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STABILITY AND BIODISTRIBUTION STUDY OF QUININE HYDROCHLORIDE NIOSOMAL FORMULATION

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ABSTRACT

Niosomal formulation found to be one of the promising approach to treat the malaria. QH loaded niosomal formulation used for the targeting the liver stages of the parasite to treat early stage malaria. The biodistribution pattern says that the QH loaded niosomes minimized the toxicity associated with plain QH solution, as were at the liver for longer period of time. Longer circulation of the niosomes showed that, incorporation of QH into the niosomes helped to increase the stability of QH by preventing it from chemical and enzymatic degradation. Lyophilized niosomal formulation (Proniosomes) were stable when stored at both refrigerated and room temperature.

Keywords: Quinine hydrochloride, Niosomes, Biodistribution.

INTRODUCTION

In vivo animal study is a reliable tool to study the response of drugs directly in the biological system. Any novel formulation development intended for humans requires the demonstration of safety and efficacy in animal models. Healthy male albino rats models provide an important means of assessing liver targeting activity for new formulations of already established drugs. Drugs and their formulations are exposed to variable atmospheric conditions throughout their shelf life i.e. during storage, shipment and handling. In addition to this, diversity of conditions with respect to temperature and humidity, in various countries, also propelled us to investigate the stability of drugs and their formulation under influence of various storage conditions. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light and establish a shelf life for the drug product at recommended storage conditions. Stability studies wherein the drug product is exposed to normal storage conditions for a period of time sufficient to cover the proposed period is the long term stability testing, plus the drug product is subjected to accelerated conditions of temperature and humidity so as to determine the shelf life and storage conditions. The studies, designed to increase the rate of chemical degradation or physical change of a drug

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substance or drug product by using exaggerated storage conditions are known as accelerated stability studies. The product is evaluated at different time points for various formulation parameters such as physicochemical characters; drug content, etc. The data obtained from these studies is used to determine the shelf life of a product at a particular storage condition. Shelf life is defined as a period till which not more than 10% of the drug is degraded. Stability studies of a drug product were carried out in the same container closure as it is going to be packed in.

MATERIALS AND METHODS

Experimental protocol for biodistribution study:

Experimental protocol was approved by CPCSA Local Institutional Animal Ethics Committee before the study was started.

The liver targeting of QH in solution and QH loaded niosomes were investigated intravenously in albino rats. Both the formulations were administrated intravenously at 10mg/kg.

1). Strain of mice used: Healthy albino rats (Bombay College of Pharmacy, Mumbai).

2). Weight of the animals: 200 ±20 grams

3). Age: 6-8 weeks

4). Number of rat / group: 6

It is as per standard experimental design.

Name of group	No. of animals
Control	6
Plain drug solution	6
Niosomal formulation containing DMPC	6
Niosomal formulation containing DCP	6

Gender of rats: Either sex

Maintenance: Animals were housed in the standard steel wire mesh bedding and door

Housing and feeding conditions:

The housing conditions were maintained as follows:

Temperature: 22º ± 3°C Humidity: 30 - 70%

Light Cycle: artificial, dark/light cycle of 12/12 hrs.

Animal feed: Animals were fed thrice a day to ensure adequate feed for the animals.

Water: Water supplied by Municipal Corporation of Greater Mumbai was provided to the animals ad libitum.

Doses used – 10 mg/kg, Intravenous.

Site of administration: Tail vain (Caudal vain)

Schedule of Drug Administration: 15 days

Procedure: Albino rats weighing between 200-250g were taken and allowed to acclimatize to laboratory conditions for thirty minutes.

1. The rats were divided into four groups of six animals each.

2. To the first group plain drug solution (QH), to the second group primaquine phosphate niosomes with DCP, third group the niosomal formulation containing DCP and DMPC was injected intravenously (through tail vein) using appropriate disposal syringe with 22 gauze, while the fourth group was served as control.

3. After each 2 hours, the 2 animals from each group was anashesized, sacrificed and the organ (liver) was excised and homogenized in PBS.

4.The homogenates were deproteinized with acetonitrile.
5.The homogenates were finally centrifuged, and filtered to remove the debris.
6.The filtrate then diluted with 100 ml PBS.
7.2ml of this solution then withdrawn with 1000 µl micropipette, which was further diluted upto 10 ml.
Analysis of the contents were carried out by HPLC method.

