CHAPTER III

EXPERIMENTAL METHODS,

RESULTS & DISCUSSIONS

3.1 Analytical Methods

3.1.1 Introduction :

The analytical methods used in studies of drug containing microspheres should, in addition to possessing the desired characteristics of accuracy, precision, reproducibility, ruggedness, etc., also possess the ability to be used in conjunction with techniques common to microspheres. The methods used should preferable be stability indicating which would when used, draw attention to any potential incompatibility between the various components of the microspheres.

3.1.2 Materials

Chloroquine phosphate sample was kindly gifted by vital pharmaceutical. Mefloquine hydrochloride was presented by Sun Pharma Advanced Research Center, Vadodara. Methanol, dichloromethane, concentrated hydrochloride acid, glacial acetic acid, sodium chloride, sodium hydroxide, disodium hydrogen phosphate, trichloro acetic acid, Potassium dihydrogen phosphate (AR) grade (S.D. fine chem.. Ltd. Boisar, Thane) Heparin sodium injection I.P. (Beparine, Biological Evans (India) limited Hyderabad.

Apparatus :

Centrifuge, (Remi Equipments, Mumbai), Ultrasonic processor Trans – o – sonic , Magnapak – 250, Chemito Spectra Scan U.V. 2600, Visible Double Beam Spectrophotometer, Weight Balance (Single Pan), K. Roy Analytical Single Pan Balance K – 12 Super, Tissue Homogenizer Teflon pastle & glass (mortar) (Remi. Equipments, Mumbai), Thermostatically controlled heating mental.

3.1.3 Estimation of Chloroquine Phosphate in 0.01N Hydrochloric acid

Chloroquine phosphate in 0.01N hydrochloric acid shows strong absorbance in the ultraviolet region of electromagnetic spectrum (Hong 1976).

3.1.3.1 Reagents and Solutions :

(A) 0.01N hydrochloric acid has prepared as per the method given in the Indian Pharmacopoeia (1985) from concentrated hydrochloric acid.

(B) Stock solutions of chloroquine phosphate 1 mg / ml solution of chloroquine phosphate in 0.01N hydrochloride acid has prepared by dissolving 10 mg of chloroquine phosphate in 10 ml of 0.01N hydrochloric acid and resulted solution was diluted 10 times with 0.01N hydrochloric acid yielded a 100 µg / ml solution of chloroquine phosphate in 0.01N hydrochloric acid.

3.1.3.2 Procedure for calibration curve :

Suitable aliquots (0.1 to 0.5 ml) of the 100 µg / ml stock solution and of the 1 mg / ml solution (0.1 to 0.2 ml) of chloroquine phosphate were pipetted into 10 ml volumetric flasks. The volume was made up with 0.01N hydrochloric acid, the contents shaken well & the absorbance measured at 343 nm using a Chemito UV- 2600 Spectra Scan Double Beam Spectrophotometer with quartz cells of 10 mm path length against 0.01N hydrochloric acid as a blank. The above procedure was repeated six times. The raw data along with the statistical analysis of the same is presented in the table 3.1. The optical characteristics for the solution of chloroquine phosphate in 0.01N hydrochloric acid are shown in table 3.2. Absorptivity of 200 nm to 400 nm for a 10 µg/ml solution of chloroquine phosphate in 0.01N hydrochloric acid is shown in Figure 3.2.

3.1.3.3 Stability & Selectivity :

The solution of chloroquine phosphate in 0.01N hydrochloric acid used for preparing the calibration curve, were observed for changes in their absorbance of the analytical wavelength, over a period of 24 hours to gain on

insight in to the stability of this method. The selectivity of this method for chloroquine phosphate was ascertained by carrying out the procedure detailed above in the presence of system interference like chitosan & ethyl cellulose at the concentrations in which they are present in the microspheres.

3.1.3.4 Accuracy & Precision:

In order to determine the precision & accuracy of the method, solutions containing known amounts of chloroquine phosphate were prepared and analyzed, using the procedure detailed above in three replicates. The analytical results obtained from these investigations are summarized in Table 3.3.

Table 3.1 Calibration curve for chloroquine phosphate in 0.01N hydrochloric acid.

Sr. No.	Concentration µg / ml	Mean Absorbance ± SD*	Regressed value
1	8	0.336 ± 0.001033	0.325
2	10	0.418 ± 0.001941	0.404
3	12	0.481 ± 0.00216	0.483
4	15	0.628 ± 0.002898	0.602
5	20	0.78 ± 0.001871	0.801

Regression Equation++ $Y = 0.0397x + 0.0069$

Correlation coefficient = 0.9989

* Mean of six values

++ n = 30

Figure 3.1 Calibration curve for chloroquine phosphate in 0.01N hydrochloric acid.

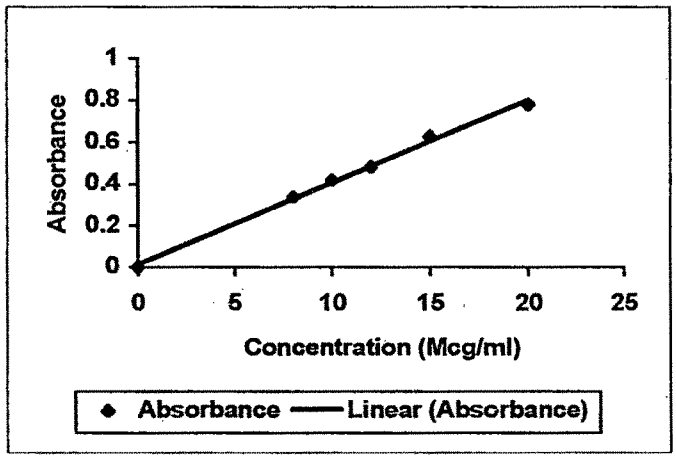

Figure 3.2 Absorptivity scan of chloroquine phosphate in 0.01N HCl (10 μg/ml)

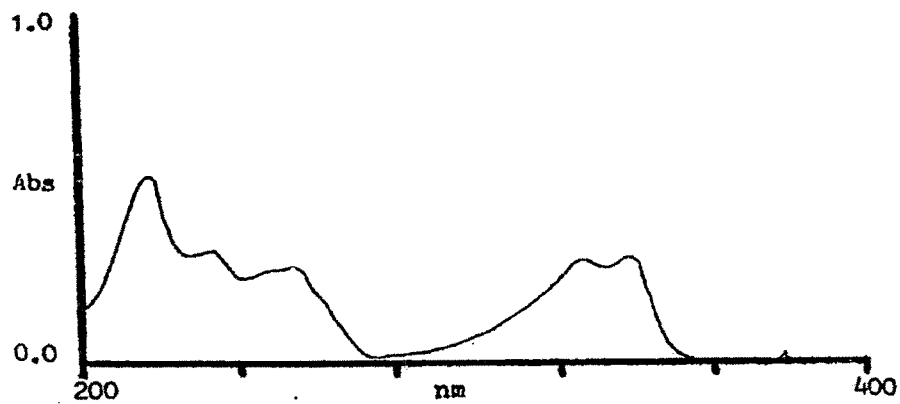

Table 3.2 Optical characteristics for chloroquine phosphate in 0.01N hydrochloride acid.

Sr. No.	Characteristics	Value
1	Absorption maxima (nm)	219,255,325,342.5*
2	Beer's law limits at 343 nm (µg /ml)	1-20
3	Apparent molar absorptivity at 343 nm (1 mol^{-1} cm^{-1})	20090.85

Table 3.3 Evaluation of accuracy & precision of the method for estimation of chloroquine phosphate in 0.01N hydrochloride acid.

Added (mg)	Found (mg) ±SD[a]	Coefficient of variation (CV)	Standard error	Confidence limits[b]
25	24.41 ± 0.98	4.05	0.0236	24.41 ± 1.109
50	50.95 ± 2.32	4.56	0.0190	50.95 ± 2.625
75	78.27 ± 3.27	4.18	0.0436	78.27 ± 3.270

a : n = 3

b = confidence limits at P = 0.95 and two degree of freedom

3.1.4 Estimation of chloroquine phosphate in phosphate buffered saline pH 7.4 (PBS)

This method was developed based on the observation that chloroquine phosphate in PBS shows strong absorbance in the ultraviolet region of the electromagnetic spectrum.

3.1.4.1 Solutions

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

(B) Stock solution of chloroquine phosphate in PBS (100 µg / ml) was prepared by dissolving 10 mg of chloroquine phosphate in 10 ml PBS and diluting 1 ml of this solution to 10 ml with PBS.

3.1.4.2 Procedure for Calibration Curve :

Suitable aliquots (0.1 – 2 ml) of the 100 µg/ml stock solution of chloroquine phosphate in PBS were pipetted in to 10 ml volumetric flasks. The volume was made up with PBS, the contents shaken well and absorbance measured at 343 nm using a Chemito UV 2600 Spactrascan using quartz cells of 10 mm path length & PBS as a blank. The above procedure was repeated six times. The measured and calculated parameters for the method are shown in table 3.4. The optical characteristics for the solution of chloroquine phosphate in PBS are shown in table 3.5. Absorptivity of 200nm to 400nm for a 10 µg/ml solution of chloroquine phosphate in phosphate buffered saline (pH 7.4) shown in Figure 3.4.

3.1.4.3 Accuracy and Precision:

Recovery studies were carried out, in triplicate, with known amounts of chloroquine phosphate, using the procedure detailed above, in three replicates to determine the precision and accuracy of the method. The analytical results obtained from these investigations are summarized in table 3.6.

Table 3.4 Calibration curve for chloroquine phosphate in PBS

Sr.No.	Conc. µg / ml	Mean Absorbance ± SD	Regressed Values
1	2	0.068 ± 0.00301	0.067
2	4	0.141 ± 0.0016	0.138
3	8	0.277 ± 0.0019	0.278
4	10	0.344 ± 0.0026	0.348
5	16	0.557 ± 0.0023	0.558
6	20	0.701 ± 0.0106	0.698

Regression Equation^{++} $Y = 0.035x + 0.0023$
Correlation coefficient = 0.9999
* Mean of six values
++ n = 36

Figure 3.3 Calibration curve for chloroquine phosphate in PBS

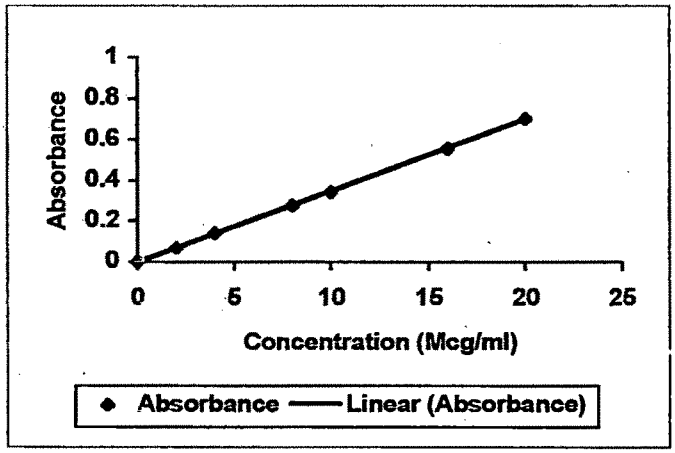

Figure 3.4 Absorptivity scan of chloroquine phosphate in PBS (10μg/ml)

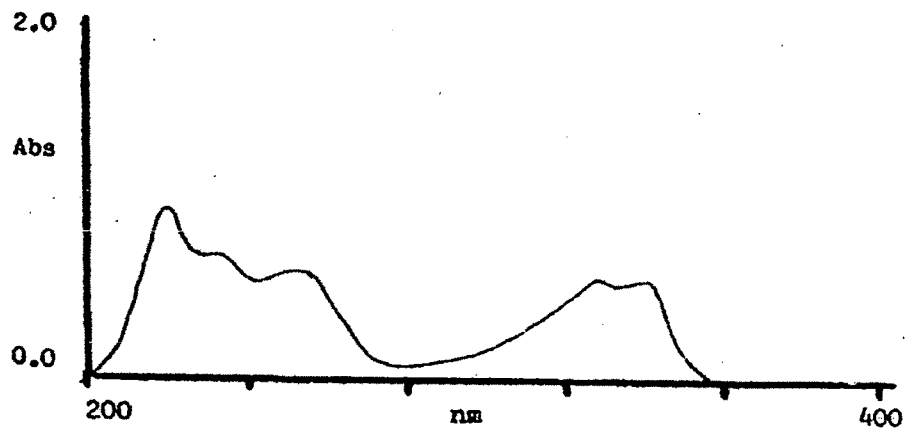

Table 3.5 Optical Characteristics for Chloroquine Phosphate in PBS

Sr.No.	Characteristics	Value
1	Absorption maxima (nm)	220,254,328 & 341 *
2	Beer's Law limits at 343 nm (µg / ml)	1-20
3	Apparent molar absorptivity at 343 nm (1 mol^{-1} cm^{-1})	18056

* Analytical wavelength ≅343 nm

Table 3.6 Evaluation of accuracy & precision of the method for estimating chloroquine phosphate in PBS

Added (mg)	Found (mg) ± SD[a]	Coefficient of variation (CV)	Standard error	Confidence limits[b]
25	24.41 ± 0.98	4.05	0.565	24.41 ± 1.10
50	50.95 ± 2.32	4.56	1.34	50.95 ± 2.04
75	78.27 ± 3.27	4.18	1.88	78.27 ± 3.11

a : n = 3
b = confidence limit at p = 0.95 & 2 degrees of freedom.

3.1.5 Estimation of Chloroquine Phosphate in rat blood & tissues :

This method was developed based on the observation that successive combined extraction was estimated by UV Visible Spectroscopy Chloroquine phosphate was estimated in rat blood, lung, liver, heart, kidney & spleen tissues.

3.1.5.1 Solutions :

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

(B) Heparin solution (100 I.U. / ml) was prepared by diluting 0.5 ml of a 5000 I.U. / ml injection (Beparine) with normal saline to 25 ml.

(C) Trichloroacetic acid (TCA) solution (5 % w/v) was obtained by dissolving 5 g of TCA in 100 ml distilled water.

(D) Sodium hydroxide (1 N) was prepared as per the procedure given in the Indian Pharmacopoeia (1985).

(E) Saturated sodium chloride solution : Sodium chloride was dissolved in water with heating to form a supersaturated solution. This solution was then cooled to room temperature & filtered to get the required saturated solution.

(F) Hydrochloride acid (0.01 N) was prepared as per the procedure given in the Indian Pharmacopoeia (1985).

(G) Stock solution of chloroquine phosphate (100 µg / ml) was obtained by dissolving 10 mg of chloroquine phosphate in 10 ml PBS and diluting 1 ml of this solution to 10 ml with PBS, obtained solution was further diluted 1 ml to 10 ml (10 µg / ml). This solution was diluted appropriately to obtain required concentration.

3.1.5.2 Tissue Preparation :

Spargue Dawley albino rats of either sex weighing between 225- 250 g, were anaesthetized by inhaling chloroform. The carotid artery was cannulated & blood collected in a heparinized vial. The organs of interest i.e. lung, liver, heart, kidney & spleen were removed, & divested of all extraneous tissue. The organs were then blotted thoroughly using filter paper, weighed and then minced with scissors. Wash of the minced tissues was then homogenized in PBS using a Teflon pestle & glass homogenizer to a concentration of 10 % w/ v of tissue.

3.1.5.3 Procedure for calibration curve

0.1 ml of blood or 0.2 ml of the tissue homogenate (lung, liver, heart, kidney & spleen) was taken in centrifuge tubes. To this was added 0.1 - 1.2 ml of appropriate stock solution of chloroquine phosphate corresponding to 200 to 1200 ng / ml of chloroquine phosphate obtained. The volume of the contents of each tube was made up to 2.5 ml with PBS. The contents were mixed by vigorous vortexing for 60 second & then the centrifuge tubes were

allowed to stand to 30 minutes for equilibrium to be attained. At the end of the time period, 0.5 ml of 5 % w/v TCA solution was added to each tube as a protein precipitant followed by addition of 1 ml of 1 N sodium hydroxide solution to render the contents alkaline (≅ PH 9), and 1 ml of saturated sodium chloride solution to prevent emulsion formation. The drug was then extracted with 2, 2 & 1 ml of dichloromethane by vigorous vortexing for 60 seconds followed centrifugation at 3000 rpm in a centrifuge for 10 minutes and collection of the bottom organic layer, containing the drug, using a syringe and needle (2 ml glass syringe, 18 gauge needle).

The combined organic extracts of each tubes were then subjected to evaporate organic solvent at 50°C on thermostatically controlled water bath. The residues were reconstituted with 0.01 N hydrochloric acid volume was made up to 10 ml with the same. The absorbance measured at 343 nm using a blank prepared in the same manner by omitting the drug. The above procedure was repeated six times. Mean absorbance values along with the regressed values (method of least squares) are shown in table 3.7 for the extracts from rat blood, lung, liver, heart, kidney & spleen respectively. The optical characteristics for these acid extract are shown in table 3.8.

