CHAPTER 6

STABILITY TESTING OF LIPOSOMAL DRY POWDER INHALER FORMULATIONS

6.1 INTRODUCTION

As industrially produced liposomes will reach the patient only after a prolonged period of time, the liposome formulation should not change its characteristics or lose the associated drug during storage or transport. In general, a shelf life of at least one year is a minimum prerequisite for a commercial product. Attention has been focused on two processes affecting the quality and therefore acceptability of liposomes (Talsma et al, 1993). First, the encapsulated drugs can leak from the vesicles into the extra liposomal compartment. Second, liposomes can aggregate and or fuse, forming larger particles. Both these processes change the disposition of the drug in vivo and thereby presumably affect the therapeutic index of the drug involved. Other physical parameters may also change drugs storage like hydrolysis of phospholipid causes the formation of fatty acids and lysophopholipids (Grit et al, 1993). Though under dehydrated storage, there is least possibility of the formulation to encounter hydrolytic degradation. Another aspect to consider is liposome oxidation (Frokjaer et al, 1984). Oxidation of unsaturated phospholipids and cholesterol may be initiated mainly by the action of light and heavy metals. Several options are available to inhibit peroxidation of lipids within membranes by addition of metal chelators such as EDTA, protection from light, oxygen free atmosphere and the addition of α - tocopherol and low temperature storage (Mowri et al, 1984).

Stability is generally considered as chemical stability of drug substance in a dosage form, however, the performance of a drug when given as a liposomal dry powder inhaler system is not only dependent upon the content of the drug substance, but also reproducible in vivo performance of the formulations. Drugs under study were considered chemically stable so the study can be focused on monitoring the drug leakage from liposomes. The stability protocol was designed as per ICH guidelines (Singh et al, 1999) for countries falling under zone III (hot, dry) and zone IV (very hot, humid).

6.2 METHOD

Comparative stability studies were carried out of the potential LDPI formulations at accelerated (40°C±2°C, 75±5% RH), intermediate storage (30°C ± 2°C, 65 ± 5 % RH controlled room temperature (25°C ± 2°C) conditions up to one year. LDPI formulations containing 1000 µg INH and 500 µg RFP were filled into gelatin capsule shells (Size "2"). These capsules were packed in HDPE bottles under nitrogen cover

and the bottle was sealed with PVC coated aluminum foil. The bottles also contained silica bags as dehumactant and were resealed with flush of nitrogen after each sampling. Set of 50 capsules from a batch were filled in the HDPE bottles for each condition. The study was done with three batches of same composition.

During sampling, one bottle containing 50 capsules was withdrawn at definite time interval, rehydrated with distilled water for 30 minutes. The LDPI formulations were also examined visually for the evidence of cacking and discoloration. The content of the capsule are tested for Assay, degradation, water content, PDR, emission and FPF. For INH, the analysis of drug content (Chapter 3, Section 3.4.3.6). For RFP, the (Chapter 3, Section 3.4.4.6). The stability results are summarized in Tables 6.1 and 6.2 (INH) and Table 6.3 and 6.4 (RFP) and PDR, liposomal mean size and FPF profiles on stability are shown in figures 6.1 and 6.2 (for INH) and 6.3 and 6.4 (for RFP).

6.3 STATISTICAL ANALYSIS

Three batches of each formulations was evaluated three times, data of nine experiments are expressed as Mean ± SEM. The data were compared using ANOVA and student's t-test and difference larger than the value at $p<0.05$ were considered significant.

Table 6.1 Stability data of INH70 LDPI formulation.

