CHAPTER 8

STABILITY STUDIES

- Leakage rate study for Rifampicin Niosomes

- Leakage rate study for Gentamicin Niosomes

- Results

- Discussion

8.1 Leakage rate study for Rifampicin Niosomes:

Stability Studies were conducted for optimized formulations Rifa 8k2 and after they seemed promising following invitro and invivo studies The Niosomal batches (Rifa $8k_2$) were prepared as described in section 5.1 2.6. Prepared Niosomes were studied for the leakage of drug and size enlargement at 5°C (refrigerated conditions) and ambient Temperatures. The study was carried out for 60 days and drug leakage from the niosomes was evaluated at definite time intervals. The study was done in triplicate for every duration. The result of the studies are shown in Table No.35 wherein the percentage of drug retained in niosomes are shown in Table No.36 for both the batches after different time intervals.

8.2. Leakage rate study for Gentamicin Niosomes:

Stability Studies were conducted for the optimized formulations, Genta 8k2 which showed promising results for targeting to the lungs following invitro and invivo studies. The Niosomal batches(Genta $2j_2$) were prepared as described in section 5.1.2.6. Prepared Niosomes were studied for leakage of drug and size enlargement at 5°C (refrigerated conditions) and ambient Temperatures. The study was

carried out for 60 days and drug leakage from the niosomes was evaluated at definite time intervals. The study was conducted in triplicate for each time points. The results of the studies are shown in Table No.35 wherein the percentage of drug retained in niosomes are shown in Table No.36 for both the batches after different time intervals.

8.3 RESULTS

TABLE NO.35

LEAKAGE RATE STUDY FOR RIFAMPICIN NIOSOMES AND GENTAMICIN NIOSOMES

Batch No.	Parameter	Time									
		1	2	4	5	16	32	40	48	56	60
Rifa $8k_2$ 5°C	Cumulative % release	-	-	-	1.25	1.82	4.02	4.75	10.03	13.04	13.04
Rifa $8k_2$ A.T.	Cumulative % release	-	2.89	7.54	10.36	20.06	32.1	46.02	70.41	73.94	77
Genta $2j_2$ 5°C	Cumulative % release	-	-	2.71	3.99	4.72	6	11.01	13.01	13.99	18
Genta $2j_2$ A.T.	Cumulative % release	-	3.98	10.27	12.72	25.03	32.1	58.31	67.98	72.07	78.03

TABLE NO. 36

LEAKAGE RATE STUDY OF RIFA $8K_2$ AND GENTA $2j_2$ NIOSOMES (% DRUG RETAINED)

Batch No.	Parameters	Time									
		1	2	4	5	16	32	40	48	56	60
Rifa $8k_2$ 5°C	% drug retained	-	-	-	98.75	98.18	95.98	95.25	89.97	86.96	86.96
	1-F	-	-	-	0.125	0.0182	0.0402	0.475	0.1003	0.1304	0.1304
Rifa $8k_2$ A.T.	% drug retained	-	97.11	92.46	89.64	79.94	67.94	53.98	29.59	26.06	23
	1-F	-	0.0289	0.0754	0.1036	0.2006	0.3206	0.4602	0.7041	0.7394	0.7700
Genta $2j_2$ 5°C	% drug retained	-	-	97.29	96.01	95.28	94	88 99	86.99	86 01	82
	1-F	-	-	0.0271	0.0399	0.0472	0.0600	0.01101	0.1301	0.1399	0.1800
Genta $2j_2$ A.T.	% drug retained	-	96.02	89.73	87.28	74.97	67.9	41.69	32.02	27.93	21.97
	1-F	-	0.0398	0.1027	0.1272	0.2503	0.321	0.5831	0.6798	0.7207	0.7803

F - fraction drug leakage.

Rifa 8k2 - 5°C

MODEL : TWO COMPARTMENT - INTRAVENOUS

No.	Time	Actual	Calculated	% Deviation
1	8	.0125	.0125	.3091
2	16	.0182	.0181	.5185
3	32	.0402	.0383	5.0579
4	40	.0475	.0557	-14.6509
5	48	.1003	.081	23.8737
6	56	.1304	.1178	10.6641
7	60	.1304	.1422	-8.2747

A = .0007
ALPHA = .0384
B = .0078
BETA = .0474
R SQUARE = -4.544
SSE = .0864
DATA POINTS = 4 3
DOSE = 1

(1) KEL = .0474
(2) HALF-LIFE = 14 6209
(3) VOLUME = 116.5522
(4) K12 = .0002
(5) K21 = .0392
(6) AUC = .1846

GENTA 2J2 - 5°C

MODEL : TWO COMPARTMENT - INTRAVENOUS

No.	Time	Actual	Calculated	% Deviation
1	4	.0217	.03	-9.8118
2	8	.0399	.034	17.5246
3	16	.0472	.0434	8.6994
4	32	.06	.0715	-16.1134
5	40	.1101	.0921	19.5678
6	48	.1301	.1188	9.5459
7	56	.1399	.1534	-8.8189
8	60	.18	.1745	3.1559

