0.2030 | 0.8381 | 0.5670 | 0.0123 | 56.5656 |
| SML5000-IC-PE | 0.0678 | 0.9285 | 0.1100 | 0.0143 | 48.4343 |
| TL | 0.1563 | 0.8837 | 0.2563 | 0.0148 | 46.7407 |
| STL5000-CC-PE | 0.1548 | 0.8813 | 0.2671 | 0.0111 | 62.4650 |
| STL2000-CC-PE | 0.1582 | 0.8566 | 0.0807 | 0.0102 | 67.7020 |
| STL5000-IC-PE | 0.1981 | 0.8643 | 0.3523 | 0.0114 | 60.7346 |
| CL | 0.1373 | 0.8931 | 0.2699 | 0.0282 | 24.5773 |
| SCL5000-CC-PE | 0.2882 | 0.7537 | 0.3682 | 0.0166 | 41.8466 |
| SCL2000-CC-PE | 0.2142 | 0.8465 | 0.5456 | 0.0142 | 48.9026 |
| SCL5000-IC-PE | 0.2711 | 0.7545 | 0.4778 | 0.0184 | 37.6202 |

**Figure 6.1** ***In vitro*** **drug release studies of conventional liposomes of methotrexate**

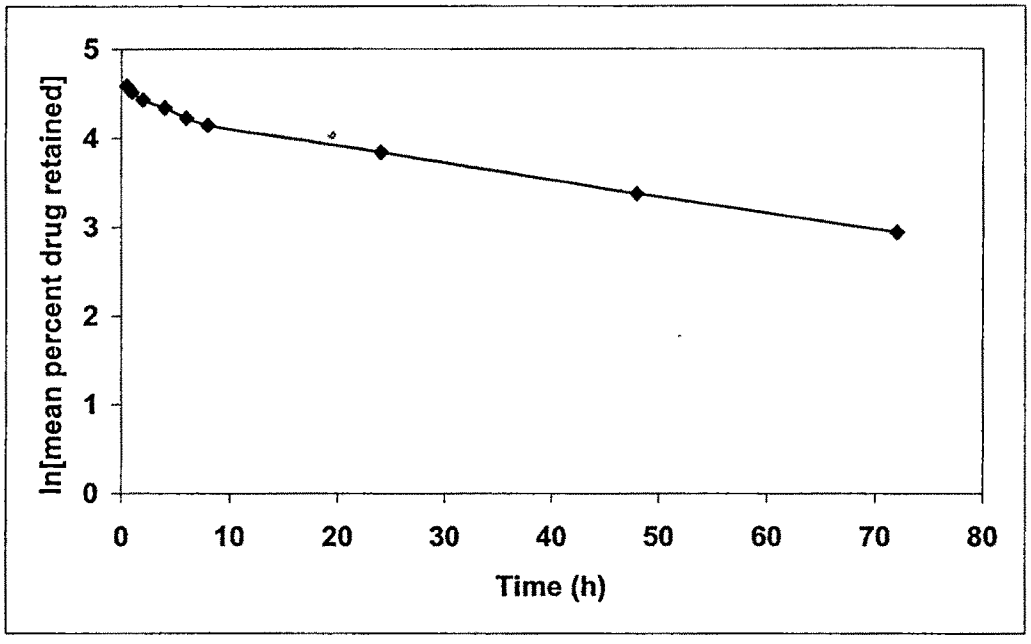



**Figure 6.2** ***In vitro*** **drug release studies of liposomes containing methotrexate sterically stabilized with mPEG5000-CC-PE**

