Chapter 7

In vitro drug release Studies

7.1 INTRODUCTION

Sustaining a unidirectional flux of the drug from the novel drug delivery 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 particulate suspensions, storage should be at high particulate concentrations.

The objectives of drug release studies are:

a) Physicochemical characterizations of the systems
b) Various aspects of system optimization such as the selection of liposome or microbubble 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, microbubbles and drug (each, separately) dilutions that the system is anticipated to undergo. If the selected dosage form is surface modified liposomes, the processes of drug release add some concerns that are of interest both academic and industrial. Kinetic studies can be used to determine whether such losses are significant and to evaluate the extent 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. 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.

7.2 EXPERIMENTAL

Disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium lauryl sulphate sodium chloride of analytical reagent (AR) grade (S.D. Fine Chem Ltd. Boisar, Thane). Distilled Water prepared in laboratory.

7.2.1 Solution

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

7.2.2 Apparatus

Magnetic stirrer (Remi Scientific Equipments, Mumbai); Teflon coated magnet (SD Fine Chem Ltd., Biosar, Thane); open-ended cellulose dialysis bag (cut off 12,000, Sigma Diagnostics, USA) having width of 35mm and diameter 21 mm.

7.2.3 In vitro drug release from liposomes and microbubbles

One end of dialysis bag (10 cm long) was tied up with thread and soaked overnight with PBS. It ensured that there would not be any leakage of the content from inside the bag before using. The bag was filled with liposomal or microbubble suspension containing 5 mg of drug (donor compartment) and suspended in 200 ml of PBS or PBS containing 0.5 %w/v of SLS (receptor compartment) in case of FLT and 6-MP in beaker, respectively. The temperature was maintained at 37 ± 0.5°C and contents were stirred using a Teflon coated magnetic bar. At predetermined intervals of time, 5 ml of aliquots were withdrawn from receptor compartment and subjected to analysis using method described in Chapter 3 as well as the same volume was replaced by fresh dissolution medium. All the experiments were repeated three times and the average values were taken.

7.3 RESULTS AND DISCUSSION

7.3.1 In vitro drug release from liposomes

Cellulose dialysis bag with a cut off of 12, 000 Daltons was selected because it will not adsorb the drug and will not be a barrier to the drug but at the same time be a complete barrier for liposomes. In order to simulate intravenous administration, PBS pH 7.4 was chosen as the external medium and the temperature was maintained 37 ±

0.5°C to simulate body condition. The volume of external medium taken was 200 ml 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. The contents were stirred using Teflon coated magnetic bar to prevent hydrodynamic diffusion layer saturation, which would interfere with drug release. The release of drug from liposomes depends on the nature of drug and lipid used in liposomes. In principle, the initiation of drug diffusion simply requires setting the driving force (an electrochemical gradient of the drug form liposomes to external medium) after which the process will proceed until equilibrium is established. Figure 7.1 and 7.2 present the % drug release of drug from pure drug and their encapsulated liposomes for FLT and 6-MP, respectively.

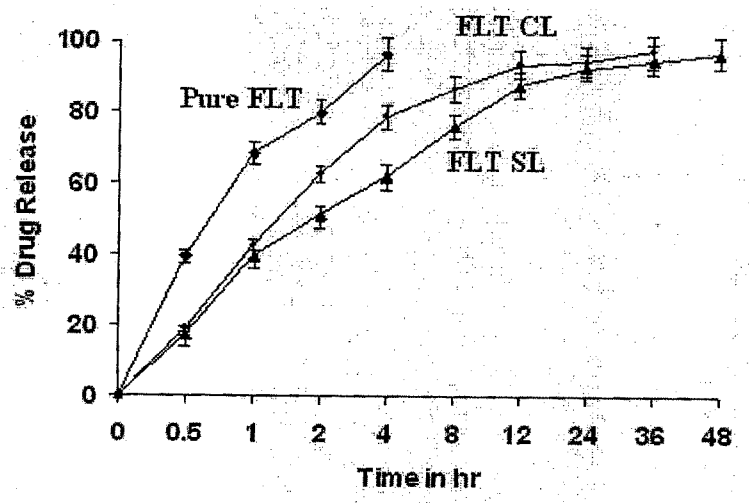

Figure 7.1: The drug release kinetic of pure flutamide and its encapsulated liposomes