A = .0206
ALPHA = .0338
B = .006
BETA = .0181
R SQUARE = -4.6046
SSE = .122
DATA POINTS = 4 4
DOSE = 1

(1) KEL = .0181
(2) HALF-LIFE = 38.3014
(3) VOLUME = 37.5765
(4) K12 = .002
(5) K21 = .0216
(6) AUC = .9408

Fig. No.21

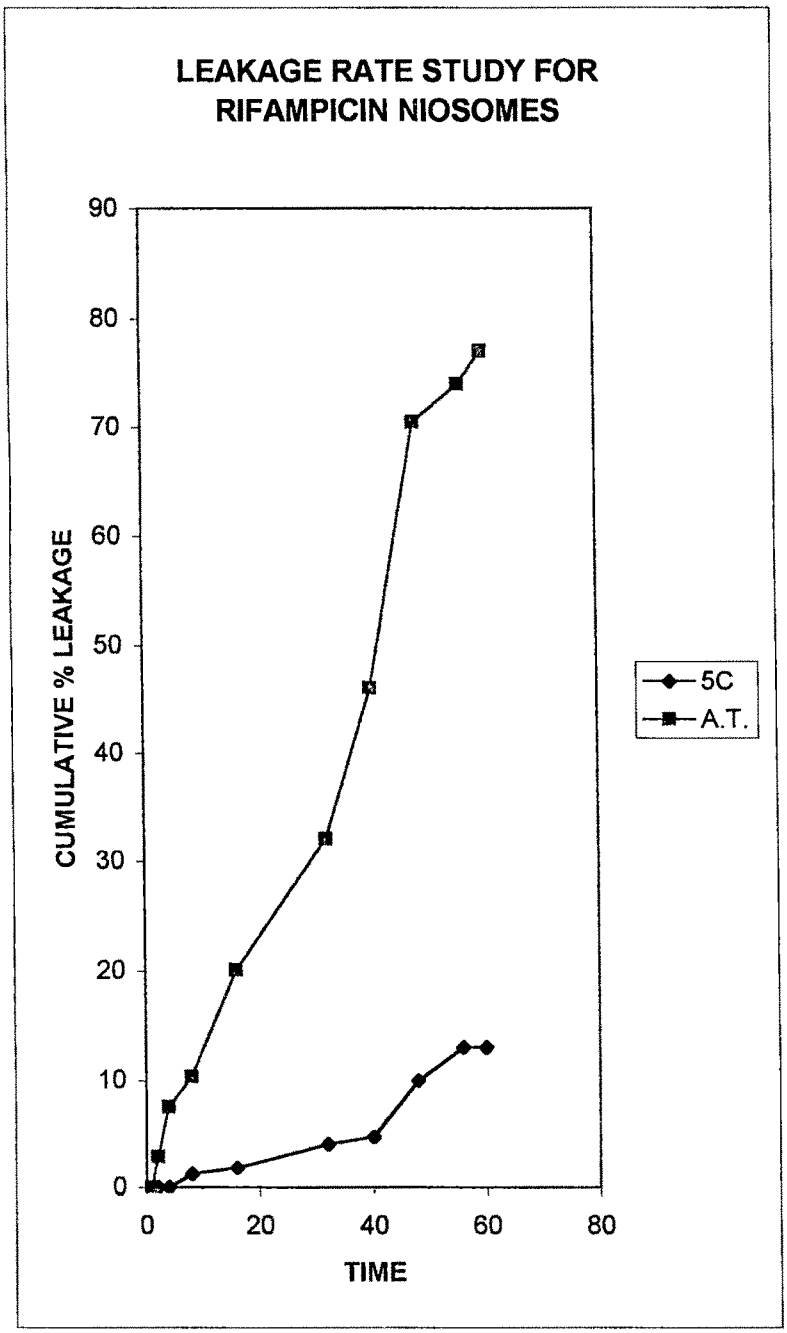
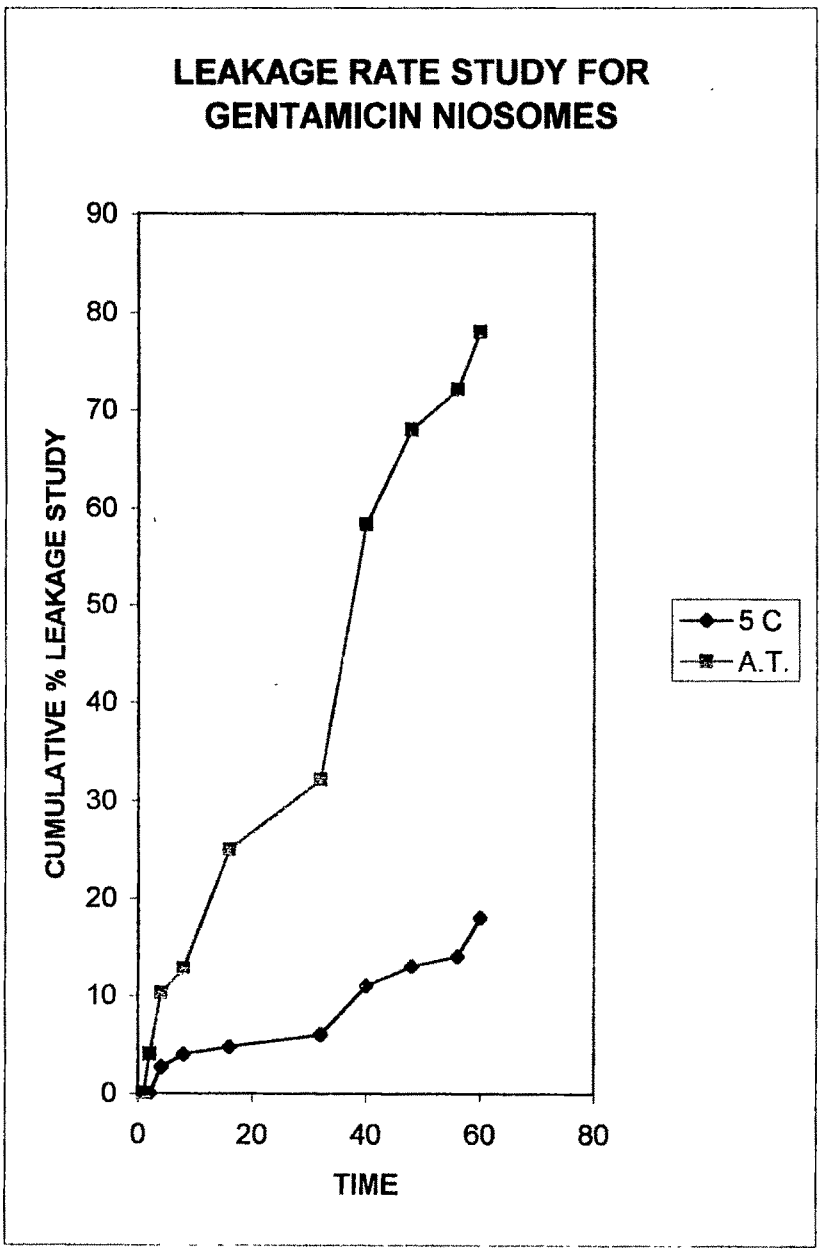