Absorptivity scan over the wavelength range of 200 to 400 nm for 10 µg / ml acid extracts from rat blood, lung, liver, heart, kidney & spleen are shown in figure 3.11 to 3.16.

3.1.5.4 Stability and Selectivity

Stability of the acid extracts, used in the preparation of calibration curves was determined by monitoring the changes in their absorbance at the analytical wavelength at predetermined intervals of time over a period of 24 hours.

The selectivity of this method for chloroquine phosphate in rat blood & tissues of interest was investigated by carrying out the procedure detailed above in the presence of the other components of the microspheres at the levels at which these materials were included in the microspheres.

3.1.5.5 Accuracy and Precision :

In order to determine the precision and accuracy of the method, blood & tissues samples containing known, added amounts of chloroquine phosphate were analyzed, using the procedure detailed above in three replicates. The analytical results obtained from these investigations are summarized in table 3.9.

Table 3.7 **Calibration Curve for chloroquine phosphate extracted from rat blood, lung, liver, heart, kidney & spleen in to 0.01 N hydrochloric acid.**

	Mean ABS (± SD)						Regressed Value					
Conc ng/ml	Blood	Lung	Liver	Heart	Kidney	Spleen	Blood	Lung	Liver	Heart	Kidney	Spleen
200	0.028 ± 0.012	0.024 ± 0.015	0.028 ± 0.008	0.022 ± 0.004	0.024 ± 0.001	0.032 ± 0.001	0.034	0.022	0.020	0.021	0.016	0.026
400	0.060 ± 0.004	0.048 ± 0.010	0.050 ± 0.010	0.040 ± 0.006	0.040 ± 0.002	0.060 ± 0.004	0.052	0.042	0.040	0.039	0.036	0.046
800	0.090 ± 0.002	0.069 ± 0.026	0.098 ± 0.002	0.071 ± 0.003	0.080 ± 0.005	0.101 ± 0.004	0.088	0.082	0.080	0.075	0.076	0.086
1000	0.110 ± 0.008	0.098 ± 0.008	0.150 ± 0.010	0.090 ± 0.007	0.120 ± 0.008	0.140 ± 0.005	0.106	0.102	0.100	0.093	0.096	0.126
1200	0.124 ± 0.007	0.130 ± 0.090	0.140 ± 0.008	0.105 ± 0.010	0.153 ± 0.009	0.160 ± 0.008	0.124	0.122	0.120	0.111	0.116	0.126

Regression Equation [++]	Correlation Coefficient
in rat blood $Y = 0.9 \times 10^{-4} x + 0.0157$	0.983
in rat Lung $Y = 0.1 \times 10^{-3} x + 0.0016$	0.9747
in rat Liver $Y = 0.0001x + 0.0003$	0.9905
in rat Heart $Y = 0.9 \times 10^{-4} x + 0.003$	0.9973
in rat Kidney $Y = 0.0001x - 0.0042$	0.9793
in rat Spleen $Y = 0.0001x + 0.0063$	0.9848

Figure 3.5 Calibration Curve for chloroquine phosphate extracted from rat blood into 0.01 N hydrochloric acid.

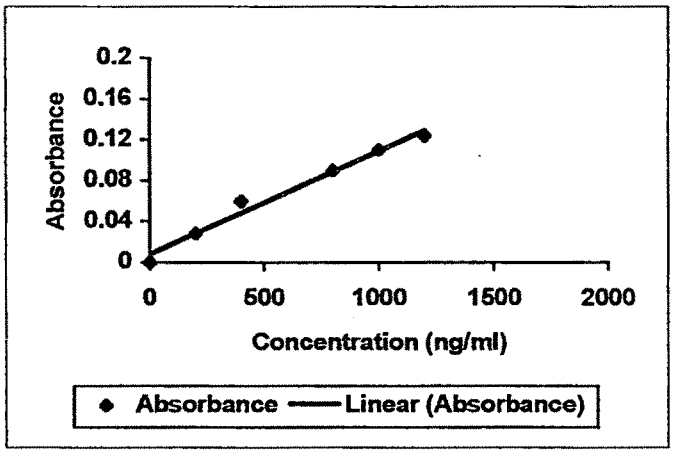

Figure 3.6 Calibration Curve for chloroquine phosphate extracted from rat lung into 0.01 N hydrochloric acid.

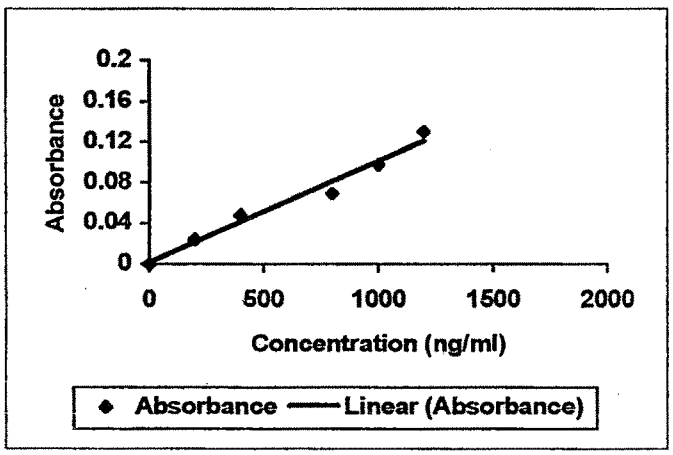

Figure 3.7 Calibration Curve for chloroquine phosphate extracted from rat liver into 0.01 N hydrochloric acid.

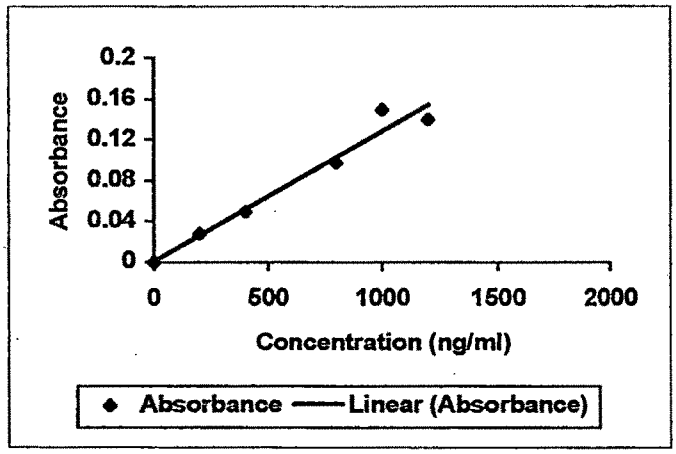

Figure 3.8 Calibration Curve for chloroquine phosphate extracted from rat heart into 0.01 N hydrochloric acid.

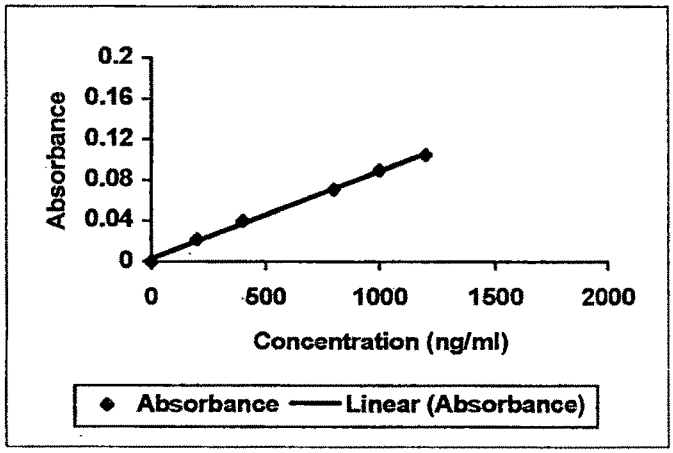

Figure 3.9 Calibration Curve for chloroquine phosphate extracted from rat kidney into 0.01 N hydrochloric acid.

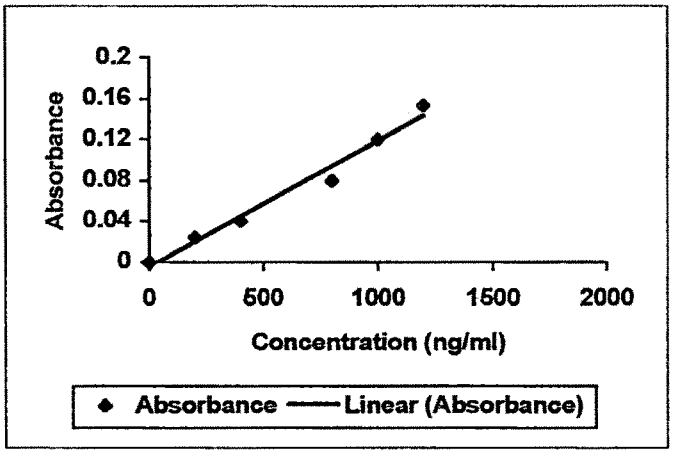

Figure 3.10 Calibration Curve for chloroquine phosphate extracted from rat spleen into 0.01 N hydrochloric acid.

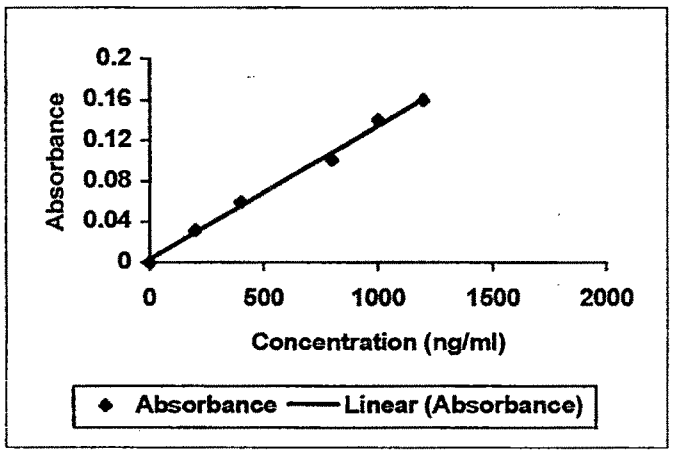

Figure 3.11 Absorptivity scan of chloroquine phosphate extracted from rat blood into 0.01N hydrochloric acid (10μg/ml)

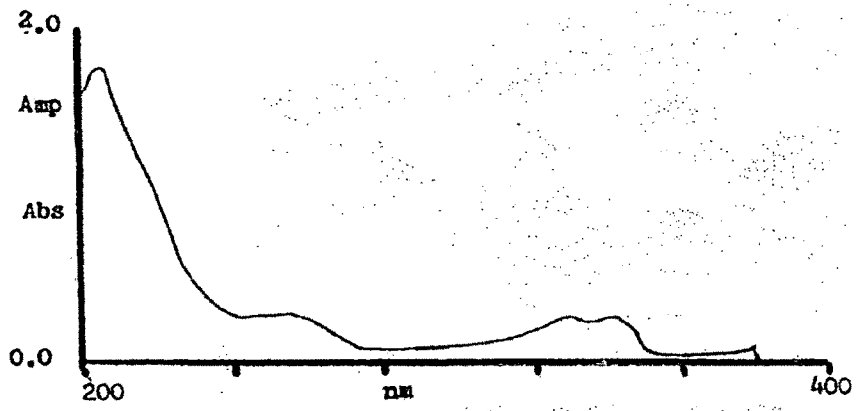

Figure 3.12 Absorptivity scan of chloroquine phosphate extracted from rat lung into 0.01N hydrochloric acid (10μg/ml)

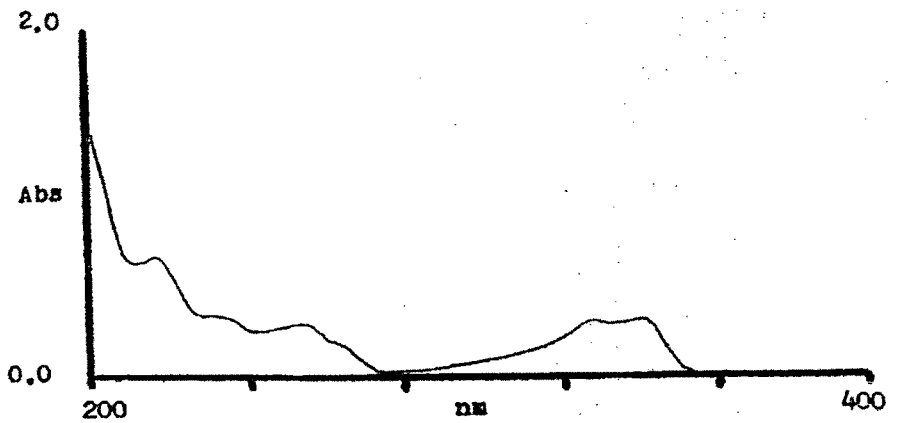

Figure 3.13 Absorptivity scan of chloroquine phosphate extracted from rat liver into 0.01N hydrochloric acid (10μg/ml)

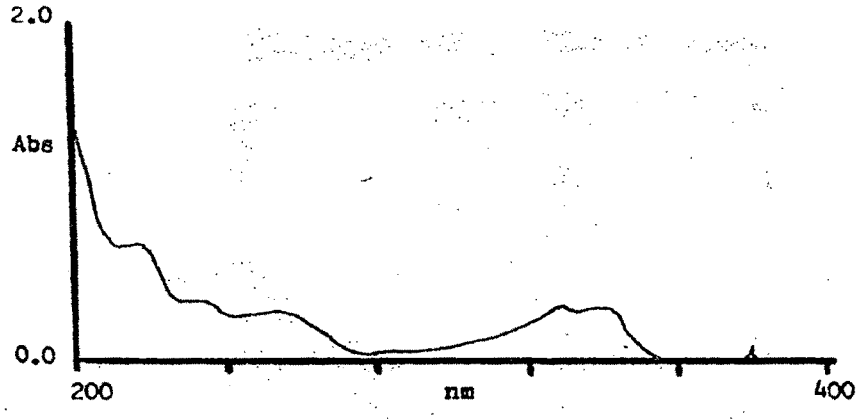

Figure 3.14 Absorptivity scan of chloroquine phosphate extracted from rat heart into 0.01N hydrochloric acid (10μg/ml)

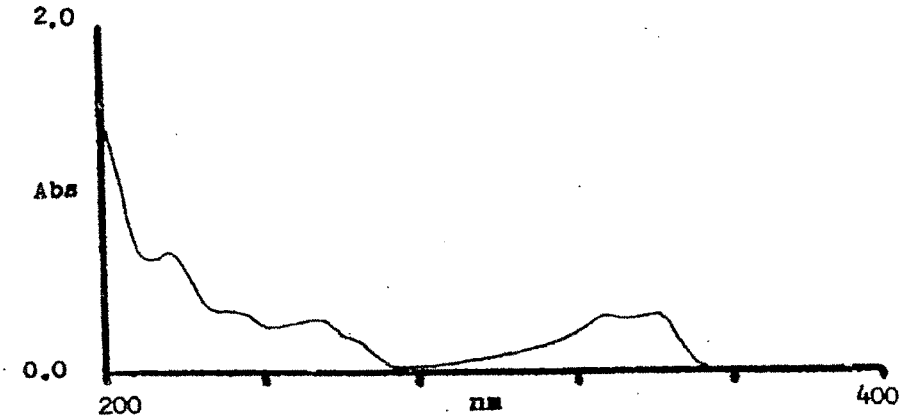

Figure 3.15 Absorptivity scan of chloroquine phosphate extracted from rat kidney into 0.01N hydrochloric acid (10μg/ml)

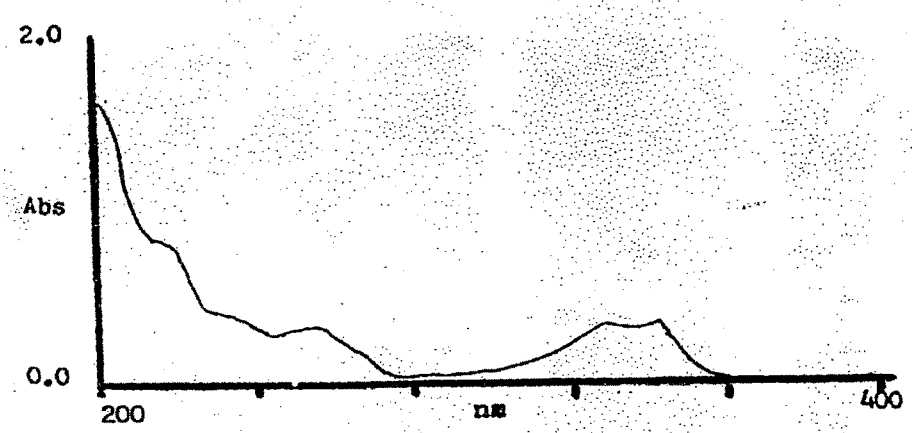

Figure 3.16 Absorptivity scan of chloroquine phosphate extracted from rat spleen into 0.01N hydrochloric acid (10μg/ml)

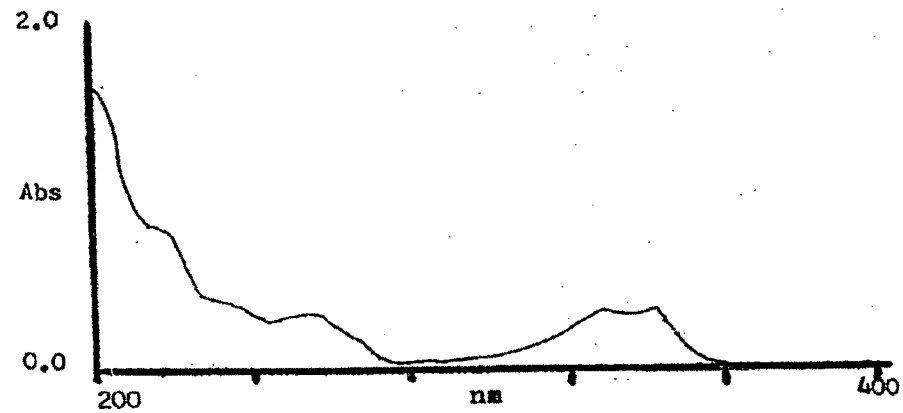

Table 3.8 Optical characteristics for chloroquine phosphate extracted from rat blood, liver, heart, kidney & spleen into 0.01N hydrochloric acid.