Stability conditions INH70	Description	Assay (%)	Degradation (%)	Water content	Percent drug retained	Liposomal size (VMD) (μm)	Emission (%)	Fine Particle Fraction (FPF)
Initial	White free flowing powder	104.23 ± 2.44	nil	5.6 ± 0.8	101.26 ± 3.42	2.75 ± 0.23	78.9 ± 2.0	35.9 ± 0.8
40°C 75% RH								
1M	White free flowing powder	102.03 ± 4.51	nil	5.3 ± 0.6	91.12 ± 3.91	2.63 ± 0.21	77.4 ± 2.3	30.7 ± 0.9
2M	White free flowing powder	98.33 ± 3.42	nil	5.7 ± 0.4	85.36 ± 4.51	3.55 ± 0.32	78.7 ± 3.1	27.4 ± 0.6
3M	White free flowing powder	98.43 ± 4.25	nil	4.9 ± 0.9	78.34 ± 4.46	6.24 ± 0.43	79.6 ± 2.4	23.7 ± 0.6
6M	Off-white free flowing powder	99.43 ± 3.32	0.52	5.2 ± 0.5	66.46 ± 5.37	8.75 ± 0.33	77.4 ± 2.6	15.6 ± 0.8
30°C 65% RH								
1M	White free flowing powder	105.33 ± 3.42	nil	5.2 ± 0.5	99.45 ± 4.36	2.65 ± 0.22	80.1± 3.5	34.4 ± 0.6
2M	White free flowing powder	103.26 ± 3.51	nil	4.8 ± 0.6	100.66 ± 4.51	3.12 ± 0.32	77.8 ± 3.4	34.8 ± 0.7
3M	White free flowing powder	100.26 ± 2.94	nil	5.5 ± 0.7	102.24 ± 3.32	2.85 ± 0.27	81.4 ± 2.8	35.2 ± 0.6
6M	White free flowing powder	99.36 ± 2.64	nil	5.5 ± 0.4	100.36 ± 4.46	2.66 ± 0.33	79.7 ± 3.4	32.8 ± 0.7
9M	White free flowing powder	101.20 ± 3.45	nil	5.3 ± 0.6	99.36 ± 3.92	3.45 ± 0.34	77.9 ± 2.9	33.8 ± 0.5
12M	White free flowing powder	100.43 ± 4.47	nil	5.2 ± 0.7	98.35 ± 4.23	3.72 ± 0.24	76.7 ± 3.3	31.2 ± 0.6
25°C (CRT)								
3M	White free flowing powder	101.21 ± 2.74	nil	5.3 ± 0.2	100.21 ± 3.02	2.64 ± 0.23	82.4 ± 2.4	34.2 ± 0.5
6M	White free flowing powder	103.45 ± 4.23	nil	4.7 ± 0.4	103.51 ± 3.22	2.55 ± 0.34	81.5 ± 3.4	34.8 ± 0.7
9M	White free flowing powder	102.61 ± 3.79	nil	5.4 ± 0.7	101.56 ± 4.42	3.25 ± 0.26	79.5 ± 2.6	35.4 ± 0.8
12M	White free flowing powder	101.65 ± 5.34	nil	5.7 ± 0.4	100.36 ± 4.46	3.45 ± 0.33	78.5 ± 3.2	34.5 ± 0.7

Table 6.2 Stability data of INH71 LDPI formulation.

Stability conditions INH71	Description	Assay (%)	Degradation (%)	Water content	Percent drug retained	Liposomal size (VMD) (μm)	Emission (%)	Fine Particle Fraction (FPF)
Initial	White free flowing powder	99.57 ± 2.54	nil	5.9 ± 0.9	99.27 ± 3.62	2.93 ± 0.30	82.4 ± 1.7	32.2 ± 0.8
40°C 75% RH								
1M	White free flowing powder	103.12 ± 3.57	nil	5.4 ± 0.5	95.12 ± 5.71	2.63 ± 0.21	84.4 ± 2.1	32.7 ± 0.5
2M	White free flowing powder	100.23 ± 3.72	nil	5.8 ± 0.7	92.12 ± 4.76	2.85 ± 0.41	80.2 ± 4.3	28.4 ± 0.4
3M	White free flowing powder	101.35 ± 3.78	nil	4.9 ± 0.8	85.36 ± 5.47	4.64 ± 0.46	78.5 ± 3.4	24.7 ± 0.7
6M	Off-white free flowing powder	98.14 ± 4.23	0.46	5.6 ± 0.6	78.24 ± 4.38	6.75 ± 0.37	76.3 ± 3.6	14.7 ± 0.6
30°C 65% RH								
1M	White free flowing powder	102.53 ± 4.22	nil	5.7 ± 0.4	103.35 ± 3.36	2.65 ± 0.24	78.1± 3.2	32.3 ± 0.4
2M	White free flowing powder	99.37 ± 4.63	nil	5.8 ± 0.4	98.57 ± 4.72	3.12 ± 0.31	82.8 ± 3.5	33.7 ± 0.6
3M	White free flowing powder	100.62 ± 3.74	nil	5.2 ± 0.7	101.34 ± 3.62	2.85 ± 0.23	75.4 ± 4.8	31.5 ± 0.8
6M	White free flowing powder	103.42 ± 3.54	nil	5.4 ± 0.4	100.66 ± 5.36	3.65 ± 0.13	81.7 ± 3.2	30.5 ± 0.5
9M	White free flowing powder	99.28 ± 4.65	nil	4.9 ± 0.8	98.23 ± 4.79	2.85 ± 0.33	79.5 ± 4.9	29.7 ± 0.7
12M	White free flowing powder	101.35 ± 4.63	nil	5.1 ± 0.6	97.35 ± 4.58	3.65 ± 0.43	77.6 ± 3.4	27.5 ± 0.4
25°C (CRT)								
3M	White free flowing powder	102.15 ± 3.34	nil	5.5 ± 0.4	100.33 ± 3.32	2.25 ± 0.28	77.4 ± 4.4	31.4 ± 0.5
6M	White free flowing powder	99.64 ± 4.33	nil	5.7 ± 0.5	101.02 ± 4.32	2.65 ± 0.43	80.7 ± 2.8	30.6 ± 0.6
9M	White free flowing powder	98.76 ± 3.09	nil	5.3 ± 0.8	102.46 ± 5.22	3.02 ± 0.42	78.4 ± 3.2	30.1 ± 0.4
12M	White free flowing powder	98.34 ± 3.44	nil	5.4 ± 0.6	99.46 ± 3.26	3.55 ± 0.34	77.5 ± 4.3	28.4 ± 0.3

Table 6.3 Stability data of RFP70 LDPI formulation.