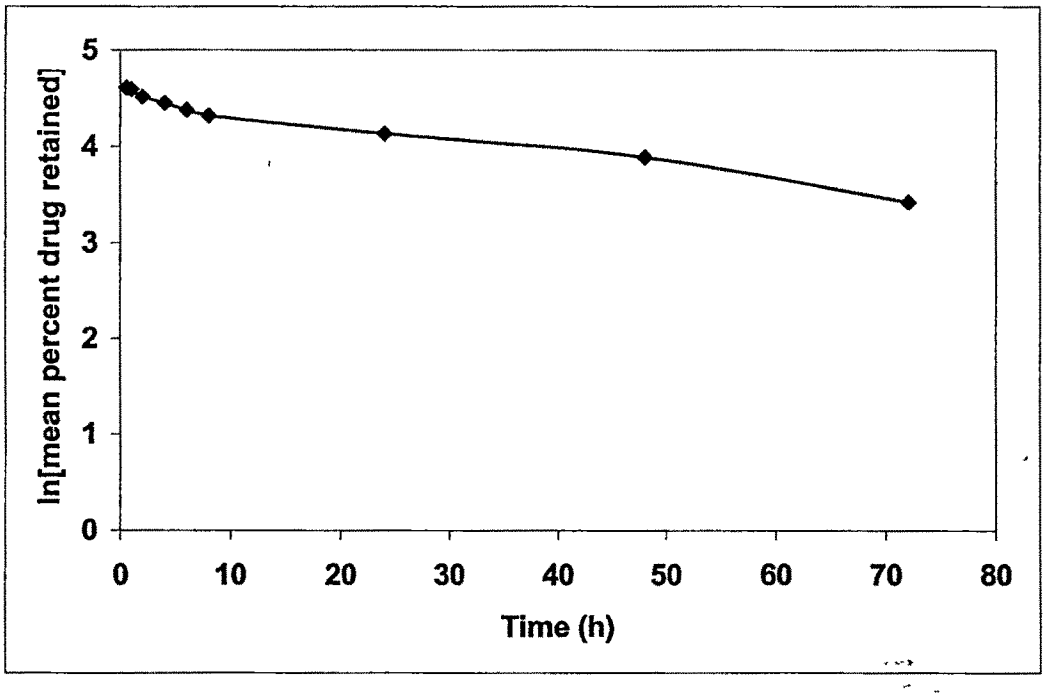


**Figure 6.3** ***In vitro*** **drug release studies of liposomes containing methotrexate sterically stabilized with mPEG2000-CC-PE**

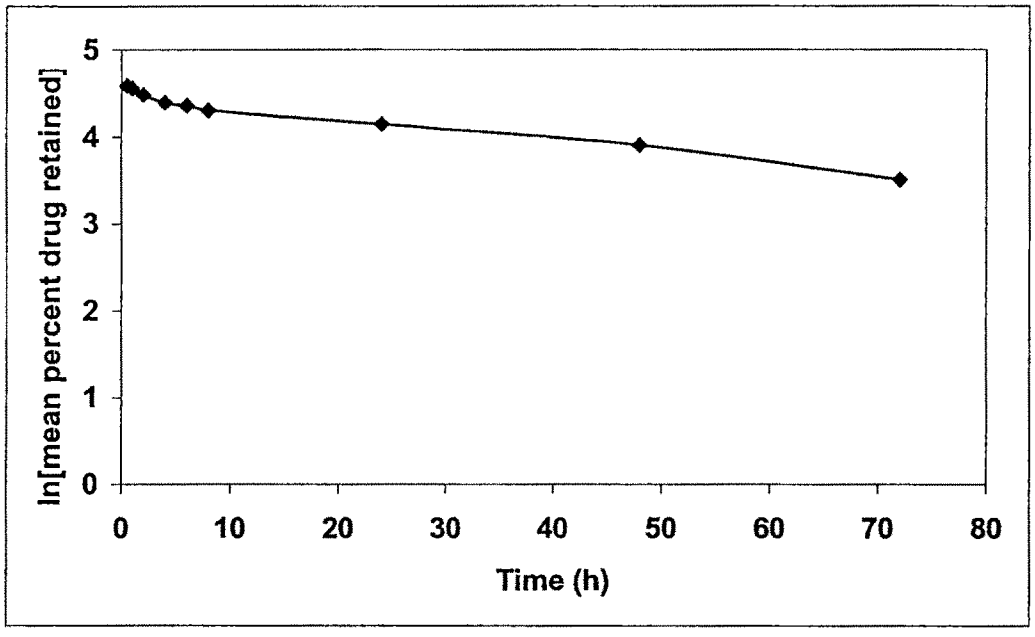



**Figure 6.4** ***In vitro*** **drug release studies of liposomes containing methotrexate sterically stabilized with mPEG5000-IC-PE**