Characteristic	Value					
	Blood	Lung	Liver	Heart	Kidney	Spleen
Absorption maxima	218, 254, 328 and 341* nm					
Beer's law limits at 343 nm (µg/ml)	**0.2 - 1.2**	**0.2 - 1.2**	**0.2 - 1.2**	**0.2 - 1.2**	**0.2 - 1.2**	**0.2 - 1.2**
Apparent molar absorptivity (S) at 343nm ($1\ mol^{-1}\ cm^{-1}$)	**53232.1**	**55807.9**	**60100.8**	**45075.63**	**65681.63**	**68686.66**

Table 3.9 Evaluation of the accuracy and precision of the method for estimating chloroquine phosphate in rat blood & lung, liver, heart, kidney &spleen

Blood

Added (µg)	Found (µg) ± SD[a]	Coefficient of variation (cv)	Standard error	Confidence limit[b]
25	22.5 ± 1.08	4.83	0.63	22.5 ± 1.22
50	47.8 ± 1.87	3.92	1.08	47.8 ± 2.11
75	73.3 ± 4.12	5.61	2.30	73.3 ± 4.66

Lung

Added (µg)	Found (µg) ± SD[a]	Coefficient of variation (cv)	Standard Error	Confidence limit[b]
25	23.8 ± 1.24	5.23	0.716	23.8 ± 1.40
50	47.5 ± 2.31	4.85	1.334	47.5 ± 2.61
75	74.5 ± 3.04	4.56	1.756	74.5 ± 3.44

Liver

Added (µg)	Found (µg) ± SD[a]	Coefficient of variation (cv)	Standard Error	Confidence limit[b]
25	22.4 ± 0.524	2.34	0.303	22.4 ± 0.592
50	46.3 ± 1.56	3.39	0.901	46.3 ± 1.765
75	71.8 ± 3.10	4.32	1.789	71.8 ± 3.507

Heart

Added (µg)	Found (µg) ± SD[a]	Coefficient of variation (cv)	Standard Error	Confidence limit[b]
25	24.8 ± 0.89	3.58	0.52	24.8 ± 1.007
50	48.3 ± 2.1	4.35	1.21	48.3 ± 2.376
75	72.9 ± 2.9	3.90	1.67	72.9 ± 3.282

Kidney

Added (µg)	Found (µg) ± SD[a]	Coefficient of variation (cv)	Standard error	Confidence limit[b]
25	23.3 ± 1.08	4.60	0.62	23.3 ± 1.222
50	51.5 ± 1.88	3.65	1.09	51.5 ± 2.128
75	72.6 ± 3.05	4.25	1.76	72.6 ± 3.450

Spleen

Added (µg)	Found (µg) ± SD[a]	Coefficient of variation (cv)	Standard error	Confidence limit[b]
25	23 ± 1.5	6.52	0.87	23 ± 1.698
50	48 ± 2.99	4.15	1.15	48 ± 2.252
75	72 ± 3.2	4.44	1.85	72 ± 3.621

a : n = 3

b : confidence limits at p = 0.95 and 2 degrees of freedom.

3.1.6 Estimation of Mefloquine Hydrochloride in methanol

This method was developed based on the observation that mefloquine hydrochloride in methanol shows high absorbance in the ultraviolet region of the electro magnetic spectrum.

3.1.6.1 Solutions :

Stock solution of mefloquine hydrochloride (mg/ml) was prepared by dissolving 10 mg of mefloquine hydrochloride in 10 ml of methanol (A) Further dilution 1 ml of 10 ml (100 µg / ml) (B)

3.1.6.2 Procedure for calibration curve :

Suitable aliquots (0.1 to 1.0 ml) of stock solutions A or B were pipetted into 10 ml volumetric flasks. The volume was made up with methanol the contents shaken well and the absorbance measured at 283 nm using a Chemito UV 2600 Spectrascan with quartz cells of 10 mm path length against methanol as a blank. The above procedure was repeated six times. Table 3.10 tabulates the raw & regressed data (method of least squares).

Absorptivity scan over the wavelength range of 200nm to 400nm for a 20 μg/ml solution of mefloquine hydrochloride in methanol is shown in Figure3.18.

3.1.6.3 Stability and selectivity

Stability of the solutions of mefloquine hydrochloride in methanol, used for preparing the calibration curve, was ascertained by observing the changes in their absorbance at the analytical wavelength, over a period of 24 hours.

The above method for estimating mefloquine hydrochloride was carried out in the presence of microsphere components.

3.1.6.4 Accuracy and Precision :

In order to determine the precision and accuracy of the method, solutions containing known amounts of mefloquine hydrochloride were prepared and analyzed, using the procedure detailed above, in three replicates. The analytical results obtained from this investigation are summarized in table 3.12.

Table 3.10 Calibration curve for mefloquine hydrochloride in methanol

Sr.No.	Conc µg / ml	Mean Absorbance ± SD	Regressed Values
1	5.0	0.094 ± 0.00388	0.0887
2	10.0	0.174 ± 0.0030	0.174
3	15.0	0.265 ± 0.0023	0.259
4	20.0	0.351 ± 0.026	0.344
5	25.0	0.426 ± 0.0201	0.429

Regression equation $^{++}$ $Y = 0.017 X + 0.0037$
Correlation coefficient 0.997
* Mean of six values ++ n = 30

Figure 3.17 Calibration curve for mefloquine hydrochloride in methanol

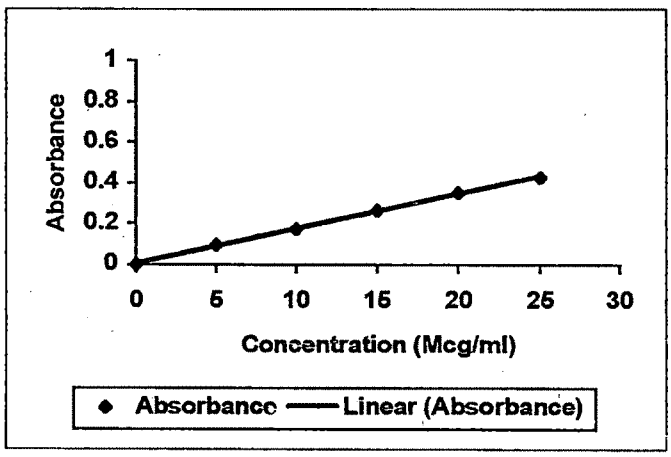

Figure 3.18 Absorptivity scan of mefloquine hydrochloride in methanol (20 μg/ml)

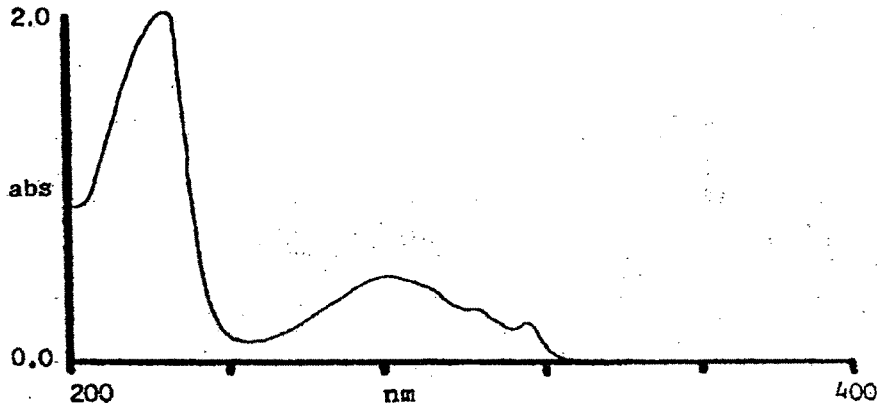

Table 3.11 Optical characteristics for mefloquine hydrochloride in methanol

Sr.No.	Characteristics	Value
1	Absorption maxima (nm)	222, 283*, 315
2	Beer's Law limits at 283 nm (µg / ml)	5 – 30
3	Apparent molar absorptivity at 283 nm (1 mol^{-1} cm^{-1})	7279

* Analytical wavelength ≅ 283 nm

Table 3.12 Evaluation of accuracy and precision of the method for estimating mefloquine hydrochloride from microspheres

Added (mg)	Found (mg) ± SD[a]	Coefficient of variation (CV)	Standard error	Confidence limit[b]
20	19.73 ± 0.32	1.62	0.0135	19.73 ± 0.362
25	25.05 ± 0.33	1.317	0.002	25.05 ± 0.370
30	29.65 ± 0.15	0.508	0.012	29.65 ± 0.169

a : n = 3
b : confidence limits at p = 0.95 and 2 degrees of freedom.

3.1.7 Estimation of Mefloquine Hydrochloride in Phosphate buffered saline pH 7.4 (PBS) :

This method was developed based on the observation that mefloquine hydrochloride in phosphate buffered saline, pH 7.4 (PBS) shows strong absorbance in the ultraviolet region of the electromagnetic spectrum.

3.1.7.1 Solutions :

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

(B) A 50 µg / ml stock solution of mefloquine hydrochloride was prepared by dissolving 5 mg of mefloquine hydrochloride in 10 ml PBS by Sonication using an ultrasonic processor trans -0-sonic Megnapak -250.

3.1.7.2 Procedure for Calibration Curve

Suitable aliquots (0.2 ml – 2.0 ml) of the 50 µg / ml stock solution of mefloquine hydrochloride in PBS were pipetted into 10 ml volumetric flasks. The volume was made up with PBS. The contents shaken well and the absorbance measured at 222 nm using a chemito U.V. 2600 Spectrascan with quartz cells of 10 nm path length against the solvent as a blank. The above procedure was repeated six times. Mean absorbance values along with the regressed values (methods of least squares) are shown in table 3.13, table 3.14 tabulate the optical characteristics for the solution of mefloquine hydrochloride in PBS. Absorptivity scan over the wavelength range of 200 nm to 400 nm for a 6 µg / ml solution of mefloquine hydrochloride in PBS is shown in Figure 3.20.

3.1.7.3 Stability and Selectivity :

Changes in absorbance of the solutions of mefloquine hydrochloride in PBS, used for preparing the calibration curve at the analytical wavelength over a period of 72 hours, was used as a means to study the stability of these solutions with respect to time mefloquine hydrochloride was estimated in the presence of the other constituents of microspheres present, in the same concentrations in which they were included in the microspheres, to obtain an understanding of the selectivity of the developed procedure for mefloquine hydrochloride.

3.1.7.4 Accuracy and Precision :

Known amounts of mefloquine hydrochloride were subjected to recovery studies, using the procedure detailed above, in three replicates, to determine the precision and accuracy of the method. The analytical results obtained from these investigations are summarized in Table 3.15.

Table 3.13 Calibration curve for mefloquine hydrochloride in PBS

Sr. No.	Conc. µg/ml	Mean Abs ± SD*	Regressed values
1	2	0.227 ± 0.0367	0.236
2	3	0.355 ± 0.00367	0.361
3	4	0.478 ± 0.0025	0.487
4	6	0.727 ± 0.0027	0.738
5	7	0.875 ± 0.0034	0.863
6	8	0.994 ± 0.0027	0.989

Regression Equation++ Y = 0.1255x - 0.0154
Correlation coefficient 0.9991
* Mean of six values
++ n = 36

Figure 3.19 Calibration curve for mefloquine hydrochloride in PBS

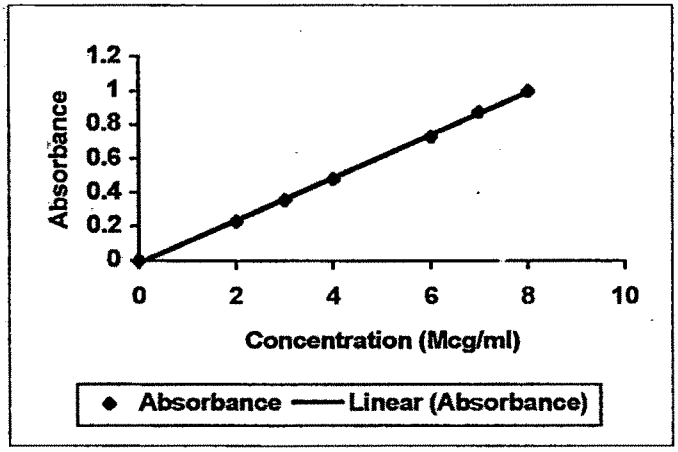

Figure 3.20 Absorptivity scan of mefloquine hydrochloride in PBS (6 μg/ml)

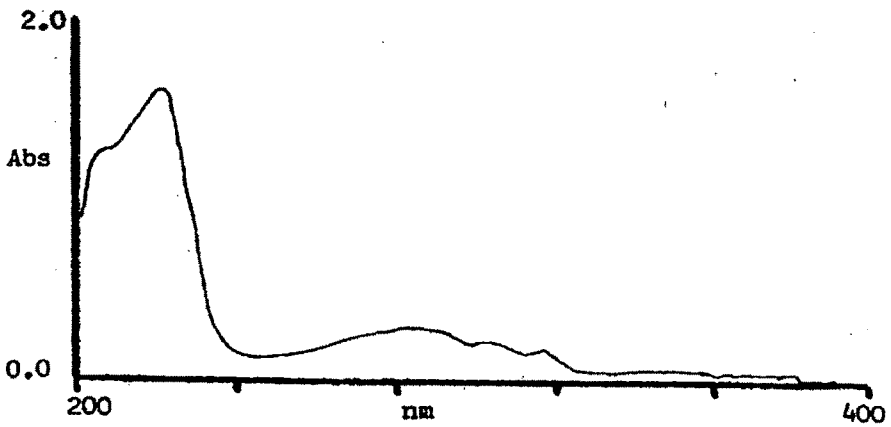

Table 3.14 Optical characteristics for mefloquine hydrochloride in PBS

Characteristic	Value
Absorption maxima (nm)	223.5*, 286.5, 302.5, 316.5
Beer's law limit at 222nm (µg/ml)	1 – 8
Apparent molar absorptivity at 222 nm (1 mol^{-1} cm^{-1})	50259.9

* Analytical wavelength ≅ 222nm

Table 3.15 Evaluation of accuracy & precision of the method for estimation of mefloquine hydrochloride in PBS

Added Mg	Found (mg) ± SD[a]	Coefficient of variation (cv)	Standard error	Confidence limit[b]
10	10.07 ± 0.55	5.4	0.3	10.07 ± 0.622
20	20.50 ± 0.95	4.6	0.55	20.50 ± 1.075
30	32.52 ± 1.50	4.6	0.86	32.52 ± 1.697

a n = 3

b : confidence limits at p = 0.95 and 2 degrees of freedom.

3.1.8 Estimation of Mefloquine Hydrochloride in rat blood & tissues

This method was developed based on the observation that successive extraction combined with derivative spectroscopy can be used in estimating mefloquine hydrochloride in blood and tissues. Mefloquine hydrochloride was estimated in rat blood, lung, liver, heart, kidney & spleen.

3.1.8.1 Solutions :

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

[B] Heparin solution (100 IU/ml) was prepared by diluting 0.5ml of 5000 IU/ml injection (Beparine) with normal saline to 25ml.

[C] Trichloroacetic acid (TCA) solution (5% w/v) was obtained by dissolving 5g of TCA in 100ml distilled water.