Stability conditions RFP70	Description	Assay (%)	Degradation (%)	Water content (%)	Percent drug retained (%)	Liposomal size (VMD) (μm)	Emission (%)	Fine Particle Fraction (FPF) (%)
Initial	Reddish free flowing powder	104.47 ± 2.34	nil	4.4 ± 1.0	99.27 ± 3.62	2.86 ± 0.33	84.1 ± 1.6	29.3 ± 0.6
40°C 75% RH								
1M	Reddish free flowing powder	99.45 ± 4.47	nil	5.2 ± 0.5	93.32 ± 5.41	2.43 ± 0.32	82.3 ± 2.3	31.4 ± 0.8
2M	Reddish free flowing powder	97.33 ± 3.92	0.23	4.8 ± 0.8	90.52 ± 4.66	3.65 ± 0.51	78.3 ± 4.3	27.2 ± 0.6
3M	Reddish free flowing powder	93.45 ± 4.68	0.46	5.4 ± 0.6	83.66 ± 5.87	5.44 ± 0.36	79.1 ± 3.6	23.7 ± 0.8
6M	Reddish brown free flowing powder	86.15 ± 5.23	0.84	5.0 ± 0.5	72.82 ± 4.57	7.65 ± 0.73	74.3 ± 4.6	17.2 ± 0.5
30°C 65% RH								
1M	Reddish free flowing powder	100.12 ± 3.68	nil	5.2 ± 0.6	102.45 ± 4.26	2.78 ± 0.44	82.3 ± 4.2	30.1 ± 0.8
2M	Reddish free flowing powder	98.86 ± 4.74	nil	4.9 ± 0.7	99.67 ± 3.92	3.23 ± 0.34	80.6 ± 4.3	29.6 ± 0.5
3M	Reddish free flowing powder	99.25 ± 3.64	nil	5.4 ± 0.3	98.44 ± 3.72	3.85 ± 0.26	77.3 ± 3.8	30.4 ± 0.7
6M	Reddish free flowing powder	97.52 ± 3.84	nil	4.4 ± 0.8	97.62 ± 5.23	3.75 ± 0.12	78.7 ± 4.2	28.4 ± 0.5
9M	Reddish free flowing powder	95.28 ± 5.65	0.32	4.7 ± 0.6	96.13 ± 4.49	3.13 ± 0.30	76.5 ± 3.9	28.3 ± 0.6
12M	Reddish free flowing powder	93.67 ± 4.67	0.47	5.4 ± 0.4	93.45 ± 4.68	3.45 ± 0.42	75.8 ± 4.4	26.4 ± 0.7
25°C (CRT)								
3M	Reddish free flowing powder	99.83 ± 4.64	nil	4.8 ± 0.7	98.64 ± 3.72	2.36 ± 0.43	78.3 ± 5.2	29.5 ± 0.7
6M	Reddish free flowing powder	97.64 ± 5.36	nil	5.6 ± 0.6	96.86 ± 4.72	2.85 ± 0.33	82.4 ± 4.6	30.4 ± 0.8
9M	Reddish free flowing powder	95.26 ± 4.29	nil	5.1 ± 0.8	95.36 ± 5.01	3.22 ± 0.23	77.3 ± 4.1	27.5 ± 0.6
12M	Reddish free flowing powder	94.34 ± 3.64	0.51	5.6 ± 0.4	94.36 ± 4.36	3.34 ± 0.28	75.5 ± 4.7	25.8 ± 0.7

Table 6.4 Stability data of RFP71 LDPI formulation.