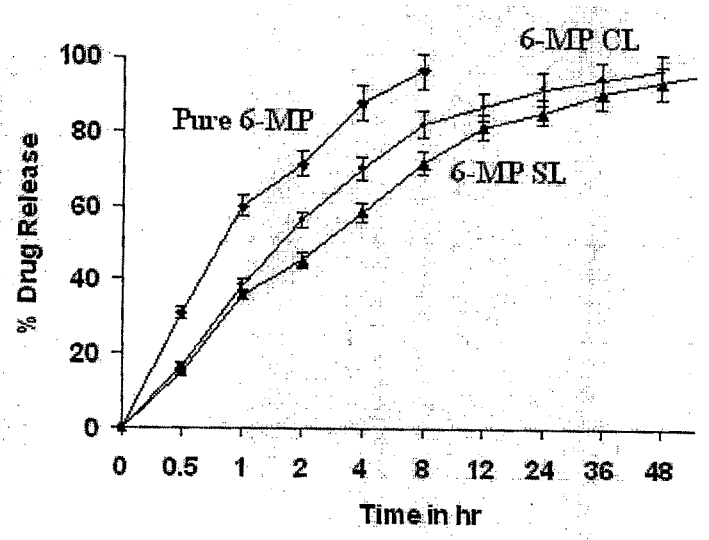

Figure 7.2: The drug release kinetic of pure 6-Mercaptopurine and its encapsulated liposomes

It was found from the drug release profiles of various flutamide dosage forms that 40 % and 30% of the drug released in just 30 min and it took 4 hr to release 96% of drug from pure FLT and 6-MP, respectively. FLT CL showed 42% of drug release initially in 1 hr. The CL showed release of drug for 36 hr while SL showed the release of drug for more than 48 hr. The drug release from FLT SL is slower than that of CL.

The data reveals that the release of drugs from encapsulated liposomes follows a biphasic pattern. In all cases, the rapid release of drug occurs initially followed by subsequent, slow and sustained release lastly. The large surface area and the enrichment of drug present in the outermost bilayer of the liposomes, which did not traverse any membrane before encountering the external medium and acted similar to free drug is responsible for initial phase. As part of the preparation procedure, liposomes are repeatedly centrifuged before storage, to remove unentrapped drug. This procedure sets up a moderate electrochemical gradient during storage. Drug from interior of the liposomes diffuses to the external environment or on surface in response to this gradient. The latter part of release profile would be due to the release of the encapsulated fraction of the drug. Initial release of 6-MP (38 % in 1 hr) from liposomes was due to the same large surface area and amount of drug present in outermost layer. This portion of the drug was associated with the bilayer due to its intrinsic hydrophobicity and the presence of PC in the bilayer, which enhanced the solubility of 6-MP in the lipidic region. The drug release from the 6-Mercaptopurine CL exhibited for 48 hr and for SL 54 hr, respectively. More than 48 hr in vitro release of 6-MP from liposomes indicated that liposomes could successfully control 6-MP release for prolonged time.

Table 7.1 and 7.2 also represent results of release studies of drug from pure drug and its liposomes for FLT and 6-MP, respectively. The presence of steric stabilizing agent in the bilayer makes the liposomal surface hydrophilic and corresponding slight decrease in the entrapment of drug present on the surface of the liposomes as compared to the corresponding conventional liposomes. The presence of hydrophilic polyethylene glycol chains on the surface of the liposomes causes the enhancement in the solubility of drugs in the immediate exterior and causes delay in the release of drug from these liposomes.