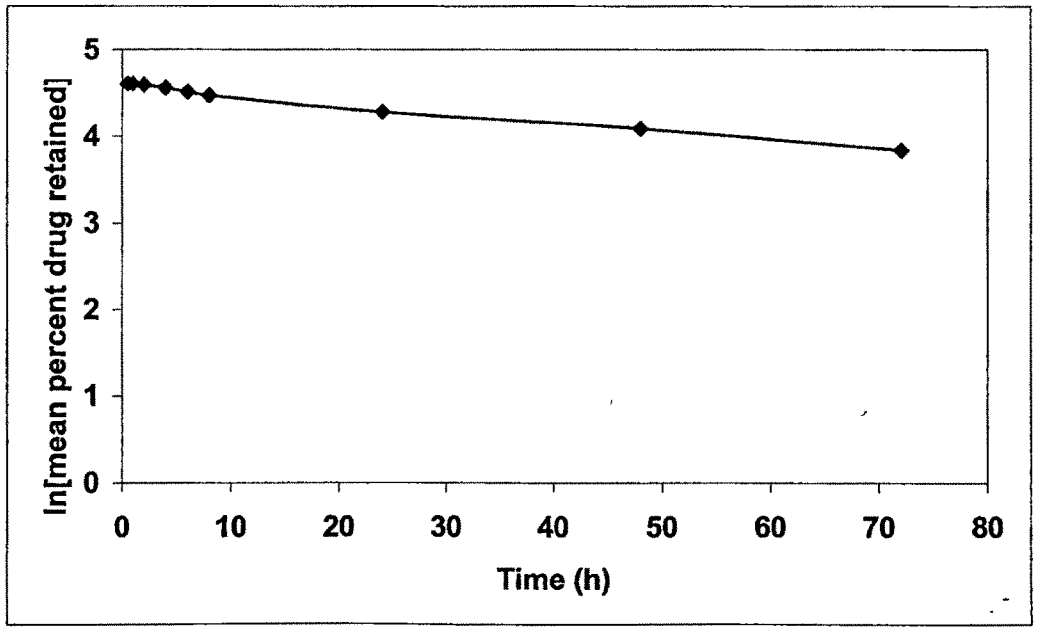


**Figure 6.5** ***In vitro*** **drug release studies of conventional liposomes of tamoxifen citrate**

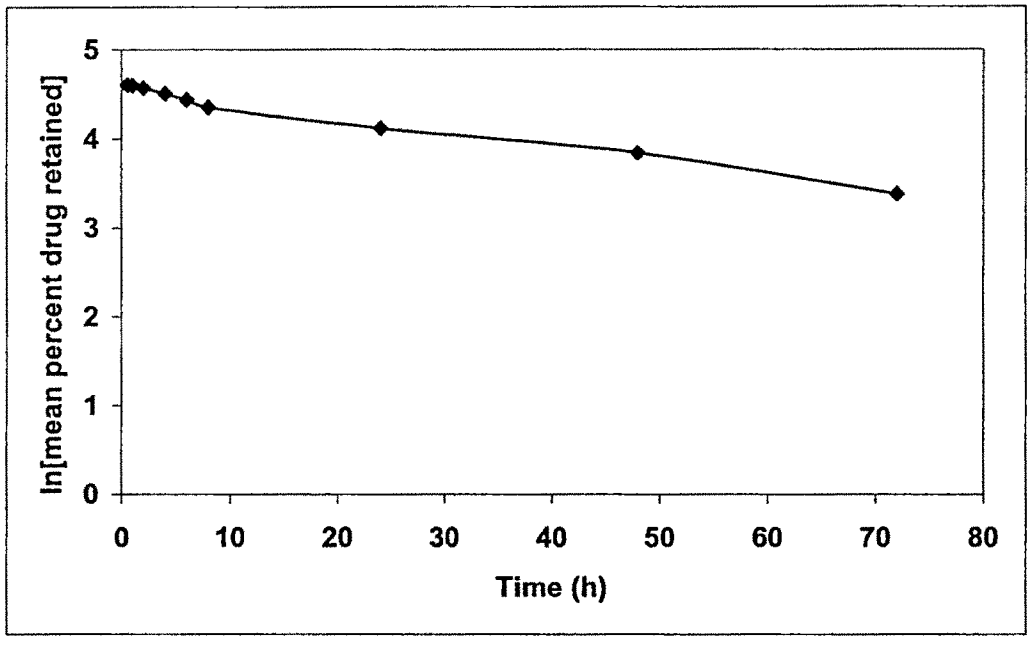



**Figure 6.6** ***In vitro*** **drug release studies of liposomes containing tamoxifen citrate sterically stabilized with mPEG5000-CC-PE**

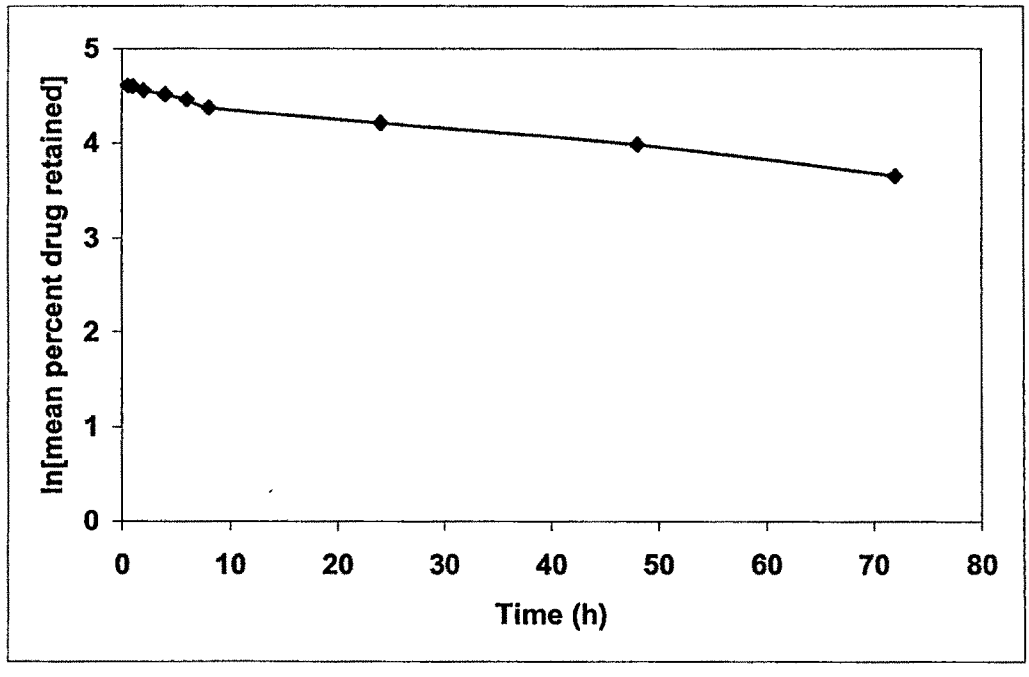


**Figure 6.7** ***In vitro*** **drug release studies of liposomes containing tamoxifen citrate sterically stabilized with mPEG2000-CC-PE**