Stability conditions RFP71	Description	Assay (%)	Degradation (%)	Water content (%)	Percent drug retained (%)	Liposomal size (VMD) (μm)	Emission (%)	Fine Particle Fraction (FPF) (%)
Initial	Reddish free flowing powder	99.67 ± 5.24	nil	5.5 ± 0.7	101.32 ± 3.53	2.83 ± 0.32	81.7 ± 1.9	29.6 ± 0.7
40°C 75% RH								
1M	Reddish free flowing powder	98.35 ± 4.57	0.31	5.7 ± 0.4	95.42 ± 4.71	2.73 ± 0.42	80.1 ± 3.1	29.3 ± 0.8
2M	Reddish free flowing powder	96.42 ± 4.72	0.43	5.2 ± 0.6	91.12 ± 4.73	3.25 ± 0.53	77.4 ± 5.3	26.3 ± 0.5
3M	Reddish brown free flowing powder	92.12 ± 4.54	0.82	5.3 ± 0.8	87.76 ± 4.67	5.74 ± 0.37	78.3 ± 4.6	24.5 ± 0.6
6M	Reddish brown free flowing powder	88.25 ± 4.56	1.42	5.5 ± 0.4	74.65 ± 5.24	6.85 ± 0.53	75.4 ± 4.7	14.56 ± 1.2
30°C 65% RH								
1M	Reddish free flowing powder	99.62 ± 4.58	nil	5.5 ± 0.4	98.54 ± 5.12	2.94 ± 0.53	80.2 ± 3.3	31.2 ± 0.4
2M	Reddish free flowing powder	98.66 ± 4.63	nil	4.8 ± 0.8	99.57 ± 4.92	2.53 ± 0.47	81.4 ± 4.2	28.4 ± 0.6
3M	Reddish free flowing powder	99.48 ± 4.54	nil	5.2 ± 0.7	98.94 ± 4.62	2.85 ± 0.45	79.3 ± 3.6	29.2 ± 0.5
6M	Reddish free flowing powder	97.63 ± 3.73	0.52	5.4 ± 0.4	95.52 ± 5.36	3.15 ± 0.32	80.5 ± 4.4	27.4 ± 0.4
9M	Reddish free flowing powder	94.32 ± 4.35	0.81	4.9 ± 0.7	92.13 ± 4.68	3.43 ± 0.36	78.5 ± 3.4	25.7 ± 0.5
12M	Reddish free flowing powder	92.47 ± 4.17	1.12	5.6 ± 0.8	90.04 ± 4.81	3.54 ± 0.31	76.8 ± 3.7	24.6 ± 0.8
25°C (CRT)								
3M	Reddish free flowing powder	99.83 ± 4.64	nil	5.8 ± 0.5	99.44 ± 3.38	2.76 ± 0.32	79.1 ± 3.8	29.7 ± 0.5
6M	Reddish free flowing powder	97.64 ± 5.36	0.46	5.4 ± 0.7	97.64 ± 4.32	2.84 ± 0.41	80.4 ± 4.3	31.2 ± 0.4
9M	Reddish free flowing powder	95.26 ± 4.29	0.75	5.3 ± 0.6	94.36 ± 4.24	3.42 ± 0.32	79.1 ± 4.4	28.3 ± 0.4
12M	Reddish free flowing powder	94.34 ± 3.64	0.92	5.7 ± 0.7	91.61 ± 3.78	3.64 ± 0.38	75.6 ± 3.7	25.6 ± 0.5

6.4 RESULTS AND DISCUSSION

The physical stability of liposomes is one of the biggest obstacles in formulation commercially viable product (Fildes et al, 1981). Liposomes should be stable for 1-2 years preferably at room temperature to be pharmaceutically acceptable with high drug retention within liposome and the particle size should be maintained during storage time, hence the drug leakage, particle size growth, in-vitro deposition characteristics, emission and the chemical stability of drugs were studied at accelerated (40°C 75% RH), intermediate (30°C 65% RH) and controlled room temperature (CRT - 25°C) conditions according to ICH guidelines (Singh et al, 1999) for countries falling under zone III (hot, dry) and zone IV (very hot, humid). The stability profiles of INH and RFP LDPI formulations are shown in figure 6.2 - 6.3 & 6.4 – 6.5, respectively.

6.4.1 Stability of INH LDPI formulations

The percentage of drug remained entrapped (PDR) for LDPI formulations of INH after six months accelerated storage was 66.46 % (INH70) and 78.24 % (INH71) was below the acceptable level, hence as per the guideline recommendation one can not assign a shelf-life of 18 months. The product was tested on intermediate storage condition in order to assign a shelf-life at CRT. The product stability in terms PDR, particle size growth, in-vitro deposition characteristics, emission and the chemical stability of drugs studies was conducted for one year at intermediate storage condition was 98.35 % (INH70) and 97.35 % (INH71), and at controlled room temperature storage was 100.36 % (INH70) and 99.46 % (INH71). There was decrease in PDR with the increase in the temperature of storage (Figure – 6.2 & 6.3). No significant difference between neutral and negatively charged LDPI formulations observed on stability (t-Test: Paired $P<0.05$). The increase in particle size (Figure – 6.2 & 6.3) and the decrease in PDR were attributed due to fusion of liposome. This fusion of liposome can be controlled by selection of higher glass transition temperature lipid composition. It is apparent that the LDPI formulations prepared using HSPC possessed glass transitions temperature < 35°C, the cause for the fusion of these liposomes at accelerated conditions might be due phase melting of lipid bilayer above this temperature. A DSC thermograph of LDPI formulation showing the glass transitions temperature is shown in figure – 6.1. The FPF of INH LDPI formulations results were observed to be in parallel to the results of liposomal size on rehydration i.e. on accelerated storage the FPF found to decrease on prolonged storage (Anova: Single Factor $P>0.05$). However the is no significant deference in FPF on

intermediate and CRT storage was observed for a year (Figure – 6.2 & 6.3) (t-Test: Paired $P<0.05$).

