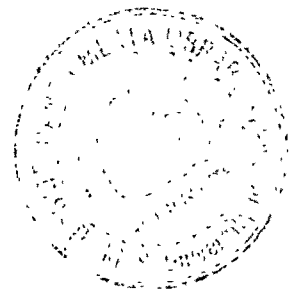


CHAPTER IV

SUMMARY & CONCLUSIONS

4.0 SUMMARY & CONCLUSIONS

4.1 Summary

Mankind has always strived to lead a better life. Though the advances in the past years have led to a healthier and longer life for humans than ever-before, effective therapies for many disease states are elusive. One such devasting illness is malaria, which in various forms, affects 300-500 million people each year & claims 3 million lives worldwide annually. Malaria kills more people than any other communicable disease except tuberculosis.

Now a days, the chemotherapy of malaria faces several challenges such as drug resistance by various strains of *P.falciparum*, high toxicity & low bio-disposibility of available drugs. Global efforts are ongoing to discover more potential antimalarial drugs. But this can be significantly affected the cost & uncertainty for discovering newer antimalarial drug, so novel drug delivery system could be better alternate to improve therapeutic efficacy for existing drugs.

A well designed controlled drug delivery system can also overcome some of the problems of conventional therapy & enhance the therapeutic efficacy of a given drug. One of the most promising drug delivery system currently being investigated is the micro-particulate drug delivery system

Microspheres of biodegradable & non-biodegradable polymers have been investigated for sustained release depending on the final application. The main advantage of microsphere based carriers is that they could be injected into the body in a suitable vehicle using a hypodermic needle. Biodegradable carrier matrix can be designed to deliver the therapeutic agent for a period ranging from a few days to few years.

The aim of present study was to make present day antimalarial therapy more convenient and effective by investigating the potential of microspheres for the drug delivery of antimalarial drugs. It was conjectured that the

intercalation of antimalarial drugs in microspheres would protect them from the kinetic process in the body. The antimalarial drugs entrapped in microspheres when injected into the body would be released from the formulation in predetermined manner. To avoid surgical problems associated with large implants, suspension of nanoparticles & microparticles are recommended for implantation in a suitable vehicle. It also offers advantage that, it eliminates the necessity for the retrieval of implanted devices following depletion.

Chloroquine phosphate is an important, cheap, widely available & well tolerated antimalarial drug used prophylactically as well as therapeutically in the treatment of all kinds of plasmodium infections in the world. The initial distribution volume of CQP is approximately one thousand times smaller than the total apparent volume of distribution. This may cause potentially fatal hypotension. However, fast parenteral administration of aqueous solutions of chloroquine may cause life-threatening toxicity. Hence, it should be given by intravenous in small divided doses.

Mefloquine is effective against multidrug resistant strains of *p.falciparum*. Mefloquine is now recommended for use alone exclusively for prophylaxis & chemotherapy of CQP resistant & multidrug resistant falciparum malaria.

Chitosan is one of the most abundantly available natural materials. It is suitable as a biodegradable matrix for the production of microparticle system owing to its biocompatibility, biodegradability, bioadhesive & low toxicity properties. The importance of cellulose ethers has increased in recent years because of easily available & cheap in comparison with other synthetic polymer.

Mefloquine hydrochloride (MQH), a lipophilic antimalarial drug & chloroquine phosphate hydrophilic antimalarials were selected as the

candidate for the present investigation. In addition, there are no parenteral formulations of MQH are available, intravenous use of CQP is associated with many untowards effect including cardiac toxicity.

Ethyl cellulose coated chloroquine phosphate microspheres were prepared by w/o emulsion solvent diffusion technique. Bulky, rough, non spherical, irregular shape microspheres were found. Span-80 & gelatin were used to form stable primary emulsion, but they were not of much use due to faster in-vitro drug release profile.

Chitosan coated chloroquine phosphate microspheres were prepared by w/o emulsification technique, cross-linked by means of aldehydes. Different parameters such as drug:polymer ratio, types of a cross-linking agent, concentration of polymer & cross-linking agent, stirring speed & hardening time etc were investigated for their effect on its characters like percentage yield, drug content, particle size, morphology & in-vitro release behaviour.

The microspheres were also optimized by 3^2 factorial design with independent variables. The optimized formulation was studied for pharmacokinetic & biodistribution using Spargue Dawley female albino rat.

The obtained microspheres were smooth & spherical when 5% w/w glutaraldehyde was used as cross-linking agent for a hardening time of 3 hours. Particle size was found to be 18.18 ± 0.71 μ (Batch CC_5) which is quite suitable for in-vivo intramuscular administration of microspheres.

Mefloquine hydrochloride microspheres were prepared by solvent evaporation technique. Different process variable like Drug:polymer ratio, solvent ratio methanol :DCM (Dichloromethane) volume of disperse phase (DCM), volume of continuous phase, concentration of PVA solution, stirring speed, incorporation of PEG into disperse phase & concentration of PEG were investigated to study their effect on characters of microspheres.