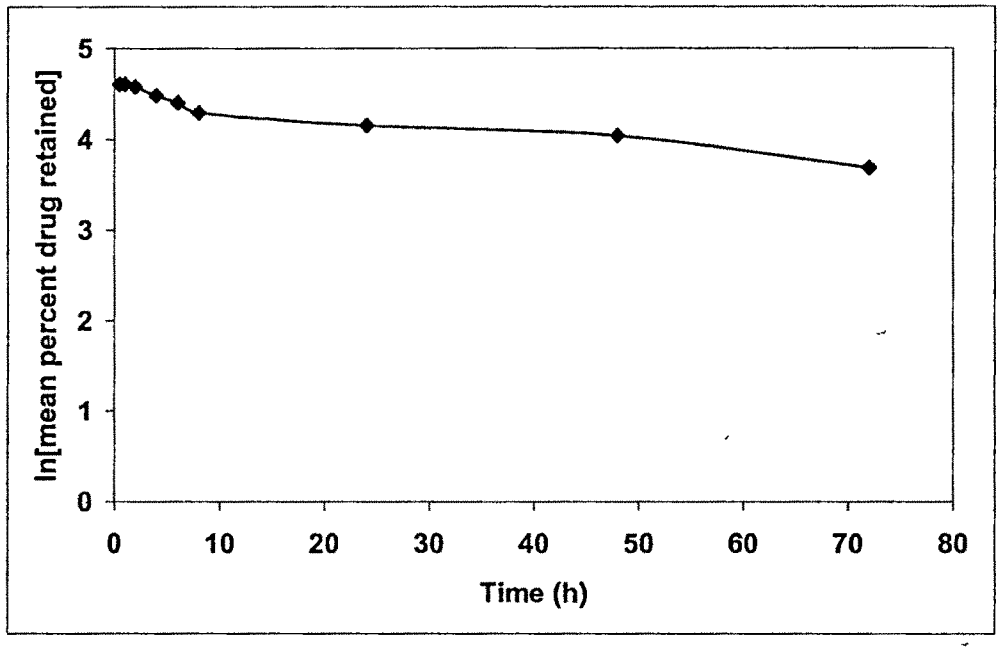



**Figure 6.8** ***In vitro*** **drug release studies of liposomes containing tamoxifen citrate sterically stabilized with mPEG5000-IC-PE**