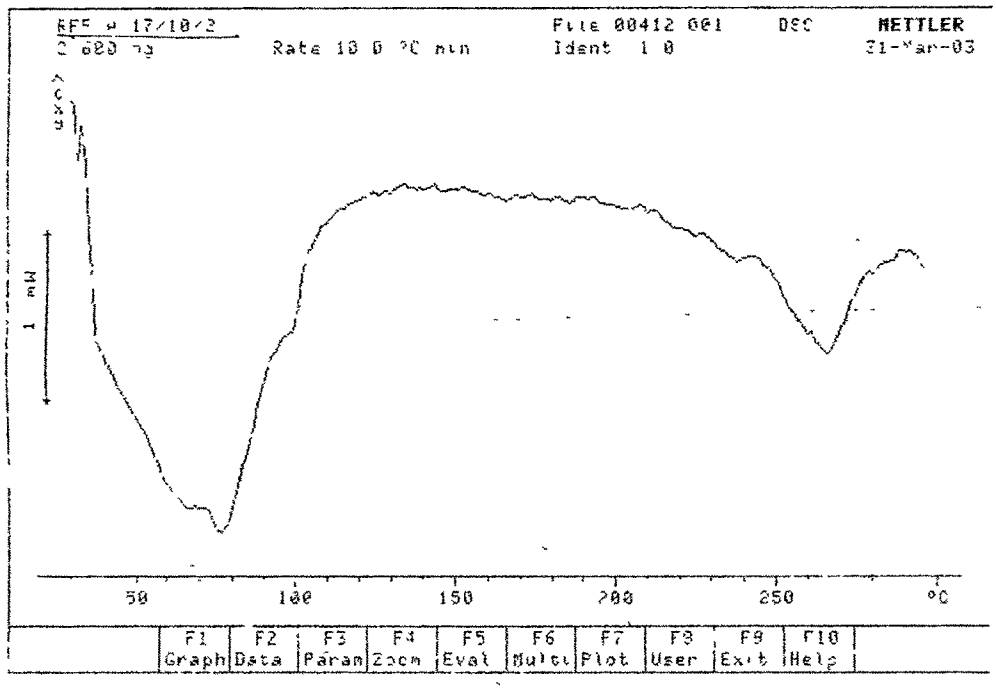

Figure 6.1: DSC thermograph of RFP LDPI formulation

6.4.2 Stability of RFP LDPI formulations

Similar to INH LDPI formulation stability, the percent drug retained (PDR) for RFP LDPI formulations after six months accelerated storage was 72.82 % (RFP70) and 74.65 % (RFP71) was below the acceptable level, hence as per the guideline recommendation one can not assign a shelf-life of 18 months. The product was tested on intermediate storage condition in order to assign a shelf-life at CRT. The product stability in terms PDR, particle size growth, in-vitro deposition characteristics, emission and the chemical stability of drugs studies was conducted for one year at intermediate storage condition. The PDR of RFP70 was observed to be 93.45 % and 90.04 % for RFP71, and at controlled room temperature storage was 94.36 % (RFP70) and 91.61 % (RFP71). Decrease in PDR with the increase in the temperature of storage was observed (Figure 6.3 & 6.4). No significant difference was observed for neutral and negatively charged LDPI formulations on stability (t-Test: Paired $P<0.05$). The increase in particle size (Figure 6.3 & 6.4) and the decrease in PDR were attributed due to fusion of liposome. This fusion of liposome can be controlled by selection of higher glass transition temperature lipid composition. It is apparent that the LDPI

formulations prepared using HSPC possessed glass transitions temperature < 35°C, the cause for the fusion of these liposomes at accelerated conditions might be due phase melting of lipid bilayer above this temperature. The FPF of INH LDPI formulations results were observed to be in parallel to the results of liposomal size on rehydration i.e. at accelerated storage the FPF found to decrease on prolonged storage (Anova: Single Factor $P>0.05$). However there is no significant deference in FPF on intermediate and CRT storage was observed for a year (Figure – 6.2 & 6.3) (t-Test: Paired $P<0.05$).

The higher PDR of INH and RFP LDPI at controlled and intermediate storage conditions may be due to selection of saturated PC that has higher glass transition temperature (40°C). There was increase in the liposomal mean particle size on storage for longer time and at higher temperature, however there was no major change in FPF was observed for both the drug LDPI formulations stored at intermediate storage and CRT. This may be due to that the fused liposomes are embedded within the low density carrier matrix, though the mean liposomal particle size increase due to fusion of liposome on rehydration, the drug dispersion and deposition was not affected significantly.

Though the PDR for INH and RFP LDPI formulations dropped below the acceptable level (90-110%) at accelerated storage conditions, the PDR at intermediate storage condition up to 12 months were within the acceptable level. Hence, as per the ICH guideline a self life of 18 months at storage condition of 25°C should be assigned for the developed INH and RFP LDPI formulations. It can be concluded that change in liposomal mean size is non significant before and after storage up to 12 months intermediate storage and controlled room temperature storage for both INH and RFP LDPI formulations. The slight increase in the liposome size may be due to aggregation on storage that is of insignificant level.

The formulations were also observed for caking and discoloration of LDPI formulations. Marginal caking or discoloration was observed for batches stored under accelerated storage conditions after 3 months. These types of observations were less visible at intermediate storage and controlled room temperature storage of both LDPI formulations.