[D] Sodium hydroxide (1N) was prepared as per the procedure given in the Indian Pharmacopoeia (1985).

[E] Saturated sodium chloride solution was prepared in the manner described in section 3.1.6.1 [E]

[F] Hydrochloric acid (0.1N) was prepared as per the procedure given in the Indian Pharmacopoeia (1985).

[G] Stock solution of mefloquine hydrochloride (50 µg/ml) & 5 µg/ml were prepared by dissolving 5mg of mefloquine hydrochloride in 100ml PBS with the aid of Sonication. It was further diluted 1ml to 10ml to get 5 µg/ml.

3.1.8.2 Tissue Preparation

Spargue Dawley albino rats of either sex, weighing between 225 – 250 gms, were anaesthetized by inhaling chloroform. The carotid artery was cannulated & blood collected in a heparinized vial. The organs of interest i.e. lung, liver, heart, kidney & spleen were removed & divested of all extraneous tissue. The organs were then blotted thoroughly using filter weighed and then minced with scissors. Each of the minced tissues was then homogenized in PBS using a Teflon pestle & glass homogenizer to a concentration of 10% w/v of tissue.

3.1.8.3 Procedure for calibration curve

0.2ml of the tissue homogenate (lung, liver, heart, kidney & spleen) or 0.1ml of blood was taken in centrifuge tubes. To this was added 0.1 – 1 ml of appropriate stock solution to obtain 150 – 750 ng / ml of mefloquine hydrochloride. The volume of the contents of each tube was made upto

1.5ml with PBS. The contents were mixed by vigorous shaking for 60 seconds allow to stand for 30 minutes to attain equilibrium conditions. At the end of this time period, 0.5ml of 5 % w/v TCA solution was added to each tube as a protein precipitant followed by centrifugation at 3000 rpm in Remi centrifuge for 10 minutes & collection of the bottom organic layer with aid of a syringe and needle (18 gauge). The combined organic extract was allowed to evaporate. Residue were reconstituted with 5ml 0.1M hydrochloric acid & subjected to derivative spectroscopy. The amplitude of the peak at 228nm of the third derivative spectrum was measured on a Chemito UV 2600 spectra scan using quartz cells of 10mm path length, against a blank prepared in the same manner as above but omitting the drug. The above procedure was repeated six times. Mean amplitude values along with the regressed values (method of least squares) are shown in Table 3.16 for rat blood, lung, liver, heart, kidney & spleen. The optical characteristics for the acid extract solutions are shown in Table 3.17 for rat blood, lung, liver, heart, kidney & spleen. Figures 3.27 to 3.32 shows the third derivative spectra for the drug extracted into 0.1N hydrochloric acid from rat blood & tissues.

3.1.8.4 Stability & Selectivity

Stability of the acid extracts, used for the preparation of the calibration curves was ascertained by observing the changes in their absorbances at the analytical wavelength over a period of 24 hours.

The selectivity of this method for mefloquine hydrochloride in rat blood, & in the tissues of interest was investigated by carrying out the procedure detailed above in the presence of potential interferences present in microspheres.

3.1.8.5 Accuracy and Precision

In order to determine the precision & accuracy of the method, blood & tissue samples containing known, added amounts of mefloquine hydrochloride were analyzed, using the procedure detailed above, in three replicates. The analytical results obtained from these investigations are summarized in Table 3.18 for rat blood & tissues.

Table 3.16 Calibration curve for mefloquine hydrochloride extracted from rat blood, lung, liver, heart, kidney & spleen into 0.1N hydrochloric acid

	Mean ABS (± SD)**						Regressed Value					
Conc ng/ml	Blood	Lung	Liver	Heart	Kidney	Spleen	Blood	Lung	Liver	Heart	Kidney	Spleen
150	0.035 ± 0.0015	0.032 ± 0.0088	0.036 ± 0.0012	0.030 ± 0.0024	0.033 ± 0.0018	0.035 ± 0.0011	0.028	0.031	0.0324	0.0304	0.031	0.027
300	0.060 ± 0.0082	0.068 ± 0.0043	0.078 ± 0.004	0.054 ± 0.0039	0.059 ± 0.0036	0.058 ± 0.0028	0.058	0.061	0.0624	0.057	0.061	0.057
450	0.108 ± 0.0098	0.099 ± 0.0064	0.120 ± 0.0019	0.101 ± 0.0058	0.089 ± 0.0044	0.099 ± 0.0024	0.088	0.0914	0.0928	0.0872	0.0914	0.087
600	0.130 ± 0.013	0.126 ± 0.0011	0.140 ± 0.0029	0.123 ± 0.0078	0.123 ± 0.0069	0.160 ± 0.0049	0.118	0.121	0.122	0.116	0.121	0.117
750	0.179 ± 0.0074	0.160 ± 0.0045	0.180 ± 0.0081	0.162 ± 0.0064	0.145 ± 0.0072	0.170 ± 0.0052	0.148	0.151	0.152	0.146	0.151	0.147

Regression Equation ++	Correlation Coefficient
in rat blood Y = 0.0002x - 0.0024	0.991
in rat Lung Y = 0.0002x + 0.0014	0.9983
in rat Liver Y = 0.0002x + 0.0028	0.9928
in rat Heart Y = 0.0002x – 0.0028	0.9926
in rat Kidney Y = 0.0002x + 0.0016	0.9976
in rat Spleen Y = 0.0002x – 0.003	0.9876

** Mean of six values

++ n = 30

Figure 3.21 Calibration curve for mefloquine hydrochloride extracted from rat blood into 0.1N hydrochloric acid

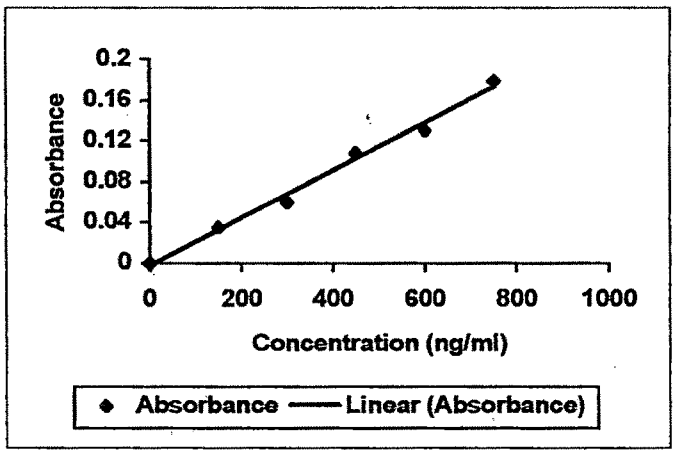

Figure 3.22 Calibration curve for mefloquine hydrochloride extracted from rat lung into 0.1N hydrochloric acid

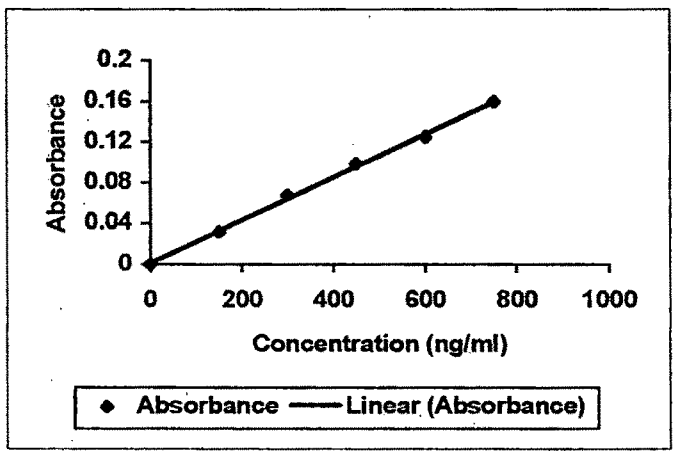

Figure 3.23 Calibration curve for mefloquine hydrochloride extracted from rat liver into 0.1N hydrochloric acid

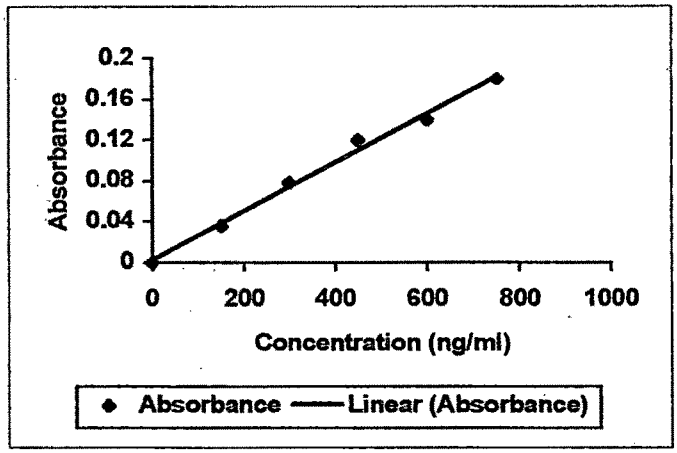

Figure 3.24 Calibration curve for mefloquine hydrochloride extracted from rat heart into 0.1N hydrochloric acid

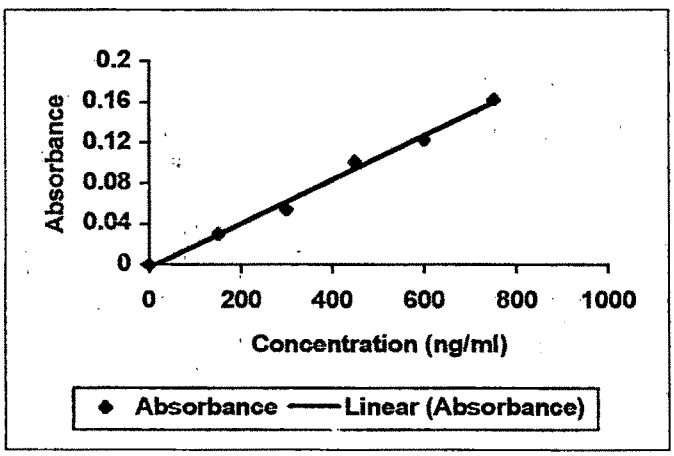

Figure 3.25 Calibration curve for mefloquine hydrochloride extracted from rat kidney into 0.1N hydrochloric acid

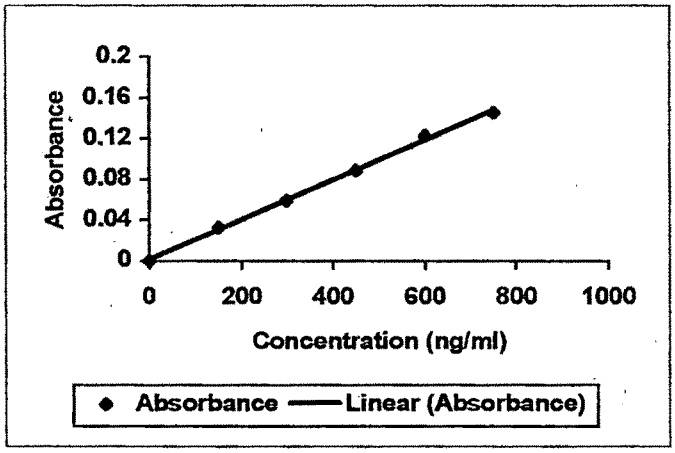

Figure 3.26 Calibration curve for mefloquine hydrochloride extracted from rat spleen into 0.1N hydrochloric acid

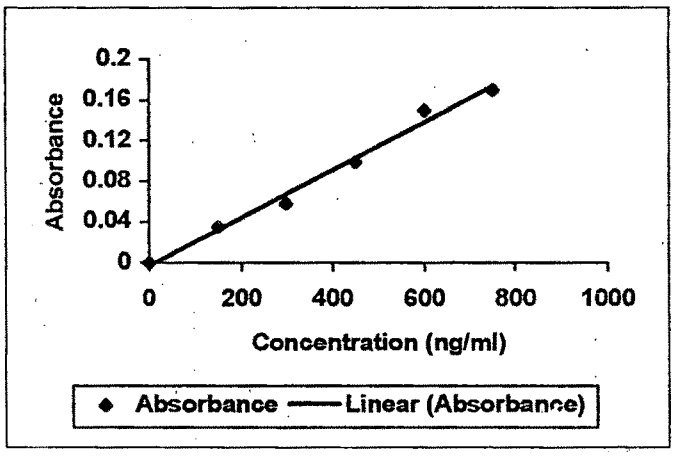

Figure 3.27 Third derivative spectrum of mefloquine hydrochloride extracted into 0.1N hydrochloric acid from rat blood (10 μg/ml)

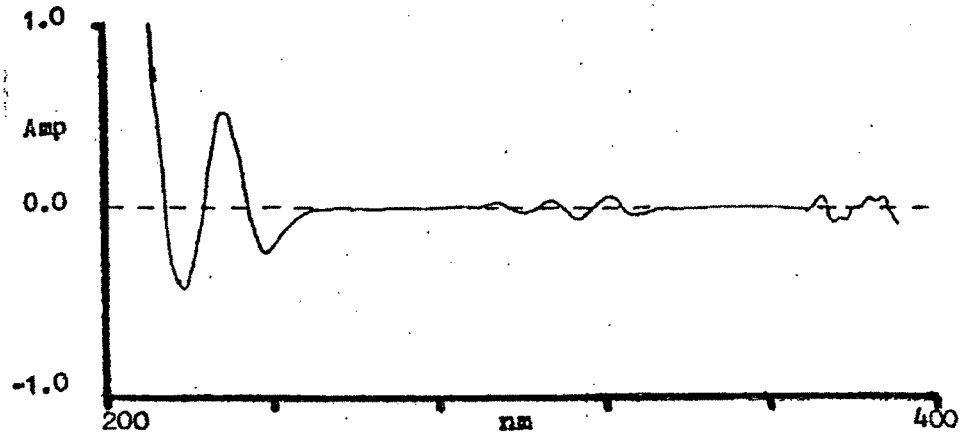

Figure 3.28 Third derivative spectrum of mefloquine hydrochloride extracted into 0.1N hydrochloric acid from rat lung (10 μg/ml)

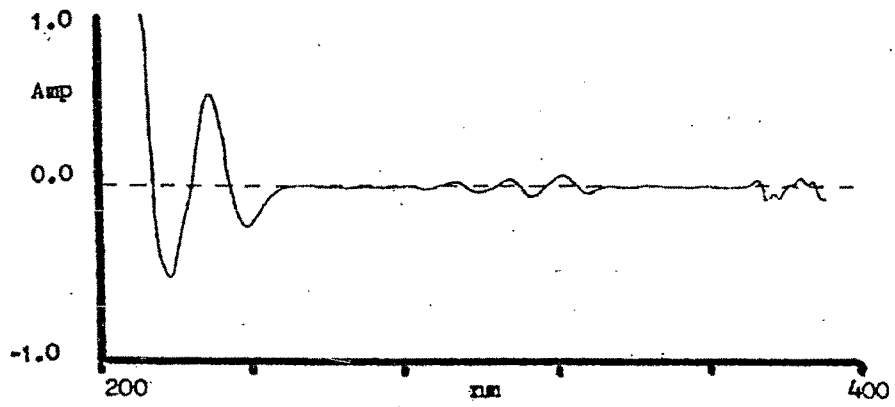

Figure 3.29 Third derivative spectrum of mefloquine hydrochloride extracted into 0.1N hydrochloric acid from rat liver (10 μg/ml)

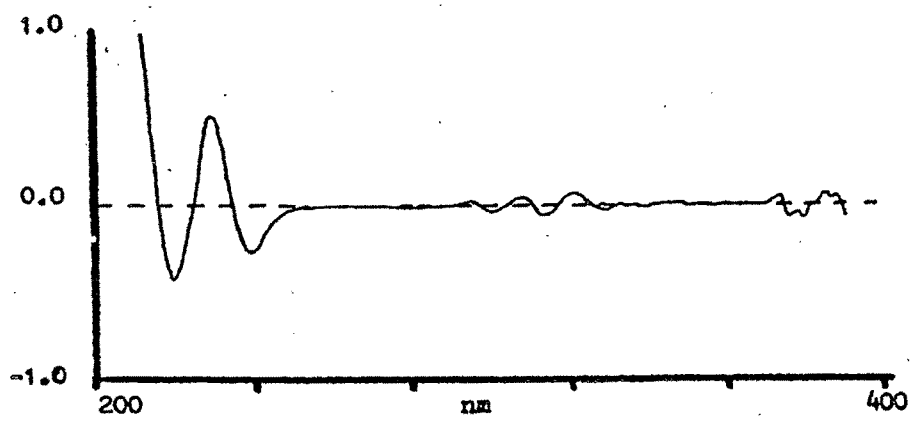

Figure 3.30 Third derivative spectrum of mefloquine hydrochloride extracted into 0.1N hydrochloric acid from rat heart (10 μg/ml)