D:P ratio significantly affected particle size & in-vitro release profile of microsphere. By increasing D:P ratio, particle size was increased & which led to decrease in release rate. Volume of disperse phase & continuous phase both variables have significant effect on particle size & morphology. The Methanol : Dichloromethane ratio also influenced morphology & particle size. When the volume of DCM reduced & volume of methanol increased, fluffy, rough, bulky, irregular, convoluted particles were formed. Concentration of PVA played important role in the formation of discrete particles.

Stirring speed was inversely affected on particle size without altering it's morphology. When PEG was incorporated in disperse phase it formed porous particles having pin-holes which induces diffusion of drug into PEG formed channels which may help the drug to diffuse to the medium.

In-vivo experiments were carried out for the free drugs (CQP/MQH) & optimized microsphere formulations (CC_5 & M18) to investigate pharmacokinetic parameters and biodistribution in female Sparague Dawley albino rats weighing between 225-250 g. Animals were divided in different groups and subgroups with code for the in-vivo study. 8 mg and 10 mg fixed doses of free drug (CQP/MQH) & their formulations (CC_5 / M18) in suspension form were administered through intramuscular route into gastrocnemius muscle of the right hind leg. Blood samples were withdrawn from the retro-orbital plexus at definite time intervals for a period of 18 days.

Blood (0.2ml) / tissue homogenates (0.2ml) was treated with 5% w/v trichloro acetic acid (0.5ml), N/10 sodium hydroxide solution (1ml), saturated sodium chloride solution (1ml). This aqueous mixture was extracted with 2,2, & 1ml of dichloromethane. The combined extract was allowed to evaporate. Residues were reconstituted with appropriate solvent. Drug concentration was determined by UV spectroscopy against its blank,

which was prepared in same manner. Pharmacokinetics parameters calculated from plasma concentration-time plots are tabulated as under :

Parameters	CQP Free drug	CQP formulation	MQH Free drug	MQH formulation
C_{max} µg/ml	18.3	8.8	16.8	8.9
T_{max} hr	2.0	3.0	2.4	3.5
$AUC_{0-\alpha}$ µg hr/ml	149.3	296.05	252.05	330.6
AUMC	1479.43	12143.9	4412	21302.96
MRT hr	10.6	24	17.5	64.5
K_a hr^{-1}	0.0417	0.0138	0.0278	0.0046
K_e hr^{-1}	0.0619	0.0108	0.0357	0.0101
MAT hr	24	72	36	216
$T_{½}$ hr	11.18	64	19.4	68.39
V_d ml	865.64	3127.59	889.07	2994.86

In-vivo profile of the microspheres containing CQP/MQH & free drug was studied by carrying out biodistribution studies in Sprague Dawley female albino rats. At definite time intervals the animals were sacrificed and organs were excised. The concentration of drugs in organs like lung, liver, heart, kidney & spleen were determined. For CQP/MQH estimation, a reported ultraviolet spectrophotometric method was used. Extraction of drug was carried out same as mentioned for whole blood by using 10% w/v tissue homogenate.

From biodistribution study, it was shown that the drug concentrations in heart and lung were higher following the administration of free drug in compare to its microsphere formulation. This may overcome drug related toxicity mainly cardic toxicity of CQP. Drug concentration in liver, spleen remains sustained for long time in steady level. Drug concentration in spleen increased with time, which was ascribed to the tendency of CQP to accumulate in RBC & the subsequent clearance of these cells by the spleen.

4.2 Conclusions :

An attempt was made to make present day antimalarial therapy more effective by intercalating antimalarial drugs in microparticulate drug delivery system. It was hypothesized that such carriers would show longer residence in blood by formation of depot at injection site. This would help to reduce side effects of these drugs and would also lead to a significantly lower dose being required for achieving therapeutic efficacy. The antimalarial agents chloroquine phosphate and mefloquine Hydrochloride were chosen as the drugs for the investigation . Microspheres of CQP and MQH were prepared using polymer like ethyl cellulose and chitosan by W/O emulsification and solvent evaporation techniques respectively. The optimization of formulation was done with parameters like polymer concentration, concentration of cross-linking agent, volume of disperse phase (DCM), volume of continuous phase(PVA solution) and condition such as stirring speed applying by 3^2 factorial design. Glutaraldehyde was used as cross-linking agent to modulate the release rate profile of drugs from chitosan microspheres.

In solvent evaporation technique, types of solvent and solvent ratios played significant role on particle size and surface morphology. Dichloromethane as solvent gave smooth, spherical and smaller microparticles.

Kinetics of drug release from microspheres were studied for all the batches containing CQP and MQH using dialysis tube covered with semipermeable membrane of which the molecular weight cut off is (12,00-14,000). The release was found biphasic in nature for both the drug (CQP & MQH). Drug release was initial rapid with comparatively higher burst effect with CQP formulation than with the formulation of MQH followed by a subsequent slow release phase. Percentage of burst calculated by

extrapolation of the second phase release profile for ethyl cellulose is given in tables 3.48a and 3.48b is summarized below for the sake of comparison.