Experimental protocol for Stability Study:

International conference on harmonization (ICH) has established a guideline for "Stability testing of new drug substances and products Q1A (R2)" [ICH, 2003]. The guideline recommends following storage conditions for drug substances intended for storage in a refrigerator. (Table 1)

Table 1: Stability testing of new drug substances and products Q1A (R2) [ICH, 2003]

Study	Storage condition	Time period
Long term	5°C ± 3°C	12 months
Accelerated	25°C ± 2°C/60% RH ± 5% RH	6 months

The present study involves investigation of the stability of the freeze dried parenteral niosomes of QH under influence of 25°C + 2°C, 60% RH ± 5% RH and 4°C + 2°C storage conditions for a period of 3 months and physical stability of QHniosomes, when stored at refrigerated condition for 15 days. The study was carried out to evaluate the effect of temperature on essential attributes of formulation such as appearance, drug entrapment and vesicle size at specified time intervals. (Table 2)

Table 2: Stability protocol for freeze dried niosomal formulations.

Condition	Storage	Sampling point points
Room temperature	Freeze dried niosomes filled in glass vials with rubber closures and sealed	0, 1, 2, 3 months
25°C with 60% RH	Freeze dried niosomes filled in glass vials with rubber closures and sealed	0, 1, 2, 3 months
5°C ± 3°C	Freeze dried niosomes filled in glass vials with rubber closures and sealed	0, 1, 2, 3 months

Evaluation of QHniosomes:

i) Physical stability:

Stability vials were observed for signs of obvious physical instability symptoms such as change in color or formation of larger aggregates.

ii) pH:

Stability of niosomal dispersion were analyzed for the changes in pH.

iii)Vesicle size:

Vesicle size of niosomal dispersion was measured for 3 weeks. Vesicle size of niosomal dispersion were measured using particle size analyzer (Beckman Coulter Inc, Miami, USA) as per the procedure described in section 6.2.3.2.

iv)Entrapment efficiency:

Drug content in reconstituted QH niosomes was determined

Table 3: Effect of storage condition on pH of niosomal dispersion

Storage In weeks → / Temp. ↓		0	1	2	3
4°C	Niosomal dispersion	7.40	7.52	8.58	8.64
25°C	Niosomal dispersion	7.42	7.59	8.68	8.83

Table:4 Effect of storage condition on vesicle size of niosomal dispersion

Storage In → / weeks ↓ Storage Temp		0	1	2	3
4°C	Niosomal formulation	219.7 nm	428.3 nm	554.5 nm	740.9 nm
25°C	Niosomal formulation	405.9 nm	440.7 nm	650.8 nm	1.35 µm

n=3; Average mean value

Table 5: Effect of storage condition on PI of niosomal dispersion.

Storage In weeks → / Storage Temp ↓		0	1	2	3
4°C	Niosomal formulation	0.60	0.76	0.96.	0.96
25°C	Niosomal formulation	0.93	1.45	1.45	2.35

n=3; Average mean value.

Table 6: Effect of storage condition on entrapment efficiency of niosomal dispersion.

Storage In weeks / Storage Temp		0	1	2	3
4°C	Niosomal formulation	59.7	55.3	54.1	48.45
25°C	Niosomal formulation	50.6	48.66	42.58	30.5

n=3; Average mean value

Table 7: Effect of storage condition on pH of niosomal dispersion

Storage In month / Temp.		0	1	2	3
4°C	Lyophilized formulation	7.40	7.42	7.42	7.43
25°C	Lyophilized formulation	7.42	7.43	7.44	7.45

n=3; Average mean value

Table 8: Effect of storage condition on vesicle size of freeze dried niosomes

Storage In month / Storage Temp		0	1	2	3
4°C	Lyophilized formulation	322.6 nm	322.67 nm	323.7 nm	324.9 nm
25°C	Lyophilized formulation	320.49 nm	324.20 nm	328.07 nm	332.07 nm

n=3; Average mean value

Table 9: Effect of storage condition on PI of freeze dried niosomes.

Storage In month / Storage Temp		0	1	2	3
4°C	Lyophilized formulation	0.70	0.73	0.71	0.72
25°C	Lyophilized formulation	0.71	0.76	0.75	0.77

n=3; Average mean value.

Table 10: Effect of storage condition on entrapment efficiency of freeze dried niosomes.

Storage In month / Storage Temp		0	1	2	3
4°C	Lyophilized formulation	60.75	60.25	59.60	59.35
25°C	Lyophilized formulation	60.75	59.3	58.4	56.04

n=3; Average mean value.

Table 11. Sterility test result of freeze dried niosomes for 3 months.