Table 7.1: The % drug released from pure FLT and its encapsulated liposomes

Time in hr	% Drug released		
	Pure FLT	**FLT CL**	**FLT SL**
0	0	0	0
0.5	39.4±1.515	19.09±1.554	17.26±0.645
1	68.32±2.902	42.73±2.914	39.62±1.524
2	79.68±3.333	62.57±3.372	51.27±2.213
4	96.24±4.563	78.55±4.511	62.32±2.678
8		86.5±4.602	75.60±3.325
12		93.24±3.724	87.24±3.684
24		94.08±4.23	92.30±4.261
36		96.79±4.394	94.23±4.521
48			96.22±4.628

Table 7.2: The % drug released from pure 6-MP and its encapsulated liposomes

Time in hr	% Drug released		
	Pure 6-MP	**6-MP CL**	**6-MP SL**
0	0	0	0
0.5	31.08±1.864	16.7815±0.654	15.281±0.645
1	60±3.251	38.41775±1.58	35.643±1.524
2	71.36±3.251	56.26±2.241	45.286±2.123
4	87.92±3.682	70.24±3.12	58.342±2.678
8	96.342±4.678	82.24±3.645	71.625±3.325
12		86.71±3.724	81.2642±3.584
24		91.77±4.23	85.324±3.684
36		94.48±4.394	90.248±4.261
48		96.34±4.365	93.241±4.521
54			96.681±4.628

7.3.2 In vitro drug release from microbubbles

Table 7.3: The % drug release of FLT from different types of microbubbles

Time in hr	% Drug released		
	MBs	**GFMs**	**AALs**
0	0	0	0
0.5	36.546 ± 1.22	28.365 ± 1.029	32.624 ± 1.489
1	58.325 ± 2.159	42.251 ± 1.796	54.248 ± 2.529
2	70.955 ± 2.938	53.254 ± 2.089	68.215 ± 3.41
4	86.522 ± 3.99	64.854 ± 2.413	76.352 ± 3.59
8	96.95 ± 4.596	75.364 ± 3.026	88.692 ± 4.516
12	-	87.212 ± 3.423	92.215 ± 4.727
24	-	90.257 ± 4.033	96.257 ± 4.788
36	-	94.251 ± 4.117	96.582 ± 4.791
48	-	96.35 ± 4.297	-

Table 7.3 and 7.4 present the % FLT and 6-MP released from different types of microbubbles after exposure of 0.5 MHz ultrasound frequency for 30 sec, respectively. It was found from the drug release profiles of various FLT and 6-MP microbubbles that 25 % of the drug released in just 30 min. FLT and 6-MP GFMs showed 40 % drug release initially in 1 hr, while % drug released from MBs was 60%.

Table 7.4: The % drug release of 6-MP from different types of microbubbles

Time in hr	% Drug released		
	MBs	GFMs	AALs
0	0	0	0
0.5	39.756 ± 1.756	26.014 ± 1.254	36.975 ± 1.568
1	61.535 ± 2.8	39.9 ± 1.416	58.599 ± 2.612
2	74.165 ± 3.37	50.903 ± 2.047	72.566 ± 3.258
4	89.732 ± 4.327	62.503 ± 2.39	80.703 ± 3.84
8	96.658 ± 4.56	73.013 ± 2.986	91.043 ±4.365
12	-	84.861 ± 3.589	93.622 ± 4.425
24	-	89.384 ± 3.993	95.362 ± 4.562
36	-	93.658 ± 4.058	96.933 ± 4.638
48	-	96.004 ± 4.253	-

Figure 7.3 and 7.4 indicated that The GFMs showed release of drug for 48 hr while AALs showed the release of drug for more than 36 hr in case of FLT and 6-MP. PLGA present in GFMs may be responsible for sustained release of both drugs.