Drug	Polymer	Burst range(%)
CQP	Ethyl cellulose	19 to 31
MQH	Ethyl cellulose	4 to 15

The above results indicates that CQP microspheres show high burst due to the fact that CQP is being water soluble, there is higher probability of migration of CQP in a hydrophobic matrix made up of ethyl cellulose resulting in higher percentage of the drug in the periphery. This may hasten the release of CQP during the initial phase followed by slow release of drug by diffusion from the core. In case of MQH which is a hydrophobic drug, the drug mixes well with hydrophobic ethyl cellulose and there are less chances of their migration to the periphery during microsphere formation. This may be the reason for very low burst (4to 15 %) observed in these batches. In fact in some batches of MQH (M5,M6,M7) release was almost monophasic with negligible burst effect.

These observation indicate that the location of the drug within the microspheres to play an important role in the drug release profile. In-vitro release was sustained for longer duration for mefloquine hydrochloride than in case of chloroquine phosphate , which may be due to the lipophilic nature of the MQH. Pharmacokinetic parameters C_{max} ,AUC, K_a, MRT, V_d is taken here to compare the release profile character of the drug administered as a free drug and its formulation. Microspheres formulation show low C_{max} , higher V_d , longer K_a and greater MRT which could reduce peak blood concentration over longer interval of time and thus reduce drug related toxicity. As per Hardman and Limbird's report, the blood CQP concentration

above 30ng/ml is optimum for antimalarial effect, while Kadir et al reports that peak level concentration of about 25μg/ml caused symptoms of severe toxicity in mice. This necessistate the maintenance of CQP level some where around 30 ng/ml to 25 μg/ml for safe and effective anti-malarial therapy. Formulation of microparticles in our present study was aimed at maintenance of blood levels above 30 ng/ml but well below 25 μg/ml , which was achieved to some extent in optimized batch where the average blood level concentration at peak was 8.8 μg/ml .

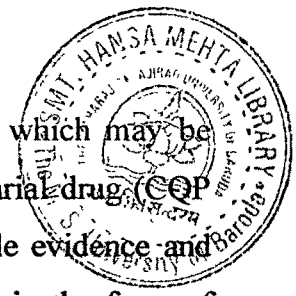
In-vivo organ distribution study of CQP microsphere formulation in albino rats showed reduction in peak tissue concentration in all the organs as compared to tissue concentration following administration of same dose of free drug. But the concentration was maintained over a long period of time (72-144hours). Following microsphere administration in comparision to maintenance to 6 to 48 hours for free drug administration. The T_p value achieved was different with different organs but they were in the period between 2 to 3 hours in case of free drug CQP and 3 to 6 hours in case of its microsphere formulation.

A very important observation made in the study was the glaring difference in the peak blood concentration of CQP in heart when administered as microsphere (4.8 μg/g) in comparision to the CQP concentration of 18.3 μg/g for free drug. This observation postulate the possibility of reduction of cardiotoxicity of CQP even when the blood level concentration is quite significant for therapeutic activity (7.9μg/ml)

Looking to the CQP accumulation in spleen the T_p value was quite high (12 hours) in both free drug and as a microsphere. This may be due to the uptake of CQP by RBC and its elimination into spleen in course of time

The bioavalibility and MRT of CQP administered as microsphere is high, (296μg hr/ml and 24 hours respectively) in comparison to free drug where bioavalibility is 149 μg hr/ml with MRT value of 10.6 hours. Plasma half of CQP calculated using blood concentration time data is 64 hours for microsphere as compared to 11 hours for free drug.

In-vivo organ distribution of MQH microsphere formulation in albino rats showed significant reduction in the peak blood concentration in most of the organs except lungs where the peak blood concentration following free drug administration was 3.8 μg/g in comparison to 3.4 μg/g in case of microsphere. It is important to note that the MQH peak concentration in heart following free drug administration was 11.8 μg/g while it was only 3.4 μg/g following administration of its microsphere. Similarly the peak blood concentration following administration of free drug and microsphere were 8.8 μg/g and 2.2 μg/g in spleen respectively. The T_p value in case of free drug was 2 to 3 hours with an exception in lungs where peak blood concentration was observed in 1 hour . MQH microsphere showed the T_p value after 3 hours in all tissues except in cases of lungs where was 2 hours. The results indicate that the possibility of reduction of cardiotoxicity of MQH is possible when administered as microsphere . The pharmacokinetics of MQH showed the characteristic of retention of the drug in the body over long time as evidenced by significant increase in MRT value (64.5 hours), when administered in the form of microsphere in comparison to 17.5 hours when administered as a free drug. The increase in bioavalibility as determined AUC was marginal, when the drug is administered as microsphere (330μg hr/ml) in comparison to free drug (252 μg hr/ml).



Thus, the present study radiate some new findings which may be exploited in improving the therapeutic efficacy of anti-malarial drug (CQP and MQH) using microspheres. The study also give possible evidence and reduction in cardiotoxicity of both drug when administered in the form of microspheres. Extensive clinical trials need to be performed to establish the efficacy of the systems in clinical practice. It is hoped that such studies will lead to the development of safe and powerful tools for combating the dreaded disease known as malaria.