LEAKAGE RATE STUDY FOR RIFAMPICIN NIOSOMES
CUMULATIVE % LEAKAGE
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
TIME
5C
A.T.

Fig. No.22


LEAKAGE RATE STUDY FOR GENTAMICIN NIOSOMES
CUMULATIVE % LEAKAGE STUDY
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
TIME
5 C
A.T.

8.4 DISCUSSION

Niosomes were evaluated for stability with respect to drug leakage. Niosomes prepared using Span 60 and cholesterol were stored in aqueous suspension form at two different temperature viz. refrigeration temperature (5°C) and ambient temperature (25°C to 35°C). The extent of drug leakage from these niosomes was determined upto a period of 60 days after removing aliquots at various time intervals. The aliquots were gel filtered and amount of drug leakage was determined by analyzing the eluent.

At 5°C the efflux of Rifampicin from niosomes prepared by thin film hydration method, exhibit biphasic drug release profile, an initial slow release phase from first day to 40th day. This may be due to the presence of saturated periferal layer in the beginning followed by diffusion of drug through the lipid layers. This pattern of release was quantitatively assessed using biphasic equation $C=A.e^{\alpha t} + B.e^{\beta t}$ which yielded the value of A, B, α and β as 0.0007, 0.0078, -0 0384 and -0 0474 respectively.

At ambient temperature the efflux of Rifampicin from niosomes exhibit monopharic drug release pattern. The rate of drug leakage was determined by monophasic equation. The leakage rate calculated was 0.0025 day^{-1} for Rifampicin Niosomes at 5°C and 0.013 day^{-1} at

ambient temp. at the end of study. This leakage rate of the drug from the niosomes was calculated from the curve plotted against fraction of the drug remaining (1-F) vs time in days.

Niosomes prepared using Tween 60 and cholesterol were stored in aqueous suspension form at two different temperature viz. refrigeration temperature (5°C) and ambient temperature (25°C to 35°C). The extent of drug leakage from these niosomes was determined upto a period of 60 days after removing aliquots at various time intervals The aliquots were dialysed and amount of drug leakage was determined by analyzing the eluent

At 5°C the efflux of Gentamicin Sulphate from niosomes prepared by thin film hydration method, exhibit biphasic drug release profile, an initial slow release phase from first day to 40th day. This may be due to the presence of saturated periferal layer in the beginning followed by diffusion of drug through the lipid layers. This pattern of release was quantitatively assessed using biphasic equation $C=A.e^{-\alpha t} + B.e^{-\beta t}$ which yielded the value of A, B, α and β as 0.0206, 0.006, 0.0338 and 0.0181 respectively.

At ambient temperature the efflux of Gentamicin sulphate from niosomes exhibit monopharic drug release pattern. The rate of drug leakage was determined by monophasic equation. The leakage rate calculated was 0.0025 day $^{-1}$ for Gentamicin Niosomes at 5°C and 0 0126 day $^{-1}$ at ambient temperature at the end of study. This leakage rate of the drug from the niosomes was calculated from the curve plotted against fraction of the drug remaining (1-F) vs time in days.