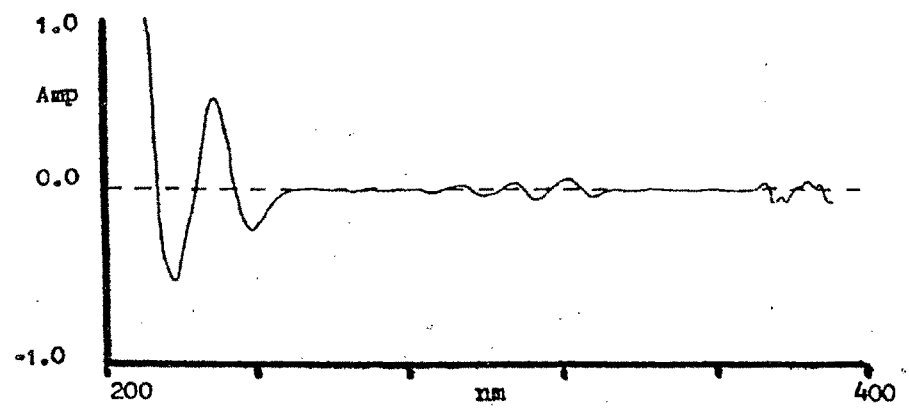

Figure 3.31 Third derivative spectrum of mefloquine hydrochloride extracted into 0.1N hydrochloric acid from rat kidney (10 μg/ml)

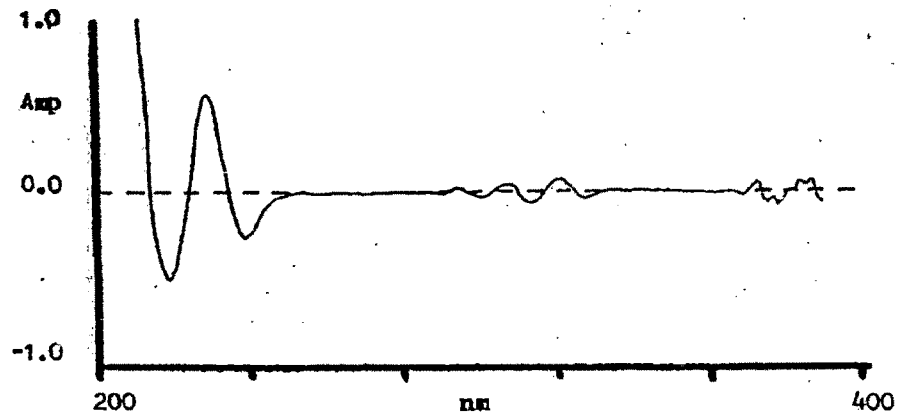

Figure 3.32 Third derivative spectrum of mefloquine hydrochloride extracted into 0.1N hydrochloric acid from rat spleen (10 μg/ml)

Table 3.17 Optical characteristics for mefloquine hydrochloride extracted from rat blood & other tissues.

Characteristic	Value					
	Blood	Lung	Liver	Heart	Kidney	Spleen
Absorption maxima	228.5*, 281.5 , 293.5, 306.5 & 320.5					
Beer's law limits at 228 nm (μg/ml)	**0.5 – 0.75**	**0.5 – 0.75**	**0.5 – 0.75**	**0.5 – 0.75**	**0.5 – 0.75**	**0.5 – 0.75**
Apparent molar absorptivity (S) at 228nm (1 mol^{-1} cm^{-1})	98998.9	88490.6	99552	89596.8	80194.67	94021.3

Analytical wavelength ≅ 228*

Table 3.18 Evaluation of the accuracy and precision of the method for estimating mefloquine hydrochloride in rat blood & other tissues.

Blood

Added (μg)	Found (μg) ± SD[a]	Coefficient of variation (cv)	Standard error	Confidence limit [b]
25	24.38 ± 1.01	4.14	0.58	2.274
50	48.85 ± 1.15	2.35	0.66	1.697
75	76.04 ± 2.0	2.63	1.54	2.26

Lung

Added (μg)	Found (μg) ± SD[a]	Coefficient of variation (cv)	Standard error	Confidence limit [b]
25	26.38 ± 1.8	6.8	1.03	2.036
50	48.04 ± 1.2	2.49	0.64	1.357
75	73.35 ± 2.5	3.4	1.44	2.828

Liver

Added (μg)	Found (μg) ± SD[a]	Coefficient of variation (cv)	Standard error	Confidence limit[b]
25	23.85 ± 0.98	4.10	0.56	1.108
50	48.30 ± 1.75	3.6	1.01	1.98
75	73.09 ± 3.1	4.2	1.78	3.50

Heart

Added (μg)	Found (μg) ± SD[a]	Coefficient of variation (cv)	Standard error	Confidence limit[b]
25	22.50 ± 1.03	4.58	0.59	1.165
50	46.80 ± 1.4	2.99	0.81	1.584
75	77.09 ± 3.1	4.02	1.78	3.507

Kidney

Added (μg)	Found (μg) ± SD[a]	Coefficient of variation (cv)	Standard error	Confidence limit[b]
25	27.5 ± 1.9	6.9	1.09	2.150
50	49.5 ± 1.2	2.4	0.69	1.357
75	72.5 ± 2.8	3.8	1.62	3.168

Spleen

Added (μg)	Found (μg) ± SD[a]	Coefficient of variation (cv)	Standard error	Confidence limit[b]
25	21.5 ± 1.8	8.37	1.04	2.037
50	46.5 ± 2.3	4.90	1.33	2.602
75	76.6 ± 2.41	3.15	1.39	2.727

3.1.9 Discussion

3.1.9.1 Estimation of chloroquine phosphate in microspheres.

The method used for estimating chloroquine phosphate in microspheres was based on a reported method wherein chloroquine phosphate in 0.01N hydrochloric acid showed strong absorbance in the ultraviolet region of the electromagnetic spectrum (Hong,1976). Chloroquine phosphate in 0.01N hydrochloric acid showed absorbance in the UV wavelength range between 200nm & 400nm (Figure 3.2), with absorption maxima at 219, 255, 328 and 342nm (Table 3.2) which agreed very well with the reported maxima at 222, 256, 328 and 343nm.

Since it was reported that measurements are most favorably made at 343nm where absorption is most intense and least affected by interfering substances (Hong,1976), this wavelength was chosen as the analytical wavelength. Absorptivity of chloroquine phosphate at 343nm was found to be 20090.85 $1mol^{-1}$ cm^{-1} (Table 3.2)

A correlation coefficient of 0.9989 (Table 3.1) indicated a strong, linear relation between the absorbance of the solution & its concentration. Beer's law was obeyed in the range of 1-20 μg/ml. The experimental data and the regression analysis performed on it (method of least squares) are shown in table 3.1. The regression equation obtained was

$Y = 0.0397x + 0.0069.$

The stability of the solutions of chloroquine phosphate in 0.01N hydrochloric acid was determined by noting the changes in the absorbance of the solutions, of different concentrations, at predetermined time intervals, over a period of 24 hours. The changes in absorbance was not significant at the level of ($P \leq 0.05$) for CQP in 0.01N hydrochloric acid for 24 hours.

In order to determine accuracy and precision of the method, known amounts of the drug were subjected to recovery studies, using the method, in

triplicate. The results of these studies are shown in Table 3.3. Accuracy of the method was ascertained by using the 't' test at each level. The computed 't' values at 20, 25 & 30 mg for the method are 0.318, 0.286 & 0.4084 respectively. These values were smaller than the tabulated 't' value of 4.30 ($P \leq 0.05$, 2 degree of freedom) indicating insignificant difference between the added and estimated quantity. The accuracy of the method is also studied by standard deviation, standard error and coefficient of variation. The low values of all these parameters (Table 3.3) signify the precision of this method.

Appreciate material balance was obtained between the amount of chloroquine added and that recovered from microspheres.

3.1.9.2. Estimation of chloroquine phosphate in phosphate buffered saline (pH 7.4) (PBS)

The estimation of chloroquine phosphate in PBS was based on the observation that chloroquine phosphate in PBS yields a characteristic curve range on being scanned in the UV wavelength range between 200 & 400 nm. The scan is shown in Figure 3.2. Absorption maxima are observed at 220, 254, 328 and 340 nm. The same method described earlier (Section 3.3.1) was used for estimating chloroquine phosphate in microspheres. Absorptivity of chloroquine phosphate was found to be 18056 l $mol^{-1}cm^{-1}$ (Table 3.5).

A correlation coefficient of 0.9999 (Table 3.4) confirmed the linear relationship between absorbance and concentration of the drug. Beer's law was found to be obeyed between 1-20 μg/ml (Table 3.5). Regression analysis was performed on the experimental data both of which are shown in Table 3.4. The regression equation was found to be

$$Y = 0.035x + 0.0023$$

The value of the slope, 0.035 (Table 3.4) indicates that the method is moderately sensitive which is well supported by the value of the apparent molar absorptivity of the compound at 343, Beer's plot is obeyed in the range of (1 - 20 μg/ml) (18056 $lmol^{-1}cm^{-1}$).

The stability of the solutions of chloroquine phosphate in phosphate buffered saline was determined by noting the changes in the absorbance of the solutions of different concentration at predetermined time intervals, over a period of 72 hours.

Accuracy and precision of the above method was determined using recovery studies of known amounts of the drug in triplicate. The results are tabulated in Table 3.6.Evaluation of the accuracy of the method was done using the 't' test at each level. The 't' values obtained from results at 25, 50 & 75 mg, for the methods were obtained 2.3, 1.45 & 1.9 respectively which is less than the table value (4.30) at the level of $P \leq 0.05$ and 2 degree of freedom. Results indicated no significant difference existed between the quantity estimated and the quantity taken for analysis.

The precision of the method was evaluated using standard deviation, confidence limit & coefficient of variation (Table 3.6). The low values of parameters indicate that the method is also precise.

Hence, the method was used to study the release of the drug from the microspheres of chloroquine phosphate in-vitro using an appropriate dialysis set up. It was found that the method gave an accurate and reproductive profile without the need to be modified.

3.1.9.3 Estimation of chloroquine phosphate in rat blood and tissues.

The method used for estimating chloroquine phosphate in rat blood & tissues i.e. lung, liver, heart, kidney & spleen was a modification of the reported procedure for the same (Hong 1976). The method involves conversion of the

salt to the base, extraction to separate it from the majority of the interfering materials and then reconstitution into an acidic solution to convert the base into a salt form and to remove any other interfering materials. Quantitative determination of the drug is then achieved by measuring the UV absorption of the acidic solution in the ultraviolet region of the electromagnetic spectrum.

The choice of dichloromethane as a solvent was dependent upon the fact that dichloromethane, being more non-polar than chloroform, would be a better solvent for chloroquine free base and also the subsequent extraction of drug into and acid would also become easy due to the limited solubility of chloroquine phosphate (the salt form) in dichloromethane extraction of the drug was performed with 2, 2 & 1ml of dichloromethane in accordance with the theory of extraction. Vortexing / shaking for 60 seconds followed by centrifugation at 3000 rpm for 10 minutes was found to be adequate for extraction as seen from the reproducibility of the readings & from the complete separation of aqueous and organic phases. The combined organic phase was then allowed to evaporate. The residues were reconstituted with 0.01N hydrochloric acid to free base into hydrochloride salt form & to eliminate impurities soluble in organic phase. The procedure was adequate in removing a majority of the impurities as can be seen from the concurrence of the absorptivity scans of the pure drug and extracted drug in acid solutions (Figure 3. 11-3.16).

Chloroquine phosphate extracted from rat blood and tissues into 0.01N hydrochloric acid showed the same absorbance characteristics in the ultraviolet region of the electromagnetic spectrum, as a solution of pure drug in the same solvent. On being scanned into the UV wavelength range between 200nm to 400nm (Figures 3.10 - 3.13), the acid extracts of the drug exhibit absorption maxima at 218, 254, 328 & 341nm (Table 3.8) which are

in close proximity to the maxima obtained for pure chloroquine phosphate in 0.01N hydrochloric acid i.e.219, 255, 328 & 342nm (Table 3.2) and to the reported maxima at 222nm, 256nm, 328nm & 343nm (Hong 1976).

This observation points to the success achieved in elimination of impurities during extraction of the drug from animal tissues. Measurements were made at 343nm as it has been reported that absorption shown by the molecule is most intense and least affected by interfering substances at this wavelength as already mentioned earlier. Absorptivity of chloroquine phosphate, extracted from rat blood, lung, liver, heart, kidney & spleen into 0.01N hydrochloric acid was calculated to be 53232.2, 65807.9, 60100.8, 45075.6, 65681.63 & 68686.66 1 $mol^{-1}cm^{-1}$ respectively at 343nm (Table 3.8). The values are quite close to each other and to the absorptivity of pure drug in acid .

Correlation coefficients for the data generated using rat blood, lung, liver, heart, kidney & spleen were 0.983, 0.9747, 0.9905, 0.9973, 0.9793 & 0.9848 respectively (Table 3.7) indicating a strong linear relationship between absorbance and concentration. Beer's law was found to be obeyed in the range of 200 to 1200 ng/ml in all types of tissues. The experimental data along with the results of regression analysis of the data are shown in Table 3.7.

$Y = 0.9 \times 10^{-4} X + 0.0157$ for drug extracted from rat blood

$Y = 0.1 \times 10^{-3} X + 0.0016$ for drug extracted from rat lung

$Y = 0.9 \times 10^{-4} X + 0.0003$ for drug extracted from rat liver

$Y = 0.9 \times 10^{-4} X + 0.003$ for drug extracted from rat heart

$Y = 0.1 \times 10^{-3} X - 0.0042$ for drug extracted from rat kidney

$Y = 0.1 \times 10^{-3} X + 0.0063$ for drug extracted from rat spleen

The low values of standard deviation of mean absorbance values of the solution used for preparing the calibration curve (Table 3.7) is a very important advantage when dealing with biological systems which are inherently quite variable. The values of the slope calculated were 0.9×10^{-4}, 0.1×10^{-3}, 0.1×10^{-3}, 0.9×10^{-4}, 0.1×10^{-3}, 0.1×10^{-3} for the curves prepared using rat blood, lung, liver, heart, kidney & spleen respectively (Table 3.7). The range of Beer's plot was 200 - 1200 ng/ml for all the curves. There were no interference of the impurities and the solvent at the wavelength of estimation of the drug.

The stability of extracts in acidic media (0.01N HCl) was ascertained over a period of 24 hours. The difference in the mean absorbance values of these extracts measured at different time intervals were insignificant which confirmed the stability of the analysis over a period of 24 hours during estimation.

The above method was used in the extraction and estimation of chloroquine phosphate in rat blood and other tissues in the presence of the other constituents of microspheres of chloroquine phosphate. No interferences from these materials were observed.

Accuracy and precision of the method was investigated by subjecting known amounts of chloroquine to recovery studies, in the presence of tissues, in triplicate using the method developed. Table 3.7 depicts the results obtained and the analysis thereof. Accuracy & precision of the method was ascertained using standard error, standard deviation, confidence limit & coefficient of variance.

3.1.9.4 Estimation of mefloquine hydrochloride in microspheres

Mefloquine hydrochloride in methanol yields a characteristic curve when scanned in the UV wavelength range between 200 to 400 nm. The scan (Figure 3.9) shows absorption maxima at 222, 283 & 315 nm (Table 3.11). Though the absorptivity of the compound was high at 222 nm, it was not selected as the analytical wavelength due to its proximity to the lower limit of UV transparent region of methanol (220nm). The absorptivity at 283 nm (7279 $1mol^{-1}cm^{-1}$, Table 3.11) was also found to be satisfactory and hence was selected as the analytical wavelength & used for further investigations. The ultraviolet spectrum of this compound can be attributed mainly to the quinoline nucleus in the mefloquine hydrochloride molecule (Lim 1985). The regression equation was found to be

$$Y = 0.017x + 0.0037$$

A correlation coefficient of 0.997 (Table 3.10) indicated that absorbance and concentration of the drug were linearly related. Beer's law was found to be obeyed between 5 to 25 µg/ml (Table 3.10). This slope of the regressed line 0.017 (Table 3.10) indicates moderate sensitivity of the method. There was little variability between the experimental & regressed values. This conclusion was based on the low value of the variance of the response variable. Absorptivity of mefloquine hydrochloride in methanol was calculated to be 7279 $1mol^{-1}cm^{-1}$ at 283nm shown (Table 3.11).

The mean absorbance values of the methanolic solutions of mefloquine hydrochloride at different concentrations of predetermined time intervals were determined. 't' test of these results showed no significant difference at level of $p \leq 0.05$ between the readings. Thus, mefloquine hydrochloride is stable over a period of 24 hours in methanol.