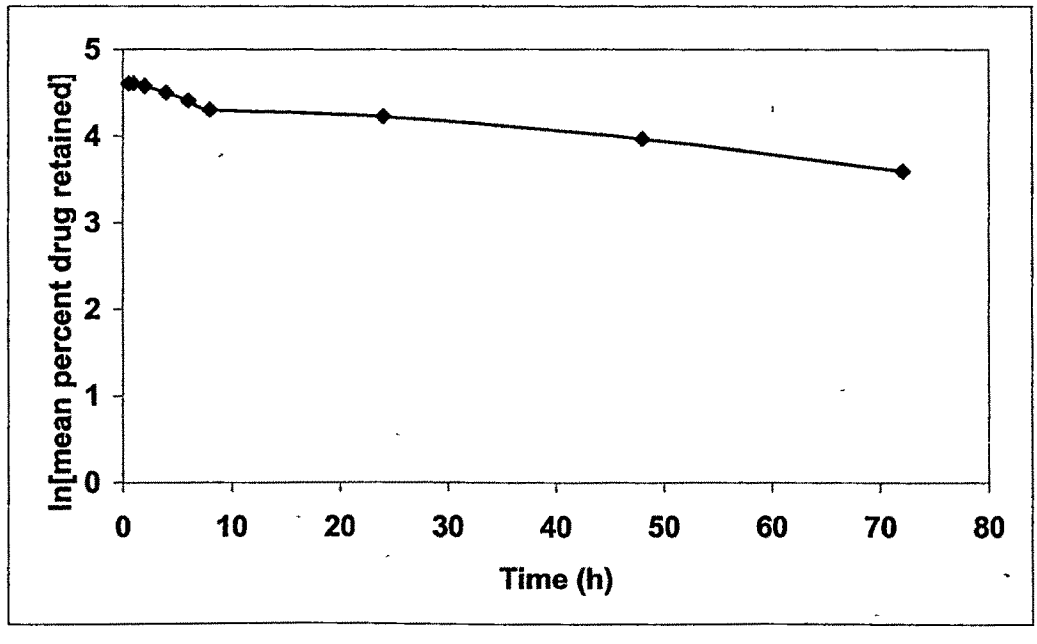


**Figure 6.9** ***In vitro*** **drug release studies of conventional liposomes of cytarabine**

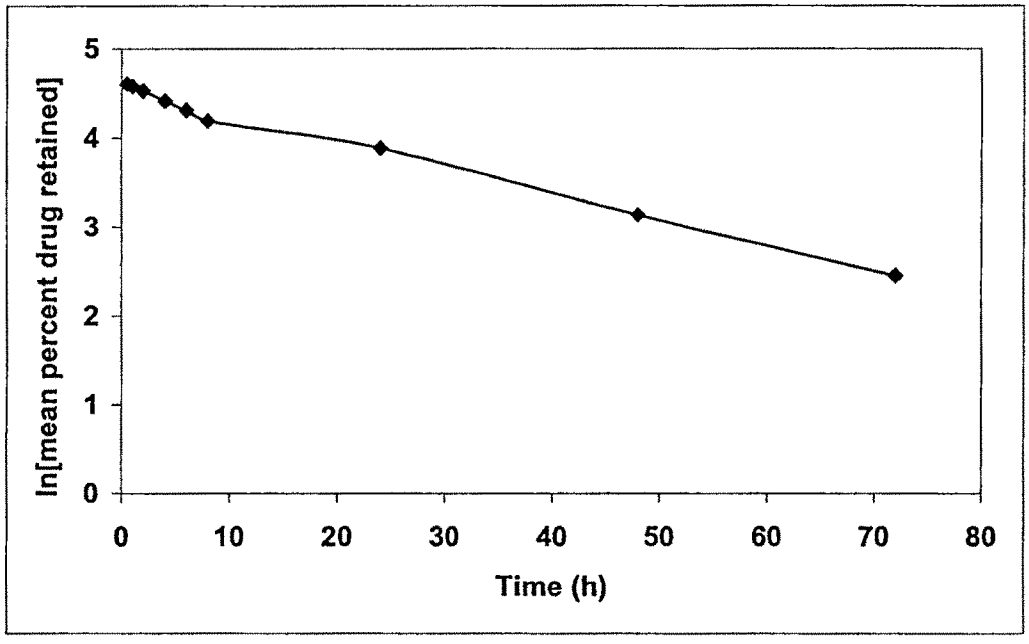



**Figure 6.10** ***In vitro*** **drug release studies of liposomes containing cytarabine sterically stabilized with mPEG5000-CC-PE**