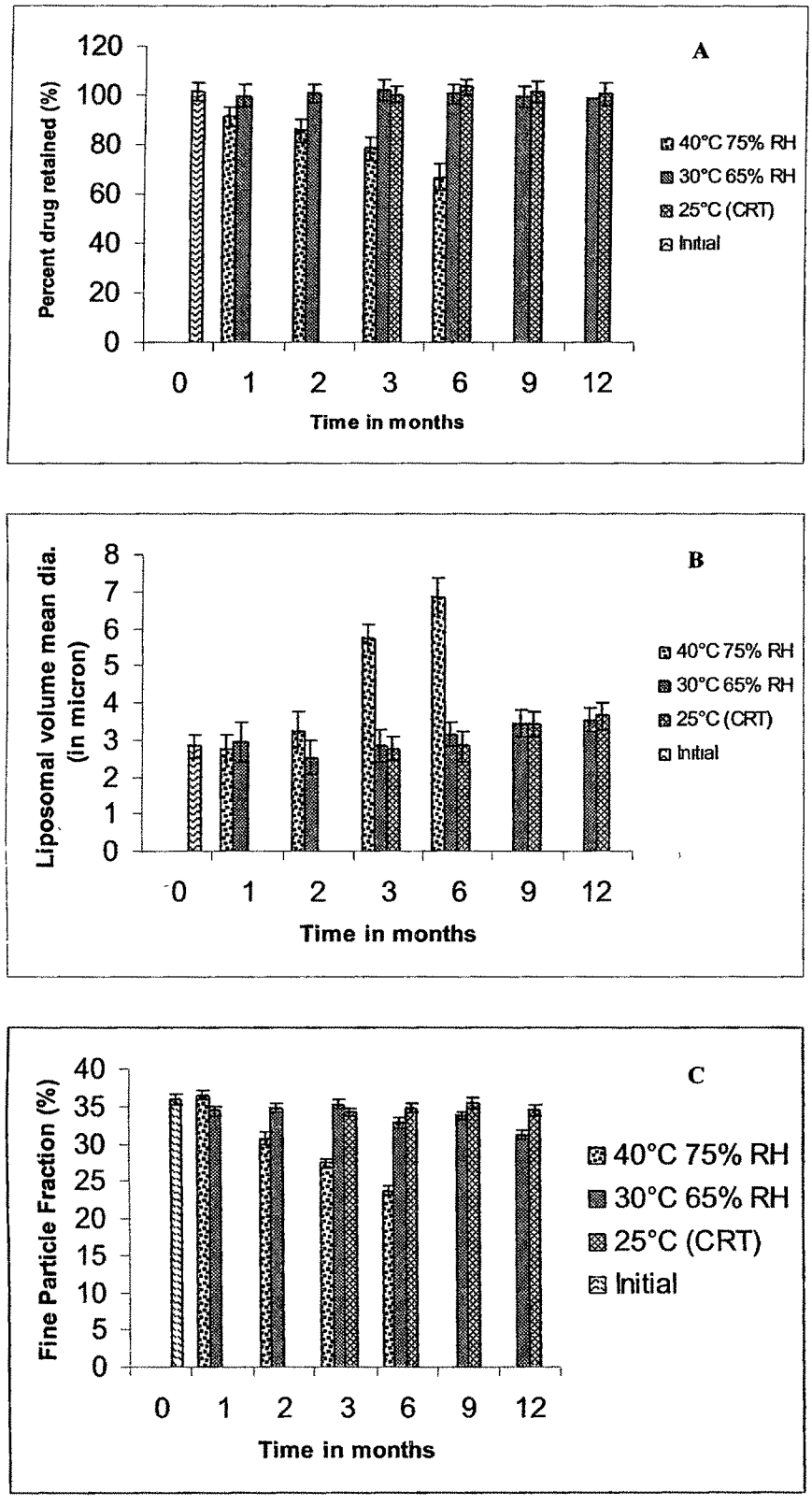

Figure 6.2 Stability profiles - INH70 A) PDR, B) Mean size and C) FPF vs. time in months