Using this method, estimation of mefloquine hydrochloride was carried out in the presence of other constituents of the microspheres. It was observed that these constituents did not interface with the estimation.

In order to determine the precision and accuracy of the method, known amounts of the drug were subjected to recovery studies, using the above method in triplicate. The results of these investigations are summarized in Table 3.12. The accuracy & precision of the method is confirmed by the low values of standard error & standard deviation, coefficient of variation. The low values of these parameters shown in Table 3.12 signify the precision of this method.

The method was used to estimate the amount of mefloquine hydrochloride entrapped in microspheres. Appreciate material balance was obtained between the amount of mefloquine hydrochloride added & that recovered from the microspheres signifying the suitability of the method for this application.

3.1.9.5 Estimation of mefloquine hydrochloride in phosphate buffer saline pH 7.4 (PBS)

Mefloquine hydrochloride in phosphate buffered saline, pH 7.4 (PBS) yields a characteristic curve when scanned in the UV wavelength range between 200 nm to 400 nm with the scan ((Figure 3.10) showing maxima at 221.5, 286.5, 302.5 and 316.5 nm (Table 3.14) led to the wavelength being chosen as the analytical wavelength. The ultraviolet spectrum of the compound, as seen above, can be attributed mainly to the quinoline nucleus in the mefloquine hydrochloride molecule (Lim 1985).

A correlation coefficient of 0.9991 (Table 3.13) indicated a linear relationship between absorbance and concentration of mefloquine hydrochloride. Beer's law was obeyed in the range of 2-10 μg/ml

(Table 3.13). Regression analysis of the experimental data was carried out. The experimental data along with the results of the regression analysis (method of least squares) are shown in Table 3.13. The regression equation $Y = 0.1255x - 0.0154$ was obtained.

This low variability of experiments is supported by the low value of standard error of the mean absorbance values of the solutions used for preparing the calibration curve. The slope value (0.1255) indicates high sensitivity of the method which is also reflected by the high absorptivity of the compound 50259.9 1 $mol^{-1}cm^{-1}$ (Table 3.14), the Beer's plot range was also narrow.

The blank (PBS) was not interfering in the absorbance measurements. The stability of the drug in PBS was ascertained over a period of 10 days. ANOVA analysis of the mean absorbance values of the solutions of different concentrations at various time intervals revealed that there was no significant difference between the readings. From this, it was concluded that mefloquine hydrochloride is stable over a period of 10 days in PBS.

Estimation of mefloquine hydrochloride was carried out, using the developed method, in the presence of other constituents of the microspheres, at the levels at which these materials were included. None of the materials interfered in any way in the estimation of the drug when the above method was used.

The results of recovery studies of known amounts of the drug carried out, in triplicate, are summarized in table 3.15 to ascertain accuracy and precision. Accuracy of the method was evaluated by 't' test. There was no significant difference at level of $P \leq 0.05$, which indicates that the amount recovered is almost equivalent to the amount added. The standard deviation, standard error & coefficient of variation of the results depict that the method is precise.

An important finding of this study was the critical role played by the solubility of the drug in the solvent. It is strongly recommended that a sonication be used for solubilization of the drug and that it is ensured that complete solution has been achieved before analysis is carried out. It was observed that the precision of the method improve at lower concentration range of estimation when solubility of the drug is not limiting factor. The method has been used in the in-vitro release study of mefloquine hydrochloride from microspheres.

3.1.9.6 Estimation of mefloquine hydrochloride from rat blood and tissues

Mefloquine hydrochloride is extracted as the free base under alkaline conditions from tissue and then re-extracted from organic solvent using hydrochloric acid. Derivative spectroscopy is used to estimate drug concentration to enable to estimate the drug in blood & tissues at low level. For preparing calibration curves, a 10% w/v suspension of tissues in PBS was used since this concentration results in uniform drug recovery, low blank values and avoids troublesome emulsion formation (Trevor et. al., 1972).

Dichloromethane would be a better solvent for mefloquine free base and also the subsequent extraction of drug into acid would also become easy. Due to the limited solubility of mefloquine hydrochloride (the salt form) in dichloromethane (Lim 1985) Extraction of the drug was performed in 3 steps with 2, 2 and 1 ml of dichloromethane in accordance with the theory of extraction. Vortexing for 60 seconds followed by centrifugation at 3000 rpm for 10 minutes was found to be adequate for reproducibility and complete separation of aqueous & organic phases. The combined organic phase was then taken for re-constitution of the drug in the form of the hydrochloride

salt into the aqueous acidic phase. However, the above procedure was not adequate in removing some impurities which showed absorbance in the same region of the ultraviolet spectrum as the drug. Derivative spectroscopy was, therefore, used to further separate absorbance due to these impurities from that of the drug.

Third derivative scan of a 10 µg/ml solution of mefloquine hydrochloride in 0.1N hydrochloric acid between 200 nm to 400 nm is shown in Figure 3.11. Absorption maxima was observed at 228.5, 281.5, 293.5, 306.5 & 320.5 nm. The third derivative scans of 10µg/ml acid extracts of mefloquine hydrochloride from rat blood, lung, liver, heart, kidney & spleen are shown in Figure 3.27– 3.32. Absorption maxima at 228.5, 281.5, 293.8, 306.5 & 320.5 nm (Table 3.17) noticed in these spectra point to the concurrence between the above spectra. Thus, these observations indicates that the above double extraction procedure coupled with third derivative ultraviolet spectroscopy was capable of complete separation of tissue-extracted drug from interfering substance & thus was suitable for estimating mefloquine hydrochloride in rat blood & tissues. Being the wavelength at which the most intense absorption was observed (228.5 ≅ 228.0) nm was taken as the analytical wavelength and all further measurements were taken at this wavelength.

Absorptivity of mefloquine hydrochloride, extracted from rat blood, lung, liver, heart, kidney & spleen into 0.1N hydrochloric acid, was calculated to be 98998.9, 88490.6, 99552, 89596.8. 80194.6 & 94021.3 1 $mol^{-1}cm^{-1}$ respectively at 228 nm (Table 3.17). The proximity of these values to each other indicate the applicability of this method to tissues of diverse nature.

Correlation coefficient for the data obtained by extraction of the drug from rat blood lung, liver, heart, kidney & spleen were calculated as 0.991,

0.9983, 0.9928, 0.9926, 0.9976 & 0.9876 respectively (Table 3.16). These values of correlation coefficients indicate that there is a strong, linear relation between absorbance and concentration. Beer's law was investigated and found to be obeyed in the range of 150 ng/ml to 750 ng/ml in all the cases (Table 3.17). The above data along with the results of regression analysis of this data is shown in Table 3.16.

The following regression equations were obtained :

$Y = 0.0002x - 0.0024$ for drug extracted from rat blood

$Y = 0.0002x + 0.0014$ for drug extracted from rat lung

$Y = 0.0002x + 0.0028$ for drug extracted from rat liver

$Y = 0.0002x - 0.0028$ for drug extracted from rat heart

$Y = 0.0002x + 0.0016$ for drug extracted from rat kidney

$Y = 0.0002x - 0.003$ for drug extracted from rat spleen.

The intercept values for blood, lung, liver, heart, kidney & spleen were -0.0024, 0.0014, 0.0028, -0.0028, 0.0016 & -0.003 respectively for the curves which indicate the method to be moderately sensitive.

The extracts were found to be stable in acidic condition over a period of 24 hours as evidenced by 't' test studies of the mean absorbance values at pre-selected time intervals, where the values did not significantly differ from each other. Using the above method, mefloquine hydrochloride was extracted from rat blood and other tissues in the presence of other constituents of the microspheres at the levels at which they are incorporated in the microspheres. It was found that the use of derivative spectroscopy effectively prevented interference of these constituents in the estimations of the analytical wavelength.

Accuracy & precision of the method was investigated by subjecting known amounts of mefloquine hydrochloride to recovery studies, in the

presence of fixed amounts of tissues, in triplicate using the method developed.

Accuracy & precision of the method was ascertained using standard deviation, standard error, coefficient of variance & confidence limit (Table 3.18).

Finally, the method was applied for the estimation of mefloquine hydrochloride from rat blood, lung, liver, heart, kidney & spleen following administration of free drug & formulation both.

3.1.10 References

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3.2 Preparation of microspheres

3.2.1 Introduction :

A wide range of micro-encapsulation techniques have been developed to date. The selection of technique depends on the nature of polymer, the drug and the intended use. When preparing controlled release microspheres, the choice of the optimal method has at most importance for the efficient entrapment of the active substance.

A controlled drug release from delivery systems like microspheres, has been sought after with great vigour to combat the indiscriminate toxicity of drugs. Microspheres are homogeneous, monolithic particles in the size range of about 0.1 – 1000 μm and are widely used as drug carriers for controlled release.

The solvent evaporation method involves the emulsification of an organic solvent (usually methylene chloride) containing dissolved polymer with an excess amount of aqueous continuous phase containing dissolved/dispersed drug with the aid of agitator. The concentration of the emulsifier present in the aqueous phase affects the particle size and shape. When the desired emulsion droplet size is formed, the stirring rate is reduced and evaporation of the organic solvent is realized under atmospheric or reduced pressure at an appropriate temperature. Subsequent evaporation of the dispersed phase solvent yields solid polymeric microparticles entrapping the drug. The solid microparticles are recovered from the suspension by filtration, centrifugation, or lyophilization. Phase separation is a non-aqueous method that is suitable for encapsulation of both water-soluble and water-insoluble drugs.

3.2.2 Materials

Chitosan as a gift sample procured from Central Fisheries Research Institute, Kochi, India having an intrinsic viscosity 8.013 dl/g at 25°C & deacetylation value 61% Ethyl Cellulose, A.R. grade gifted by Sun Pharma. Advanced Research Center. Glacial acetic acid, dichloromethane (DCM), liquid paraffin, arachis oil , Poly Vinyl Alcohol (PVA) Van Organics, India. PEG polyethylene glycol 400, 2000 & 4000, n-Hexane, Glutaraldehyde, S.D. Fine Chem. Mumbai. Gelatin Glaxo Lab India.

3.2.3 Equipments

Weight balance K-Roy analytical single pan balance K-12 super Mechanical stirrer, centrifuge – Remi Motors Equipment, Mumbai & Vaccum Pump Toshnival, Madras.

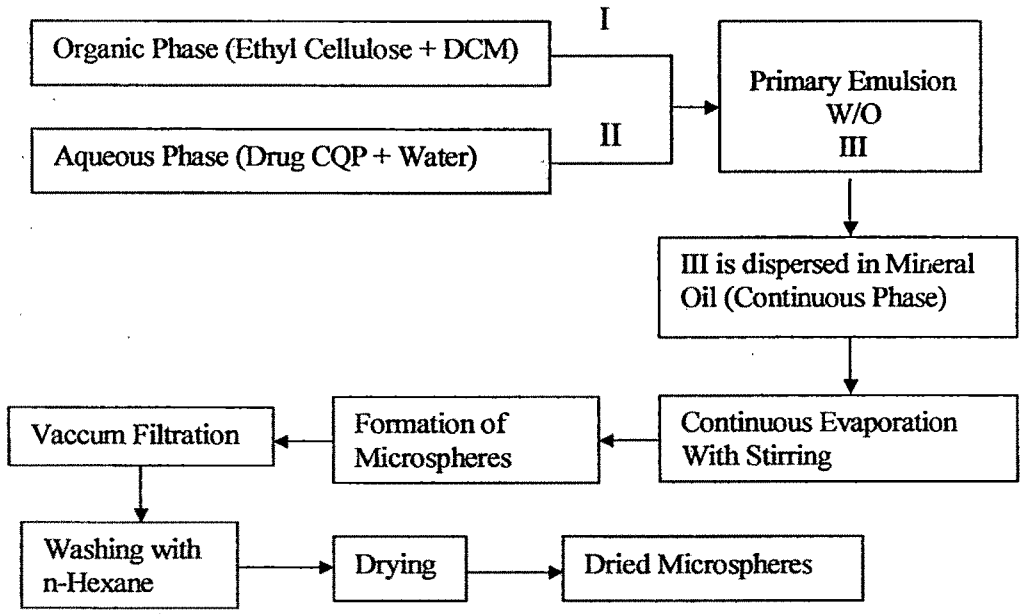
3.2.4 Preparation of ethyl cellulose coated chloroquine phosphate microsphere.

Ethyl cellulose microspheres containing CQP were prepared by "Emulsion solvent diffusion technique" as described by Fernandez et al 2000 and Yang et al.2000.

Ethyl cellulose (500 mg) was dissolved in 9.5ml DCM. Drug was dissolved in 0.5ml distilled water and was added to organic phase and stirred to form w/o emulsion. This primary emulsion was poured into 100ml of PVA (0.5%w/v) solution with continuous stirring until complete evaporation of solvent. Microspheres formed were filtered & washed under vaccum with appropriate solvent. Following modification was done to improve stability and payload in subsequent batches.

- Primary emulsion was not stable. So Span-80 & gelatin were introduced as emulsifying agent for improving its stability.

- To improve drug loading, liquid paraffin was used as continuous phase.

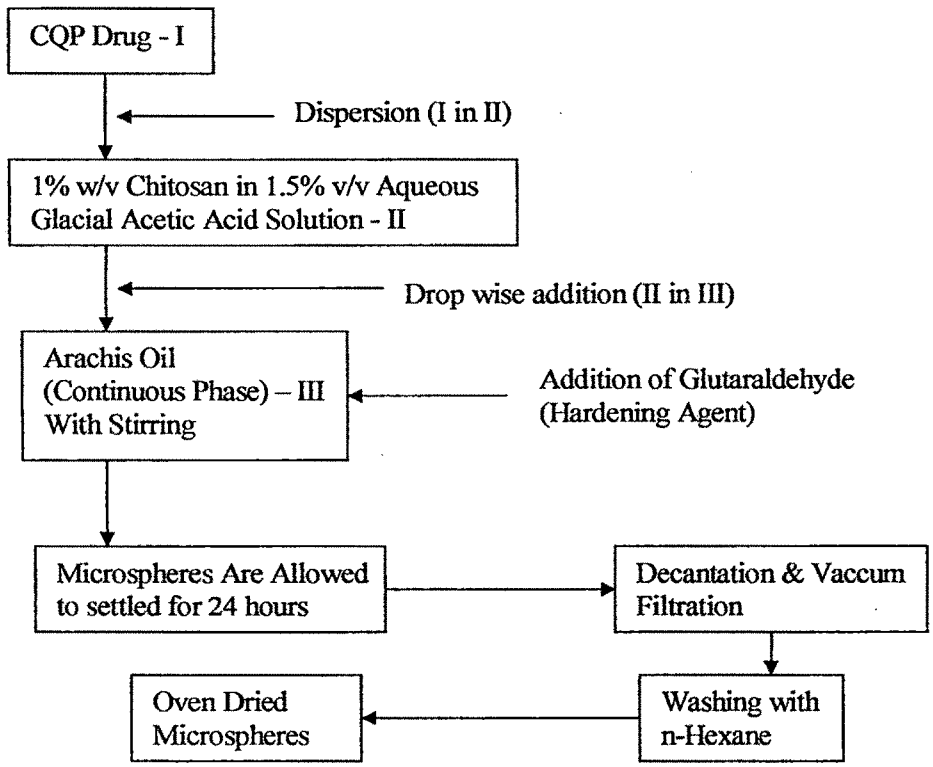


3.2.5 Preparation of chitosan coated Chloroquine Phosphate microspheres

Microspheres were prepared by w/o emulsification technique followed by hardening using chemical cross linking agents.

A 1.0 % (w/v) chitosan solution was prepared by stirring chitosan into 1.5% (v/v) acetic acid solution in a 100ml beaker with aid of stirring (Fwu-long et al., 1997). To the chitosan solution(10ml), 100mg chloroquine phosphate powder was added & mixed well. The final solution was dispersed in 100ml arachis oil without adding emulsifying agent. The resulting mixture was stirred at 1000 rpm for 30 minutes to form fine (w/o) emulsion. Then to the aqueous phase of emulsion the hardening agent was added to produce the microspheres. Hardening of microspheres was done by chemical cross-linking with glutaraldehyde (GA) & stirred continuously at 1000 rpm for 3 hours. Microspheres formed were allowed to sediment over a period of 24

hours. The resultant microspheres were harvested by vaccum filtration, washed with 70 ml n-Hexane and were dried in oven at 40°C (Kumbar et al. 2002, Akbuga & Bengisad 1999, Lim & Wan 1998)



3.2.6 Preparation of ethyl cellulose coated mefloquine hydrochloride microsphere (Khidr, et al, 1998, Shishoo et. al. 2002, Lin & Vasavadu, 2000 Gabor et. al., 1999, Millard et al., 2000)

Ethyl cellulose (250mg), mefloquine hydrochloride(250mg) & PEG were dissolved in 10ml of DCM. Resulting solution was poured into the aqueous phase (100ml of 0.5% w/v PVA solution). The mixture was stirred until complete evaporation of organic solvent (1.5 – 3 hours). Microspheres were collected by filtration under vaccum, washed with distilled water & allowed to dry under vaccum at room temperature for one week. Several batches of microspheres were prepared varying the following parameters.