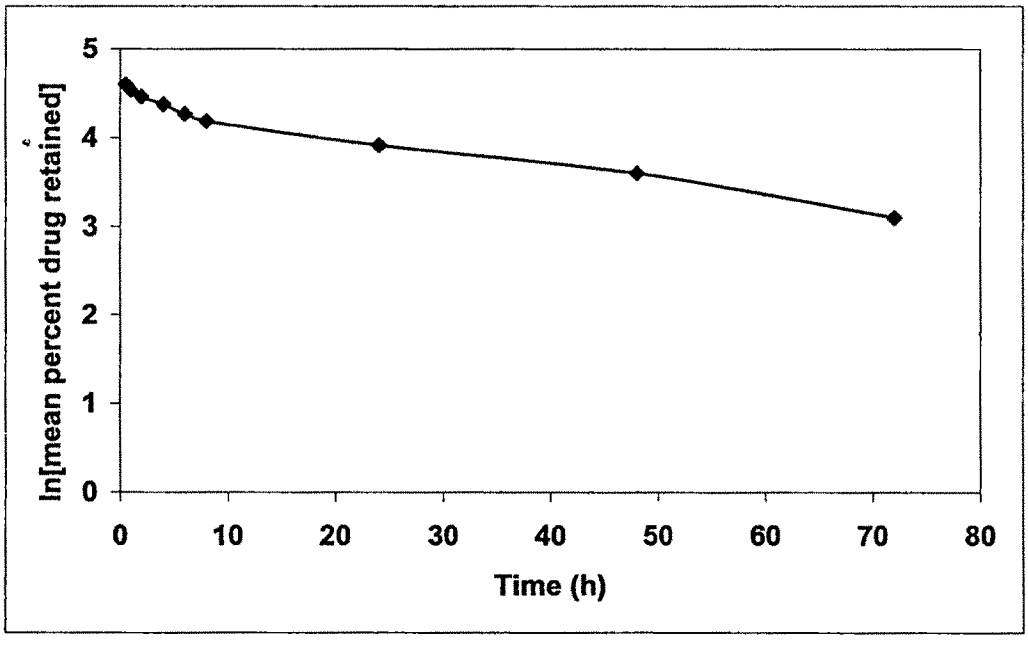


**Figure 6.11 *In vitro* drug release studies of liposomes containing cytarabine sterically stabilized mPEG2000-CC-PE**

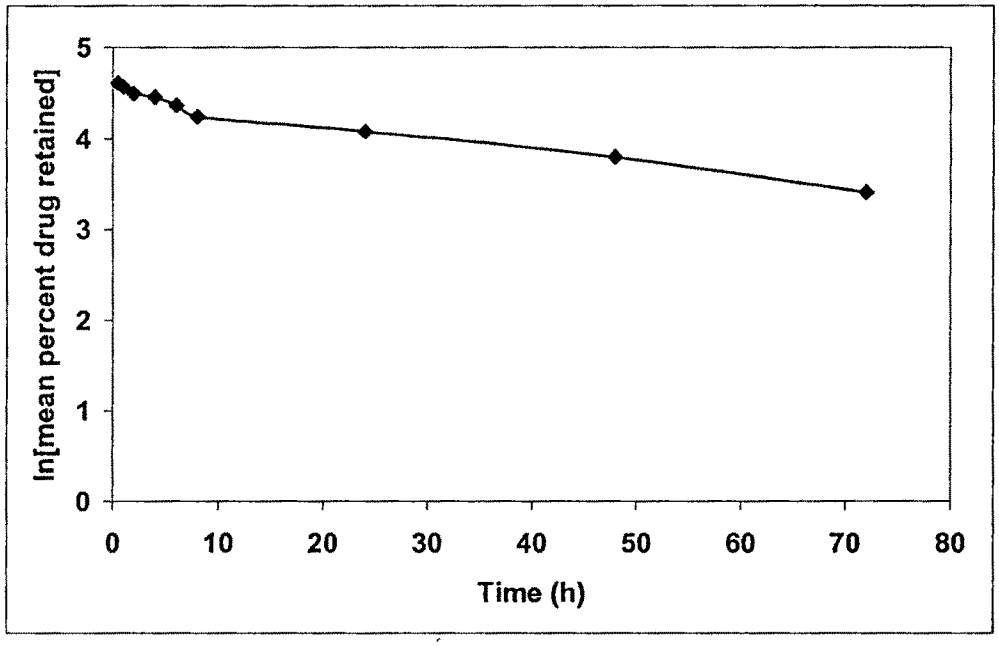
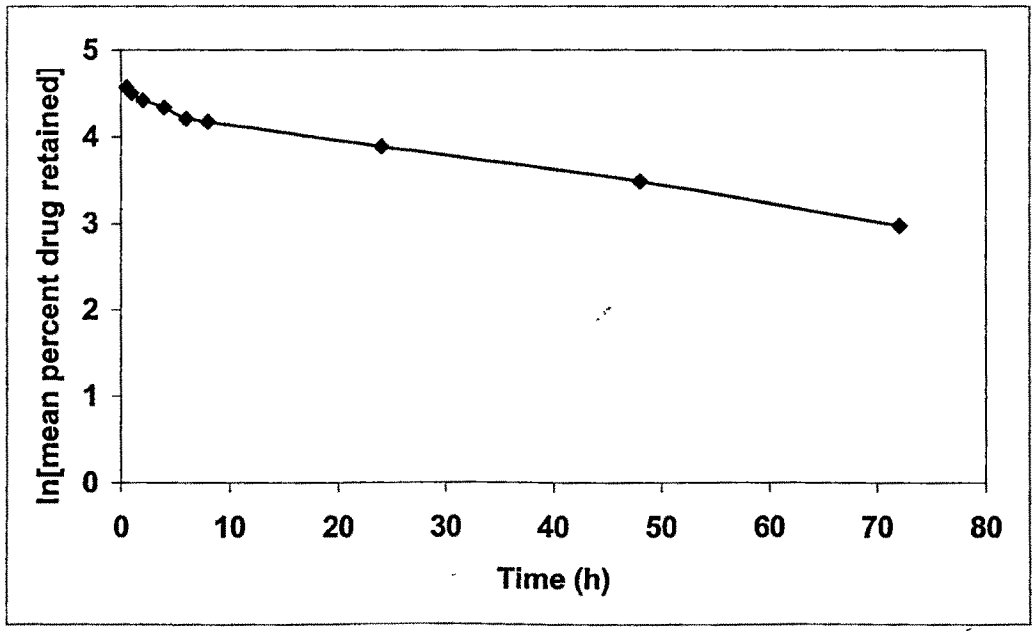



**Figure 6.12 *In vitro* drug release studies of liposomes containing cytarabine sterically stabilized with mPEG5000-IC-PE**



## 6.3 RESULTS AND DISCUSSION

### 6.3.1 METHODOLOGY

Drug release from intact liposomes into the media within which the liposomes are placed (or suspended) is essentially a process of diffusion in a heterogeneous system; the latter made of several phases of water and lipid bilayers in case of multilamellar vesicles.

In principle, the initiation of drug diffusion simply requires setting the driving force (i.e. an electrochemical gradient of the drug from its liposomal location to the external medium) after which the process will proceed until equilibrium is established (or re-established). If the liposomal system had been previously subjected to "cleaning procedures", using semi-permeable matrices, to eliminate unentrapped drug, the electrochemical gradient is already in existence and the diffusion process already operative prior to the experiment. In these studies, separation of unentrapped drug was done using centrifugation, which minimizes the occurrence of the above condition to some extent. To trigger the drug release process sufficiently, a reduction in the drug concentration at the external medium has to be brought about. One of the several means which have been suggested to generate this reduction: precipitation, enzyme-catalysed degradation and dilution of the liposomal system into the medium of choice such as buffer or body fluid (real or simulated), the last one was chosen since it is by far, the easiest and most general means to generate such a drug concentration reduction.

Critical to the successful execution of such studies is the quantitative separation of liposomes and the external medium in aliquots withdrawn from the reaction mixture at designated time points. Classic means for the quantitative separations of particles from their suspension medium, such as centrifugation and filtration are often compromised by drug loss to the filtration matrix or by centrifugation procedures that are too slow with respect to the time that has elapsed between samplings. Additional experimental constraints are the drug and liposome assays. The dilution, which is essential for the onset and maintenance of the gradient, can result

in lipid and drug levels in the separated fractions of the withdrawn aliquots that fall below the limits of detection and/or quantitation.