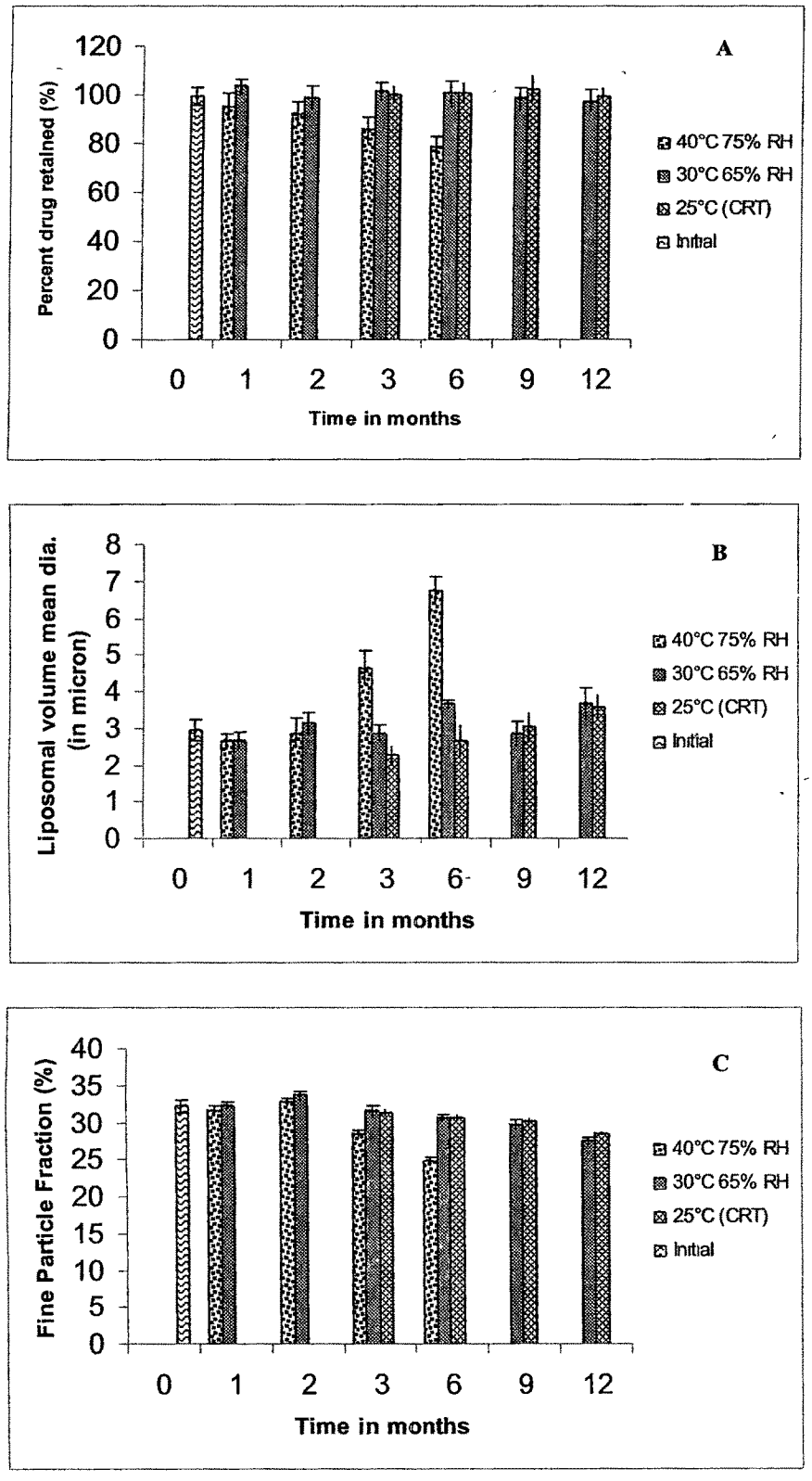

Figure 6.3 Stability profiles - INH71 A) PDR, B) Mean size and C) FPF vs. time in months.