Test	Control		Conclusion
	Negative (-)	Positive (+)	
No growth	No growth	Growth	Sterile
Growth	No growth	Growth	Non sterile
Growth	No Growth	Growth	Repeat
No growth	No growth	Growth	Repeat

n = 3

RESULT AND DISCUSSION

The analysis of the drug were carried out by HPLC to measured the % of QH accumulated in the liver with respect to time in hours. The liver uptake of the niosomal formulation containing DCP and DMPC was found to be 90 % of administered dose of QH within the 2 hours after the initiation of study, while in case of niosomal formulation containing DCP showed 60% drug in liver. At 4 th and 6th hour the accumulation of Q Hniosomes containing DMPC and DCP were 78% and 70% respectively. The Q Hniosomal formulation containing only DCP showed 45% and 32% of QH in liver cells at 2nd hours and 4th respectively. Scavenger receptors in the kuffer of liver were responsible for liver uptake of niosomes containing negative charge on their surface. The cells were the binding site for negatively charge niosomal formulation as were reported in literature (Fengliu.et al, 1995). Incorporation of QH into the niosomes helped to increase the stability of QH by preventing it from chemical and enzymatic degradation which further helped to increase residence time. The percent uptake of drug from plain drug solution were 56.5% and 49% at the time point of 2nd and 4th hour. At the end of 6 hour the percent drug in liver from the plain drug solution was not detectable, indicating it`s elimination from liver, as the half life of drug was 3-4 hours. (Alving. et a1, 1978), (Dijkstra et al. 1984), (Gregoriadis and Ryman et al , 1972).

The One way ANOVA (Non-parametric) applied for the significance of the results. Dunnet`s t-test applied for plain drug solution, niosomal formulation containing DCP and niosomal formulation containing DCP and DMPC. This results indicated that the DMPC containing niosomal formulation accumulate at the liver cells and thus increased the drug concentration there by relapsing the parasitic stages of infection of malaria. Due to the negative charge on the niosomal membrane the niosomes binds to the receptors of parenchymal cells and hence the combination of the QH loaded niosomal formulation containing DCP and DMPC where found mainly in liver as compared to other organs (Muramatsu K.et al,1995). Statistically significant response was observed, when compared with the control group as the p value was less than 0.001 for all six hours of study as shown in table 1. In case of the niosomes containing DCP, liver uptake were found to be 60%, 45%, 32% respectively at the time interval of 2, 4 and 6 hours. Statistically significant response was observed for these results as p value was less than 0.005, at the confidence level of

95%. It was found that upon intravenous injection, drug-containing niosomes were rapidly removed from the blood and taken up to a large extent by endocytotic reticuloendothelial system-derived elements of the liver, compared to the free drug form. From the table no.1 it could be stated that QH entrapped within niosomes exhibited a prolonged plasmatic half-life, which was more than 6 hours whereas of free QH it was found to be less than 3 hours, showed that the niosomes restricted the enzymatic degradation of PQH and thus increase the amount of drug at the site of action. These results revealed that the QH had more half life when formulated in niosomal formulation and also had prolonged effect on the targeted site. On the other hand the toxicity of PQH reduced drastically as compared to free drug solution.

Stability studies:

i) Physical stability:

The stability samples stored at all conditions showed, visible signs of physical instability at the end of 3 weeks. Niosomes aggregates were formed in these batches which were confirmed by optical microscopy.

ii) pH:

pH of niosomal dispersion was evaluated which is shown in Table 3. pH of QH loaded niosomal dispersion were found to be changed when analyzed for 3 weeks at both the temperature conditions. The increased in the pH of niosomal dispersion over the storage period could be due to the drug leakage from niosomes, which is basic in nature. The niosomal dispersion was found to be unstable for the 3 days at both the storage temperature. (Table 3)

iii) Vesicle size:

Effect of storage condition on vesicle size of niosomal dispersion shown in table 4 &5. From the Table 4 it was observed that niosomal dispersion were in the range of 219-740 nm and 528 nm -1.35 µm when stored at 4^0C and 25^0C respectively over the period of 3 weeks. The significant increased in the particle size of niosomal dispersion observed at 4^0C as well as 25^0C could be due to aggregation of vesicles to form the bigger particles, which consequently decreased the stability of niosomes. The niosomal dispersion when analyzed at both 40C and 250C showed the significant change in PI value as shown in Table 5 revealed that the niosomal membrane starts disrupting and vesicles gets aggregating, which reflected in higher PI values as shown in Table 4

iv) Drug entrapment:

From the Table 6, it was observed that there was decreased in entrapment efficiency of niosomal dispersion at both 40C and 250C could be due to the leakage of the drug from niosomal membrane, as membrane starts disrupting. The drug leakage at 4^0C was found to be considerable less than leakage at 25^0C. This indicates that the niosomal dispersion have better stability when stored at refrigerated condition than 25^0C.