(1) Drug : polymer ratio

(2) Volume of DCM

(3) Solvent ratio of Methanol to DCM

(4) Continuous phase volume

(5) Concentration of PVA solution

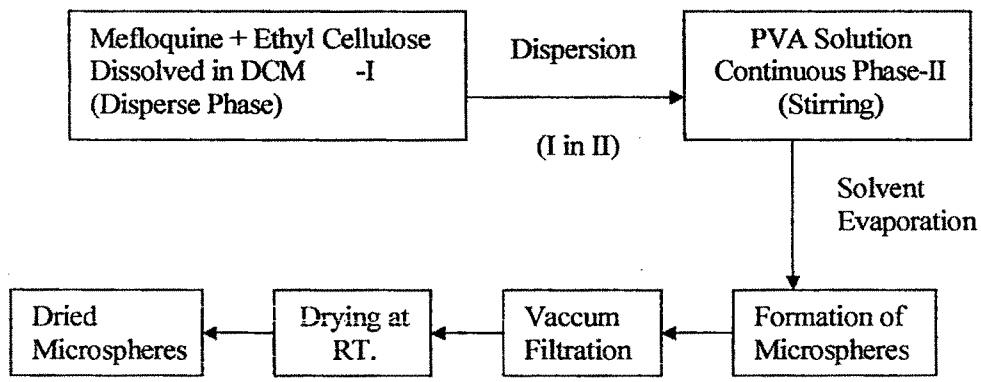
(6) Stirring speed

(7) Employment of PEG into disperse phase

(8) Concentration of PEG-2000

Selection of solvent for Ethyl Cellulose :

Ethyl cellulose is freely soluble in dichloromethane, Methanol & chloroform, Badmeir & M C Grinity (1988) suggested that dichloromethane is preferred over other solvents since it is more soluble in water (1.96%). Rapid solidification of microspheres occur due to faster diffusion of CH_2Cl_2 in the outer phase resulting entrapment of higher percentage of the water soluble drug in the microspheres.



3.2.7 Factorial Design

Introduction :-

- The goal of pharmaceutical **F** and **D** center is to develop an acceptance pharmaceutical formulation in the shortest possible time using minimum number of man-hours and raw materials.
- The formula developed by the **F** and **D** center is then tried at the pilot plant scale and manufacturing scale. Ideally, minor changes are to be made during scale-up. It is therefore very essential to study the formulation from all perspectives.
- In addition to the art of formulation, statistical techniques are available that can aid in the pharmacist's choice of formulation components which can optimize one or more formulation attributes.
- It is well known that traditional experiments involve a good deal of efforts and time, especially where complex formulations are to be developed. A very efficient way to enhance the value of research and to minimize the process development time is through design experiments.
- Factorial designs are used in experiments where the effects of different factors or conditions on experiments are to be elucidated.
- A factor is assigned variable such as concentration, temperature, lubricating agent, drug treatment or diet. Factor may be qualitative or quantitative.
- The levels of a factor are the value or designation assigned to the factors. For example, the levels are 1% and 1.5% for the factor **"concentration"**.
- The runs or trail that comprise factorial experiments consists of all combinations of all factors at all levels.

- The effect of a factor is the change in response caused by varying the levels of the factor. The main effect is the effect of factor averaged over all levels of the other factors.
- The important objective of a factorial experiment is to characterize the effect of changing the levels of factor or combination of factors on the response variable. Predictions based on results of undersigned experiments will be less variable than those, which could be obtained in designed experiment in particular factorial design.
- The optimization procedure is facilitated by construction of an equation that describes the experimental results as function of the factor levels. A polynomial equation can be constructed, where the coefficients in the equation are related to the effects and interactions of the factors. The equation constructed from 3^2 factorial experiment is the following form.

$$Y = B_0 + B_1X_1 + B_2X_2 + B_{12}X_1X_2 + B_{11}X_1X_1 + B_{22}X_2X_2$$

Where,

Y = the measured response

X_i = the level of i^{th} factor

B_1, B_{12}, B_{11} = The coefficients from the response of the formulation in design.

B_0 = Intercept

- The magnitude of the coefficients represents the relative importance of each factor. Once the polynomial equation has been established, an optimum formulation can be found out by grid analysis. With the use of computers, a grid method can be used to identify optimum regions and response surfaces may be depicted. A computer can calculate the

response based on equation at many combinations of factor levels. The formulation whose response has optimal characteristics based on the experiment's specifications chosen.

Advantages of factorial design :

A. In the absence of interaction, factorial design has maximum efficiency in estimating main effects.

B. If, interactions exist, **F** and **D** are necessary to reveal and identify the interactions.

C. Since factors effects are measured over varying levels of other factors, conclusions apply to a wide range of conditions.

D. Maximum use is made of the data since all main effects and interactions are calculated from data.

E. Factorial designs are orthogonal.

F. More information is obtained with less work and effects were measured with maximum precision.

The result of a factorial experiment may be used

a) To help interpret the mechanism of an experimental system.

b) To recommend of implement a practical procedure of set of condition is an industrial manufacturing operation.

c) As guidance for further experimentation.

In most situations, where one is interested in the effect of various factors or conditions on some experimental outcome, factorial design will be optimal.

Formulation of Batches :

The statistical problem solving approach uses a series of small carefully designed experiments. We sometimes call the statistical approach **'strategic experimentation'** or iterative problem solving strategy. We also call this the

'Stop, Look and Listen' approach to experimentation. Analyze the result of few experiments and plan the experiment, namely a screening experiments where from many factors affecting the process few important factors are identified, then optimization experiment where a predictive model is build for the few factors in the region of optimum and finally a verification experiment where the result is confirmed at the predictive settings. (REF) In the present work, factorial design was used for the development of the effective, functional and perfect dosage form. The help of systematic formulation approach is taken to get detailed knowledge of the formulation.
A 3^2 factorial design was used to optimize process variables that were thought to affect the release of drug and loading of the drug.

3.2.8 References

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3.3 Characterization & in-vitro evaluation of microspheres.

3.3.1 Introduction :

Both physical and chemical characteristics of microspheres influence their in-vivo & in-vitro behaviour . Chemical & physical characterizations are very important for a meaningful comparison of different batches prepared to study process variable effect. Biological characterization helps to ensure safety of use in human.

Studies of drug release from microsphere systems are directed towards issues that are relevant to the in-vitro- in-vivo correlation. In-vitro analysis helps to guide

(1) Minimizing the loss of encapsulated drug on route from site of administration to the site of drug action.

(2) The ability to match the rate of release to the requirements of the therapy.

(3) In-vitro result helps in optimization & selection of formulation for further in-vivo study.

3.3.2 Materials

Disodium hydrogen phosphate, potassium dihydrogen phosphate, SD Fine Chem. Ltd. Mumbai; hydrochloric acid, sodium chloride of Analytical Grade (AR).

Solutions :

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

Equipments :

Magnetic stirrer (Remi Equipment, Mumbai), Microscope (Veego Scientific Device, Mumbai), Chemito Spectra Scan UV 2600 visible double beam

spectrophotometer, Olympus microscope BX 40 (Olympus Optical Co. Ltd. Japan) Stage & occular micrometer Erma, Japan.

3.3.3 Characterization of microspheres :

The prepared microspheres containing chloroquine phosphate (CQP) & mefloquine hydrochloride (MQH) were characterized for the following attributes :

3.3.3.1 Percentage Yield

Percentage yield of microspheres was calculated by practical yield divided by actual theoretical yield. The total microspheres yield was calculated gravimetrically on the basis of polymer & drug recovery.

$$\% \text{ Yield} = \frac{\text{Weight of microsphere obtained}}{\text{Weight of (Drug \& Polymer)}} \times 100$$

3.3.3.2 Particle size

The mean particle size of the prepared microspheres was obtained by using a ordinary microscope with aid of optical micrometer. A dilute suspension of microspheres was prepared in light liquid paraffin which was mounted on slide. The diameter was measured with least count of optical micrometer 13.33 μm & 3.125 μm correspond to 45x & 10x (high power & low power)

3.3.3.3 Morphology

Morphology was investigated from photomicrographs taken using an Olympus – BX40 microscope at a magnification of 2500X. Plates 3.1 to 3.10. show the photographs of the various microspheres.

3.3.3.4 Drug content :

Spectrophotometric methods were used for determination of drug contents.

$$\text{Drug content \%} = \frac{\text{Actual amount of drug recovered in Microspheres}}{\text{Amount of drug loaded in Microspheres}} \times 100$$

A weighed quantity of chitosan / ethyl cellulose coated CQP microspheres were extracted with 0.01N hydrochloric acid in glass stoppered conical flask at 37°C for 1 hour. The mixture was filtered, absorbance was measured at 343nm after appropriate dilution.

Microspheres of mefloquine hydrochloride content was determined by the method same as above but for the solvent was replaced by methanol & absorbance was measured at 283nm.

3.3.3.5 Drug Release Profile

The in-vitro release test of CQP from (chitosan/EC) microspheres was conducted in phosphate buffer saline 7.4 pH at 37°C. Drug CQP loaded microspheres were placed into the dialysis tube, which was fabricated by using cylinder with both side open. One side was covered with semipermeable membrane (molecular weight cut off is 12,000- 14,000). The covered side of dialysis tube was dipped 2cm into 250 ml beaker after replacing microspheres, which act as the receptor compartment, containing 100 ml PBS. Stirring was maintained with magnetic stirrer. Aliquots of solution (1ml) were withdrawn at designated times & same volume of fresh medium was supplemented. Samples were analyzed by UV-spectroscopy. Each determination was carried out in triplicate & the release results are plotted as the cumulative percentage of drug content in dissolution media Vs time.

3.3.4 Results & Discussion

3.3.4.1 Results

Table 3.19 Summary of the properties of various batches of Ethyl Cellulose coated Chloroquine Phosphate Microspheres

Batch No.	Conditions			Response				
	D:P Ratio	Disperse phase	Continuous phase	% Yield ±SD	% Drug Content ±SD	P.S.* µm ±SD	t_{50}@ Min.	t_{70}@ Min.
EC_1	1:1	100mg EC in 9.5ml DCM + 100mg CQP in 0.5ml water	100ml 0.5% w/v PVA aq. solution	55.6 ± 1.5	12 ± 2.3	13.35 ± 0.63	40'	60
EC_2	- do -	100mg EC in 9.5ml DCM + 100mg CQP in 0.5ml water	100ml liquid paraffin	68.0 ± 2.3	58 ± 1.5	68 ± 1.5	60'	240
EC_3	- do -	100mg EC in 9.5ml DCM + 100mg CQP in 0.5ml water + 1% Span-80 (EA)	100ml liquid paraffin	69.95 ± 1.56	67.9 ± 2.4	69.95 ± 1.2	48	220
EC_4	- do -	100mg EC in 9.5ml DCM + 100mg CQP in 0.5ml water + 2% Span-80 (EA)	100ml liquid paraffin	71.0 ± 3.1	76.8 ± 3.8	31.7 ± 0.56	55	190
EC_5	- do -	100mg EC in 9.5ml DCM + 100mg CQP in 0.5ml water + 3% Span-80 (EA)	100ml liquid paraffin	81 ± 3.6	71.8 ± 2.8	33.4 ± 0.48	58	185
EC_6	- do -	100mg EC in 9.5ml DCM + 100mg CQP in 0.5ml water + 5% Gelatin (EA)	100ml liquid paraffin	85 ± 4.1	80 ± 3.3	40 ± 0.6	50	170
EC_7	- do -	100mg EC in 9.5ml DCM + 100mg CQP in 0.5ml water + 10% Gelatin (EA)	100ml liquid paraffin	82.18 ± 3.8	86 ± 3.6	46 ± 0.75	52	180
EC_8	- do -	100mg EC in 9.5ml DCM + 100mg CQP in 0.5ml water + 15% Gelatin (EA)	100ml liquid paraffin	85 ± 4.2	84.26 ± 2.3	49 ± 0.98	55	180

D : P Drug : Polymer Ratio

* Particle size measured using optical microscope

@ Time for 50% and 70% release in 7.4 pH PBS.

Table 3.20 Summary of properties of various batches of Chitosan coated Chloroquine Phosphate Microspheres

B. No.	Composition	Conditions	% Yield ±SD	Drug Content ±SD (%)	P.S. μm ±SD	t_{50} Min.	t_{70} Min.
CC_1	Drug : Polymer Ratio (1:1) (100mg+100mg) in 1.5% acetic acid aq. solution Arachis oil as continuous phase. Formaldehyde (1ml) as hardening agent	Hardening time - 3hrs., Stirring speed – 1000 rpm Volume of continuous phase 100ml Volume of disperse phase 10ml	72 ± 3.32	60.20 ± 2.35	105.5 ± 2.7	55	210
CC_2	Hardening agent – formaldehyde (2ml)	-do-	79 ± 2.6	64.91 ± 2.58	98.5 ± 3.55	63	180
CC_3	Hardening agent – formaldehyde (3ml)	-do-	70 ± 3.6	65.90 ± 4.2	106.8 ± 3.71	90	284
CC_4	Hardening agent – formaldehyde (5ml)	-do-	81 ± 4.2	63.38 ± 4.4	125.8 ± 5.79	144	210
CC_5	Hardening agent – Glutaraldehyde (5%)/100mg Chitosan 1:1 D:P ratio	-do-	67.5 ± 3.3	81.8 ± 5.5	18.18 ± 0.71	214	405
CC_6	1:2 D:P ratio	-do-	82.4 ± 2.4	78.05 ± 3.9	15.8 ± 0.79	261	435
CC_7	1:3 D:P ratio	-do-	80 ± 2.85	91.8 ± 5.1	28.85 ± 1.32	180	285
CC_8	1:4 D:P ratio	-do-	92 ± 3.6	85.92 ± 4.8	48.65 ± 2.45	240	360
CC_9	1:5 D:P ratio	-do-	79.66 ± 3.3	84 ± 4.3	62.85 ± 3.36	228	321

B. No.	Composition	Conditions	% Yield ±SD	Drug Content ±SD (%)	P.S. μm ±SD	t_{50} Min.	t_{70} Min.
CC_{10}	1:1 D:P ratio 0.5% Chitosan solution	-do-	62.5 ± 2.95	81.68 ± 3.85	16.5 ± 0.81	159	243
CC_{11}	1:1 D:P ratio 1.5% Chitosan solution	-do-	83.5 ± 4.2	71.1 ± 3.2	26.63 ± 1.25	240	354
CC_{12}	1:1 D:P ratio 2% Chitosan solution	-do-	88.13 ± 3.7	73.12 ± 3.3	33.39 ± 1.3	252	351
CC_{13}	1:1 D:P ratio 5% hardening agent (Glutaraldehyde) 1% Chitosan solution	Stirring speed – 500 rpm	81.5 ± 3.9	76.2 ± 3.2	26.2 ± 1.3	240	420
CC_{14}	-do-	Stirring speed - 1500 rpm	86.8 ± 3.6	67.08 ± 1.5	23.33 ± 1.2	168	321
CC_{15}	-do-	Stirring speed - 2000 rpm	84.5 ± 4.2	82.19 ± 4.2	15.59 ± 0.48	143	300
CC_{16}	-do-	Hardening time –1hr.	84.5 ± 3.5	67.08 ± 3.2	53.5 ± 1.1	60	135
CC_{17}	-do-	Hardening time –2hr.	88.76 ± 4.6	80.67 ± 4.1	37.75 ± 1.04	63	158
CC_{18}	-do-	Hardening time –4hr.	89 ± 4.4	83.7 ± 4.4	17.5 ± 0.83	141	255
CC_{19}	-do-	Hardening time –5hr.	86 ± 4.3	77.15 ± 3.6	21.12 ± 1.62	165	280
CC_{20}	1:1 D:P Concentration of Crosslinking agent – 10% Glutaraldehyde	Stirring speed – 1000 rpm Hardening Time – 3hr. Volume of continuous phase 100ml Volume of disperse phase 10ml	78 ± 3.6	81.18 ± 4.3	49.95 ± 1.9	261	450
CC_{21}	1:1 D:P Concentration of Crosslinking agent – 15% Glutaraldehyde	-do-	76.0 ± 4.2	76.1 ± 4.1	57.64 ± 2.9	189	300

Total 500mg – Drug + Polymer

Table 3.21 Summary of Ethyl Cellulose Coated Mefloquine Hydrochloride Microspheres