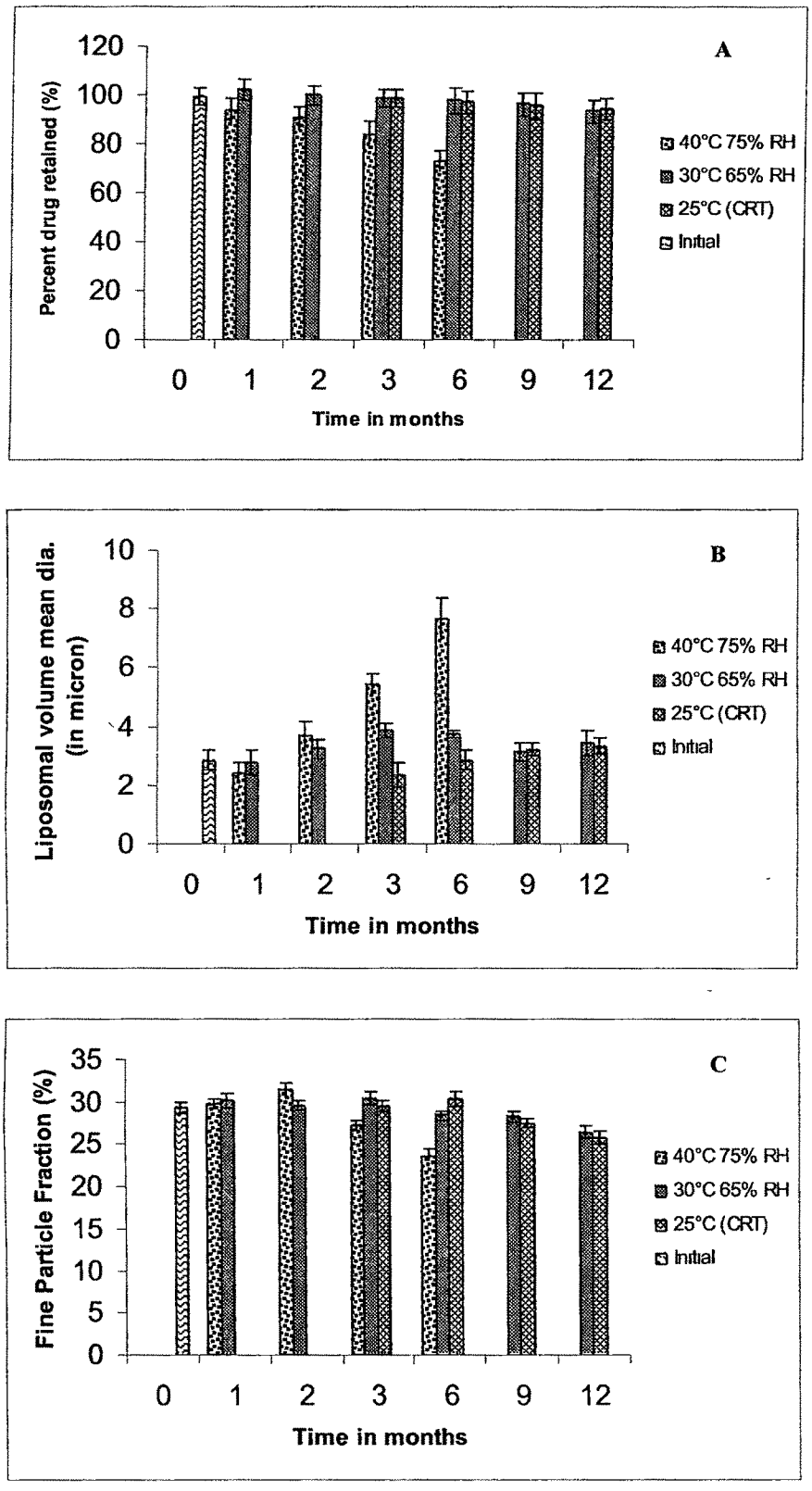

Figure 6.5 tability profiles – RFP70 A) PDR, B) Mean size and C) FPF vs. time in months

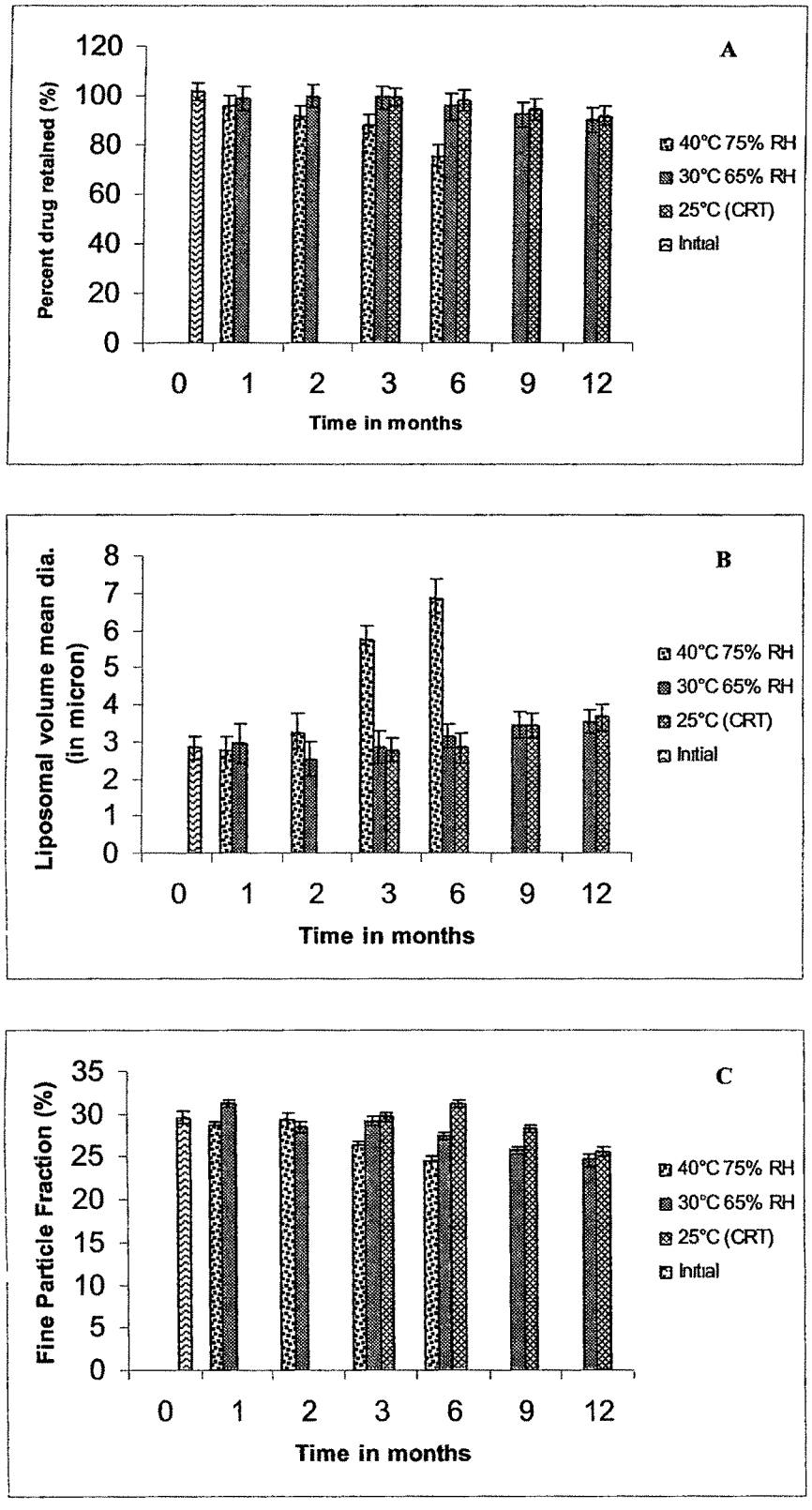

Figure 6.5: Stability profiles – RFP71 A) PDR, B) Mean size and C) FPF vs. time in months.

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