Freeze dried niosomal formulation was placed at various storage conditions as per ICH guidelines and the samples were evaluated at different time intervals over a period of three months for visual changes, resuspendability, particle size, polydispersity index (PI) and entrapment efficiency.

i) Visual changes:

The stability samples stored at all conditions did not show any visible signs of physical instability at the end of three months. There were no major aggregates formed in these batches and the powder was found to retain its free flowing characteristics without any change in the color.

ii) Resuspendability:

Freeze dried formulations at all conditions were found to be redispersible with manual shaking.

iii) pH:

pH of QH loaded lyophilized niosomal formulation was evaluated which is shown in Table 7. pH of QH loaded lyophilized niosomal formulation does not showed the noticeable changes, when analyzed for 3 months at both the temperature conditions.

iv) Vesicle size and PI:

Table 8 indicate vesicle size of lyophilized PQH niosomal formulation for 0, 1, 2 and 3 month time intervals. From the Table no.8 there was no significant change in vesicle size of lyophilized niosomal formulation over the period of 3 month. This showed that lyophilized niosomes had better stability over niosomal dispersion at both temperature i.e. 4^0C and 25^0C respectively. The PI of the freeze dried formulation were in the range 0.70 - 0.77 showed that the particles were of uniform size and well separated from each other as shown in table 9. These results, revealed that lyophilized formulation were stable.

v) Drug entrapment:

Table 10 indicates drug entrapment of lyophilized niosomal formulation and niosomal dispersion for 0, 1, 2 and 3 month time intervals. Results indicated in table 10 revealed that the lyophilized niosomal formulation kept at 4°C during 3 month period, showed no significant changes in entrapment efficiency. At 25^0C over the period of 3 month there were no noticeable changes in entrapment efficiency. Retention of drug in lyophilized niosomal formulation over the 3 month was observed which could be attributed to swelling of niosomal membrane without rupturing it during lyophilization process, which were observed from the slightly increased in particle size of freeze dried niosomal formulation.

vi) Sterility study:

At the end of 14 days of incubation in the respective nutrient broth, no growth was found in any of the sample for all the 3 months. This assured the sterility of the QH loaded samples and also further confirms ensure parenteral safety of the developed system, as shown in Table 11.

CONCLUSION

From the above biodistribution study results we could conclude that, niosomal formulation found to be one of the promising approach to treat the malaria. QH loaded niosomal formulation used for the targeting the liver stages of the parasite to treat early stage malaria. The results showed that the final niosomes were stable when stored in buffers and plasma .i.e were not degraded in plasma shown by the more accumulation of the formulation in liver stages in lager concentration. From the biodistribution pattern we could conclude that the QH loaded niosomes minimized the toxicity associated with plain QH solution, as were at the liver for longer period of time. Longer circulation of the niosomes showed that, incorporation of QH into the niosomes helped to increase the stability of QH by preventing it from chemical and enzymatic degradation. DMPC and DCP containing QH and loaded niosomal formulation showed the prolong released and liver targeting and hence ultimately increased the efficacy of the developed formulation as compared to conventional formulation. The stability studies of QH loaded lyophilized formulation revealed that there is a no measurable change observed in particle size, PI, entrapment efficiency, pH, and sterility, while in the niosomal dispersion measurable changes observed in the physical stability, pH, vesicle size, PI and entrapment efficiency kept under 4ºC and 25ºC conditions. But dispersion stored at 40C were found to be stable for 2 month. From these result it could be concluded that QH loaded lyophilized niosomal formulation (Proniosomes) were stable when stored at both refrigerated and room temperature.

REFERENCES

1. Uchegbu I.F., Florence A.T. (1995), “Advanced Colloidal Interface Science”, 58, 10-24.

2. Alving A.S., Eichelberger C.B., Jones R., Whorton C.M. (1978), "Textbook on Adverse Drug Reaction", Macmillan, USA.
3. Murdan S., Gregoriadis G., Florence, A.T., (1996), Non-ionic surfactant based organogels incorporating niosomes, *STP Pharmaceutical Science*, 6, 44–48.
4. ICH, 2003. Q1A stability testing of new drug substances and products, in Proceedings of the International Conference on Harmonization, Geneva.
5. IP(2007), "Indian Pharmacopoeia", The Indian Pharma-copoeial Commission Ghaziabad, Volume 1, 2 and 3.
6. Arica B., Ozer A.Y., Ercan M.T., Hincal A. A.(1995), Characterization, in vitro and in vivo studies on primaquinediphosphate liposomes., *Journal of Microencapsulation*., 12, 469–485.

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