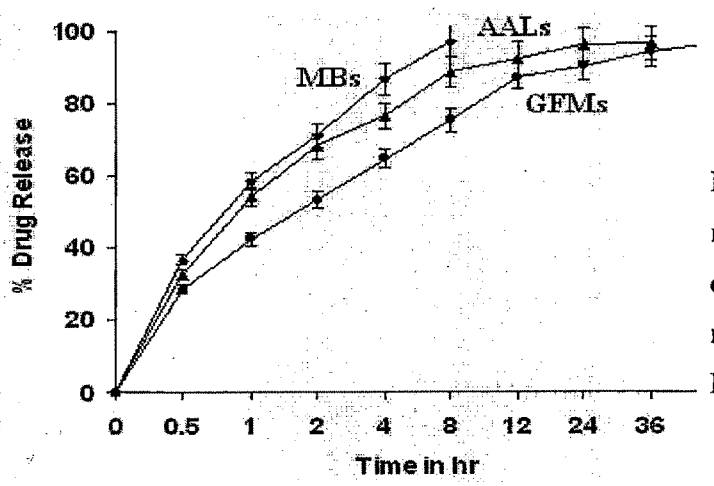

Figure 7.3: The drug release kinetic of different types of microbubbles of 6-MP

The slower release rate of the drug from PLGA could be due to hydrophobic interaction between the drug and the polymer as it has higher lactide content. The drug release from GFMs is slower than that of MBs because MBs have gas core stabilized by monolayer of lipid, which may damage after exposure of ultrasound. The data reveals that the release of drugs from GFMs and AALs follows a biphasic pattern, too.

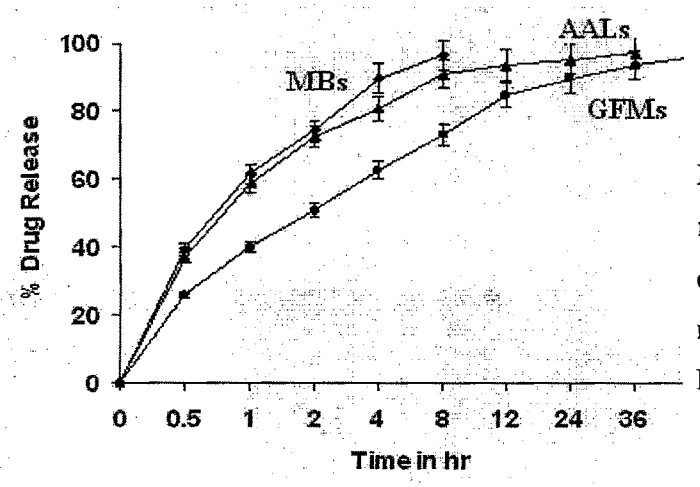

Figure 7.4: The drug release kinetic of different types of microbubbles of 6-MP

In all cases, the rapid release of drug occurs initially followed by subsequent, slow and sustained release lastly. The large surface area and the enrichment of drug present on the surface of GFMs or in the outermost bilayer of the AALs, which did not traverse any membrane before encountering the external medium and acted similar to free drug. More than 36 hr in vitro release of FLT and 6-MP from AALs and GFMs type of microbubbles indicated that both could successfully control FLT and 6-MP release for prolonged time from respective microbubbles.

7.4 CONCLUSION

Considering the above observations made with liposomes and microbubbles, following conclusion can be drawn:

All the batches of liposomes (conventional and sterically stabilized) and AALs have the potential for sustained drug delivery. Incorporation of sterically stabilizing agent into FLT and 6-MP liposomes and AALs causes a significant increase in the half-life of the drug release in comparison to conventional liposomes and other types of

microbubbles. It is suggested that increasing the rigidity of the liposomal bilayer by using saturated lipids may resolve the problem of altered permeability. These observations also emphasized the importance of considering the hydrophobicity or hydrophilicity of the drug in determining membrane composition. It was shown here from % drug released from GFMs and AALs that an appropriate delivery system could be designed based on the physical properties of the drugs and using the existing delivery system.