Elimination altogether of the need for separation together with significant reduction of the risks of falling below detection limits is offered by the use of appropriate dialysis set-ups. The wide variety among available semi permeable dialysis membranes makes it possible to select, according to specifications of the investigated system, a membrane that will not adsorb the drug and will not be a barrier to the drug but at the same time be a complete barrier to the liposomes. Cellulose tubing with a cut off of 12,000 Daltons fulfilled the above criteria and thus was chosen as the membrane for the set up in the present study. In order to simulate the conditions of intravenous administration, phosphate buffered saline, pH 7.4 was chosen as the external medium or the medium of the receptor compartment. The volume of the external medium was fixed at 200ml since this would provide the dilution essential for the onset and maintenance of the electrochemical gradient necessary for drug release without compromising the accuracy of the drug analysis in terms of limits of detection and/or quantitation. The temperature of the external medium was kept at 37°C ± 2°C for simulation of the body conditions. Under these temperature conditions and the amount of time involved (~ 3 days), appreciable evaporative losses are possible from the external medium, which may drastically change the concentration of the drug estimated. In order to minimize the occurrence of this situation, the system was covered with aluminium foil. The contents of the receptor compartment were stirred using a Teflon coated bar magnet to prevent hydrodynamic diffusion layer saturation, which would interfere with drug release. The theoretical approach developed by Eyring and depicted as equation (4) was chosen for analysing the data of drug diffusion from the liposomes. This approach is suitable since it allows drug release from homogeneous (unencapsulated drug) and heterogeneous (liposome-associated and liposome encapsulated drug) systems to be dealt with simultaneously. It yields parameters that allow the direct determination of the fraction of encapsulated drug and of the half-life of drug release (Margalit and Yerushalmi, 1996).

### 6.3.2 GENERAL DISCUSSION

Studies of drug release from conventional and sterically stabilized liposomal systems containing methotrexate, tamoxifen citrate and cytarabine were performed using the above described dialysis set-up. It was expected that such studies would lead to insights regarding the effects of 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. Correlation of the generated data with that obtained from animal studies can identify factors that are instrumental in gaining control of the release. This information can be utilized for further refinement of systems, which can fulfill the aim of providing anticancer therapy with improved therapeutic efficiency.

Tables 6.1 to 6.12 contain the experimental data along with the results of non-linear regression of the data of the release of methotrexate, tamoxifen citrate and cytarabine from conventional 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 6.12. Calculated kinetic parameters for various systems are tabulated in Table 6.13.

Examination of the above data overall reveals that the release of methotrexate, tamoxifen and cytarabine from both 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 $f_l$).

### 6.3.3 RELEASE STUDIES OF CONVENTIONAL LIPOSOMES CONTAINING METHOTREXATE, TAMOXIFEN CITRATE AND CYTARABINE

Table 6.1 (and Figure 6.1) presents the release of methotrexate from conventional liposomes. From the values of the kinetic parameters (Table 6.13), it can be seen that around 31% 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 methotrexate is released at a rate of $0.3318h^{-1}$. 72% of the drug present in the encapsulated form is released at a rate of $0.0186h^{-1}$, 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 for drug release ($t_{1/2}$) from the conventional liposomes of methotrexate is around 37h. This, therefore, indicates that methotrexate containing liposomes have the potential for use as sustained drug delivery systems.

Table 6.5 (and Figure 6.5) depict the results of the release studies conducted for conventional liposomes containing tamoxifen citrate. Around 16% of the total drug associated with the system is in the unencapsulated form (Table 6.13). The reasons given for the presence of free drug in the above liposomes can be attributed to the conventional liposomes containing tamoxifen citrate. The unencapsulated tamoxifen citrate is released at a rate of $0.2563h^{-1}$. 88% 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 for drug release ($t_{1/2}$) from the conventional liposomes of tamoxifen citrate is around 47h. This, therefore, indicates that tamoxifen citrate containing liposomes also have the potential for use as sustained drug delivery systems.

Table 6.9 (and Figure 6.9) represents the results of release studies conducted for conventional liposomes containing cytarabine. Around 14%, of the total drug associated with the system, is in the unencapsulated form as shown in Table 6.13. 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 methotrexate. The high aqueous solubility of cytarabine makes this gradient more pronounced than in case of methotrexate and tamoxifen citrate. 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 $f_l$ = 0.8931$h^{-1}$), 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 cytarabine in the lipidic region. The high rate of release of this unencapsulated fraction (0.2699 $h^{-1}$) points out the hydrophilic nature of cytarabine when compared with the same parameter in case of conventional liposomes containing methotrexate and tamoxifen citrate. The rate of release of the encapsulated fraction is 0.0282$h^{-1}$, the greater rate compared to that for conventional liposomes containing tamoxifen citrate, drawing attention to the greater fluidity of the bilayer in case of conventional liposomes containing cytarabine and to the importance of the hydrophilicity of cytarabine. The half-life for drug release ($t_{1/2}$) from conventional liposomes containing cytarabine is around 25h indicating that these liposomes can be used as sustained drug delivery systems.

### 6.3.4 RELEASE STUDIES OF LIPOSOMES CONTAINING METHOTREXATE, TAMOXIFEN CITRATE AND CYTARABINE STERICALLY STABILIZED WITH POLYETHYLENE GLYCOL DERIVATIVES

Sterically stabilized liposomes of methotrexate were prepared using methoxy polyethylene glycol derivates (mPEG5000-CC-PE, mPEG2000-CC-PE and mPEG5000-IC-PE) and the results of release studies of these liposomes are shown in Tables 6.2-6.4 (and Figures 6.2-6.4) respectively. The presence of these agents in the bilayer brings about a moderate increase in the entrapment of methotrexate with a corresponding slight decrease in the amount of drug present on the surface of the liposomes (the unencapsulated fraction) as compared to the corresponding conventional liposomes. In all probability, this increased entrapment is by virtue of the increase of solubility of methotrexate in the aqueous compartment. The conclusion is well supported by the lower rate of release of encapsulated methotrexate ($k_l$ = 0.0137$h^{-1}$, 0.0123$h^{-1}$ and 0.0143$h^{-1}$ for SML5000-CC-PE, SML2000-CC-PE and SML5000-IC-PE respectively) as compared to that of conventional liposomes ($k_l$ = 0.0186$h^{-1}$) 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 for drug release from these liposomes ($t_{1/2}$ = 50.47h, 56.57h and 48.43h for SML5000-CC-PE, SML2000-CC-PE and SML5000-IC-PE respectively) as compared to that of conventional liposomes ($t_{1/2}$ = 37.29h). The rate of release of free methotrexate from these liposomes is increased almost 2-3 folds compared to that of the conventional liposomes ($k_f$ = 0.4778$h^{-1}$, 0.5670$h^{-1}$, 0.1100$h^{-1}$ and 0.3318$h^{-1}$ in case of SML5000-CC-PE, SML2000-CC-PE, SML5000-IC-PE and ML respectively). The presence of the hydrophilic polyethylene glycol chains on the surface of the liposomes causes an enhancement in the solubility of methotrexate in the immediate exterior. Enhancement of the diffusion gradient is thus brought about and drug rapidly diffuses from the surface of the liposomes to the aqueous environment. The high rate points out to the high hydrophilicity associated with these steric stabilizing agents.

Sterically stabilized liposomes of tamoxifen citrate were prepared using methoxy polyethylene glycol derivates (mPEG5000-CC-PE, mPEG2000-CC-PE and mPEG5000-IC-PE) and the results of release studies of these liposomes are shown in Tables 6.6-6.8 (and Figures 6.6-6.8) respectively. The presence of these agents in the bilayer does not bring about change in the entrapment of tamoxifen citrate except in case of STL5000-IC-PE as compared to the corresponding conventional liposomes. The release rate of encapsulated tamoxifen citrate ($k_l$ = 0.0111$h^{-1}$, 0.0102$h^{-1}$ and 0.0114$h^{-1}$ for STL5000-CC-PE, STL2000-CC-PE and STL5000-IC-PE respectively) was not significantly different as compared to that of conventional liposomes ($k_l$ = 0.0148$h^{-1}$), which is possibly due to the hydrophobic nature of the drug due to which the drug stays in the bilayer. 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 for drug release from these liposomes ($t_{1/2}$ = 62.47h, 67.70h and 60.73h for STL5000-CC-PE, STL2000-CC-PE and STL5000-IC-PE respectively) as compared to that of conventional liposomes ($t_{1/2}$ = 46.74h). The rate of release of free tamoxifen citrate from these liposomes is almost similar to that of the conventional liposomes ($k_f$ = 0.2671$h^{-1}$, 0.0807$h^{-1}$, 0.3523$h^{-1}$ and 0.2563$h^{-1}$ in case of STL5000-CC-PE, STL2000-CC-PE, STL5000-IC-PE and TL respectively) except in case of sterically stabilized liposomes containing tamoxifen citrate using methoxy polyethylene glycol (mol wt 2000).

Tables 6.10 –6.12 and Figures 6.10 –6.12 along with Table 6.13 give an insight into the effects of the incorporation of the methoxy polyethylene glycol derivatives on the release of cytarabine from liposomes. Methoxy polyethylene glycol derivates, when included in the bilayer of the liposomes, increases the percentage of unencapsulated drug ($f_f$ = 0.2882$h^{-1}$, 0.2142$h^{-1}$ and 0.2711$h^{-1}$ for SCL5000-CC-PE, SCL2000-CC-PE and SCL5000-IC-PE respectively) as compared to conventional liposomes ($f_f$ = 0.1373$h^{-1}$). 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 slow rate of drug release from the liposomes (entrapped drug)

($k_l$ = 0.0166$h^{-1}$, 0.0142$h^{-1}$ and 0.0184$h^{-1}$ for SCL5000-CC-PE, SCL2000-CC-PE and SCL5000-IC-PE respectively as compared to the conventional liposomes with $k_l$ = 0.0282) and the increase in half life for drug release ($t_{1/2}$ = 41.85h, 48.90h and 37.62h for SCL5000-CC-PE, SCL2000-CC-PE and SCL5000-IC-PE respectively as compared to 24.58h for CL) indicates that cytarabine containing sterically stabilized liposomes have the potential for use as sustained drug 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 sterically stabilizing agents into liposomes of methotrexate, tamoxifen citrate and cytarabine causes a significant increase in the half-life of the drug release 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 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. It is suggested that increasing the rigidity of the liposomal bilayer by using saturated lipids may resolve the problem of altered permeability in case of cytarabine containing liposomes. 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 (Tables 6.1-6.12) and the fulfilment 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.

## 6.4 REFERENCES

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