(a) Effect of D : P ratio

B. No.	D:P Ratio	% yield ± SD	Drug content ± SD (%)	Particle size (μm) ± SD	T_{50} (days)	T_{70} (days)
M1	1:1 (♦)	72.00 ± 1.8	79.00 ± 2.37	9.80 ± 0.16	7	9
M2	1:2	75.58 ± 3.6	81.18 ± 4.5	12.50 ± 0.26	9	12
M3	1:3	78.50 ± 3.4	81.98 ± 3.3	16.86 ± 0.371	9	14
M4	1:4	80.18 ± 4.2	84.18 ± 2.9	22.12 ± 0.33	10	15
M5	1:5	82.15 ± 3.2	85.15 ± 4.6	43.62 ± 1.8	11	15

(♦) Optimized D:P ratio was selected for further investigation

(b) Effect of solvent Ratio :: Methanol : DCM

B. No.	Solvent ratio	% yield ± SD	Drug content ± SD (%)	Particle size (μm) ± SD	T_{50} (days)	T_{70} (days)
M6	Methanol :DCM (1:0)	75 ± 1.73	78.18 ± 3.1	55.58 ± 1.1	12	--
M7	Methanol :DCM (1:1)	73.18 ± 2.1	76.15 ± 2.92	40.38 ± 0.99	9	15
M8	Methanol :DCM (1:1.5)	74.45 ± 2.4	75.58 ± 2.54	38.89 ± 1.01	8	13
M9	Methanol:DCM(0:1) (♦)	72.00 ± 1.8	79.00 ± 2.37	9.80 ± 0.16	7	9

(♦) Optimized D:P ratio & solvent blend selected for further investigation.
[D:P ratio 1:1, solvent blend methanol : DCM (0:1)]

(c) Effect of disperse phase volume

B.No.	Volume of disperse phase	% yield ± SD	Drug content ± SD (%)	Particle size (μm) ± SD	T_{50} (days)	T_{70} (days)
M9	10 ml DCM (♦)	72.00 ± 1.8	79.00 ± 2.37	9.80 ± 0.16	7	9
M10	20 ml DCM	68.86 ± 2.28	69.95 ± 2.51	9.60 ± 0.17	6	9
M11	5 ml DCM	75.89 ± 3.42	76.63 ± 2.98	12.35 ± 0.25	7	10

(♦) Optimized D:P ratio (1:1), solvent blend methanol : DCM (0:1) & volume of disperse phase (10ml DCM)

(d) Effect of PVA concentration in aqueous continuous phase

B. No.	Con. of continuous phase	%yield ± SD	Drug content ±SD (%)	Particle size (μm) ± SD	T_{50} (days)	T_{70} (days)
M12	0.1% PVA solution	74.47 ± 1.62	80.88 ± 3.1	10.82 ± 0.08	7	8
M13	0.5% PVA solution (♦)	72.00 ± 1.8	79.00 ± 2.37	9.80 ± 0.16	7	9
M14	1.0% PVA solution	76.63 ± 1.85	80.05 ± 4.1	14.35 ± 0.32	8	13

(♦) Optimized D:P ratio (1:1), solvent blend methanol : DCM (0:1), volume of disperse phase (10ml DCM) & 100ml of 0.5% PVA aqueous solution as continuous phase

(e) Effect of continuous phase volume

B. No.	Volume of continuous phase	%yield ± SD	Drug content ± SD (%)	Particle size (μm) ± SD	T_{50} (days)	T_{70} (days)
M15	100ml PVA solution (♦)	72.0 ± 1.8	79.0 ± 2.37	9.8 ± 0.16	7	9
M16	50ml PVA solution	69.80 ± 2.1	77.80 ± 3.2	15.85 ± 0.79	8	11
M17	200ml PVA solution	67.53 ± 1.92	68.83 ± 2.89	6.82 ± 0.62	6	9

(♦) Optimized D:P ratio (1:1), solvent blend methanol : DCM (0:1), volume of disperse phase (10ml DCM), 100ml of 0.5% PVA aqueous solution as continuous phase & volume of continuous phase 100 ml

(f) Effect of Stirring Speed

B. No.	Stirring speed	%yield ± SD	Drug content ± SD (%)	Particle size (μm) ± SD	T_{50} (days)	T_{70} (days)
M18	1000 rpm (♦)	72 ± 1.8	79 ± 2.37	9.8 ± 0.16	7	9
M19	2000 rpm	72.63 ± 2.3	74.48 ± 3.3	7.9 ± 0.18	6	9
M20	3000 rpm	68.83 ± 2.1	67.35 ± 2.4	6.9 ± 0.30	6	8

(♦) Optimized D:P ratio (1:1), solvent blend methanol : DCM (0:1), volume of disperse phase (10ml DCM), 100ml of 0.5% PVA aqueous solution as continuous phase, volume of continuous phase 100 ml & stirring speed 1000 rpm

(g) Effect of PEG molecular weight

B. No.	Types of PEG (10 %)	%yield ± SD	Drug content ± SD (%)	Particle size (μm) ± SD	T_{50} (days)	T_{70} (days)
M21	PEG-400	76.8 ± 2.8	74.5 ± 3.1	12.12 ± 0.41	5	6
M22	PEG-2000 (♦)	70.83 ± 3.3	77.88 ± 2.4	13.89 ± 0.62	5	7
M23	PEG-4000	73.83 ± 3.8	76.65 ± 1.95	18.5 ± 0.82	6	8

(♦) Optimized D:P ratio (1:1), solvent blend methanol : DCM (0:1), volume of disperse phase (10ml DCM), 100ml of 0.5% PVA aqueous solution as continuous phase, volume of continuous phase 100 ml, stirring speed 1000 rpm & 10% PEG 2000

(h) Effect of concentration of PEG- 2000

B. No.	Concentration of PEG 2000	%yield ± SD	Drug content ± SD (%)	Particle size (μm) ± SD	T_{50} (days)	T_{70} (days)
M24	5% PEG	74.85 ± 4.2	78.18 ± 3.6	14.12 ± 0.374	6	8
M25	10% PEG	70.83 ± 4.4	74.5 ± 3.1	13.5 ± 0.458	5	7
M26	15% PEG (♦)	78.88 ± 3.9	76.85 ± 4.5	15.5 ± 0.605	5	6

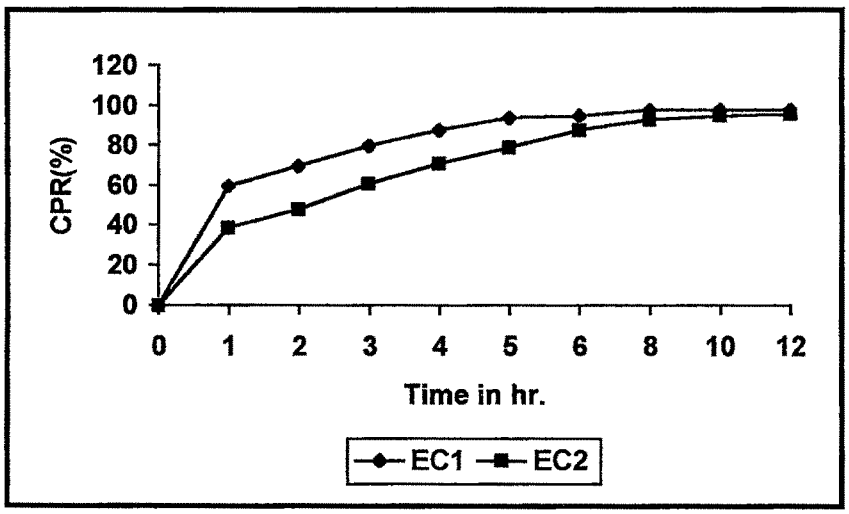
(♦) Optimized D:P ratio (1:1), solvent blend methanol : DCM (0:1), volume of disperse phase (10ml DCM), 100ml of 0.5% PVA aqueous solution as continuous phase, volume of continuous phase 100 ml, stirring speed 1000 rpm & 15% PEG 2000

Table 3.22 The effect of types of continuous phase on in-vitro release profile of ethyl cellulose coated CQP microspheres.

Time in hr.	EC_1		EC_2	
	mg	CPR ± SD	Mg	CPR ± SD
1	0.719	60 ± 2.45	2.262	39 ± 1.2
2	0.842	70 ± 1.12	2.784	48 ± 1.3
3	0.960	80 ± 3.44	3.540	61 ± 1.5
4	1.056	88 ± 2.90	4.160	71 ± 2.3
5	1.128	94 ± 3.60	4.580	79 ± 2.4
6	1.140	95 ± 4.80	5.100	88 ± 1.6
8	1.176	98 ± 1.20	5.390	93 ± 1.5
10	1.176	98 ± 1.20	5.510	95 ± 2.1
12	1.176	98 ± 0.50	5.570	96 ± 2.3

CPR : Cumulative Percentage Release

Figure 3.33 Comparative in-vitro release profile of batches EC_1 and EC_2



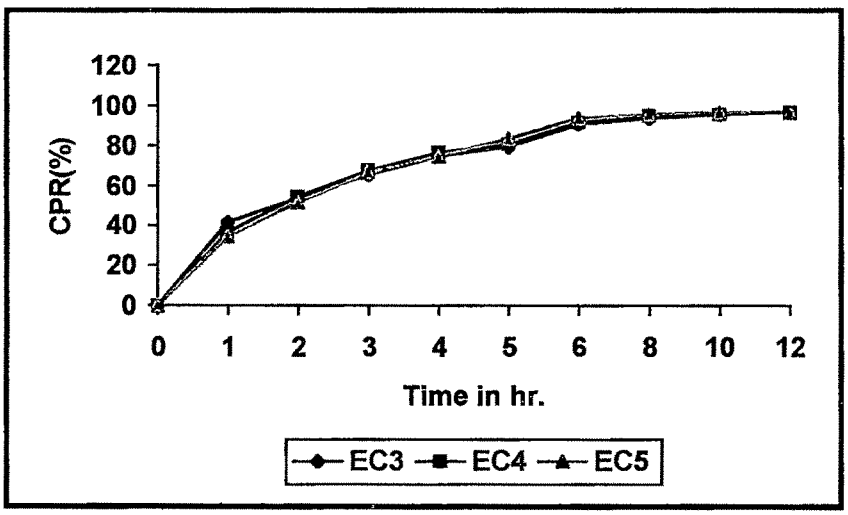
CPR : Cumulative Percentage Release

Table 3.23 The effect of concentration of Span-80 as an emulsifying agent on in-vitro release profile of ethyl cellulose coated CQP microspheres.

Time In hr.	EC_3		EC_4		EC_5	
	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD
1	2.86	42 ± 2.3	2.69	37 ± 2.1	2.51	35 ± 1.6
2	3.67	54 ± 3.3	3.89	55 ± 1.4	3.74	52 ± 2.3
3	4.48	66 ± 3.1	4.48	68 ± 2.6	4.81	67 ± 3.2
4	5.09	75 ± 3.2	5.45	77 ± 2.5	5.39	75 ± 3.3
5	5.43	80 ± 2.6	5.73	81 ± 2.2	6.03	84 ± 2.8
6	6.18	91 ± 2.5	6.52	92 ± 2.1	6.75	94 ± 2.5
8	6.38	94 ± 2.2	6.73	95 ± 1.3	6.89	96 ± 3.1
10	6.52	96 ± 1.9	6.79	96 ± 1.6	6.96	97 ± 2.8
12	6.59	97 ± 1.4	6.86	97 ± 1.5	6.96	97 ± 3.3

CPR : Cumulative Percentage Release

Figure 3.34 Comparative in-vitro release profile of batches EC_3 to EC_5



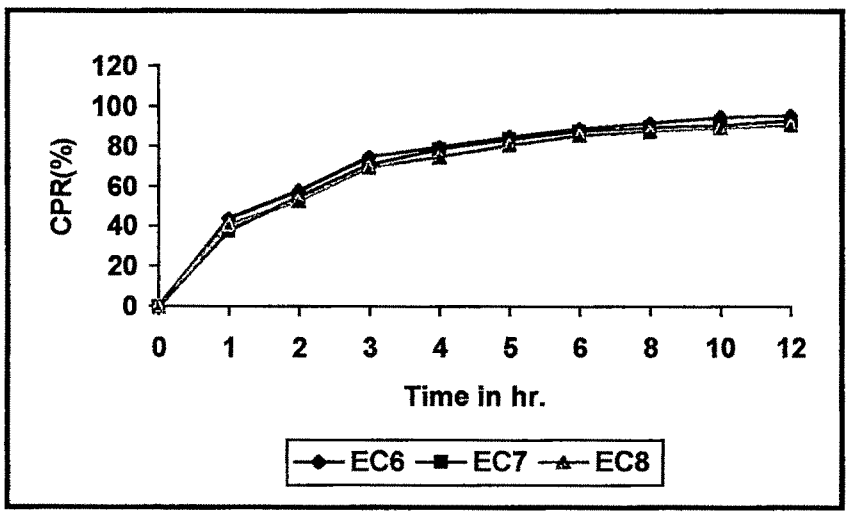
CPR : Cumulative Percentage Release

Table 3.24 The effect of concentration of gelatin as an emulsifying agent on in-vitro release profile of ethyl cellulose coated CQP microspheres.

Time in hr.	EC_6		EC_7		EC_8	
	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD
1	3.52	44 ± 2.5	3.27	38 ± 1.1	3.45	41 ± 1.2
2	4.64	58 ± 3.6	4.73	55 ± 1.4	4.46	53 ± 2.1
3	5.96	75 ± 3.3	6.11	71 ± 3.1	5.89	70 ± 3.3
4	6.40	80 ± 3.4	6.79	79 ± 2.1	6.32	75 ± 3.4
5	6.80	85 ± 2.8	7.22	84 ± 2.2	6.82	81 ± 2.1
6	7.12	89 ± 2.6	7.57	88 ± 2.5	7.24	86 ± 3.3
8	7.36	92 ± 2.5	7.74	90 ± 1.7	7.41	88 ± 1.8
10	7.60	95 ± 2.2	7.81	91 ± 1.8	7.58	90 ± 1.4
12	7.68	96 ± 1.4	7.99	93 ± 2.3	7.66	91 ± 2.1

CPR : Cumulative Percentage Release

Figure 3.35 Comparative in-vitro release profile of batches EC_6 to EC_8



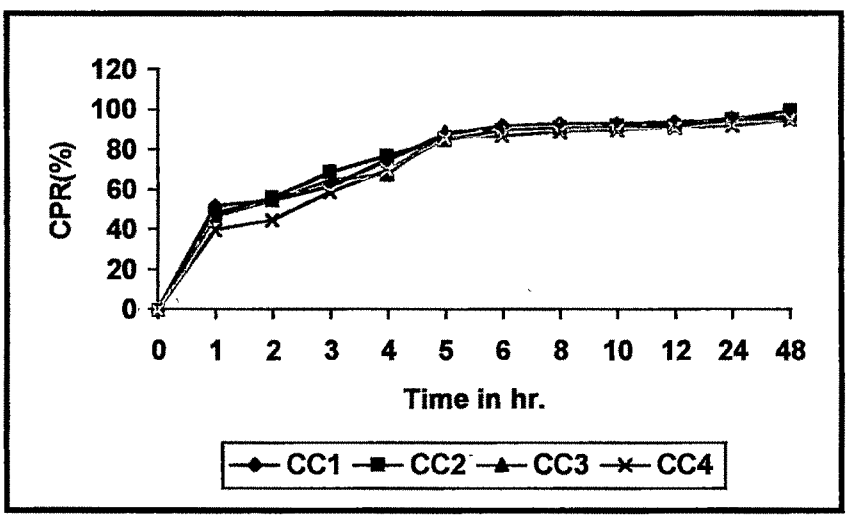
CPR : Cumulative Percentage Release

Table 3.25 The effect of formaldehyde concentration (cross linking agent) on in-vitro release profile of chitosan coated CQP microspheres.

Time in hr.	CC_1		CC_2		CC_3		CC_4	
	Mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD	Mg	CPR ± SD
1	3.80	52 ± 1.2	3.42	48 ± 0.85	3.48	47 ± 0.98	3.07	40 ± 0.85
2	4.02	55 ± 1.1	3.95	56 ± 1.05	4.07	55 ± 1.05	3.44	45 ± 1.20
3	4.43	62 ± 2.3	4.86	69 ± 2.20	4.87	65 ± 1.52	4.53	59 ± 1.10
4	5.45	75 ± 3.1	5.43	77 ± 1.60	5.05	68 ± 1.98	6.24	70 ± 1.40
5	6.40	88 ± 3.2	5.99	85 ± 2.40	6.61	89 ± 2.30	6.70	86 ± 1.50
6	6.70	92 ± 1.9	6.33	90 ± 2.30	6.69	90 ± 2.10	6.76	87 ± 2.05
8	6.80	93 ± 2.1	6.39	91 ± 1.48	6.79	91 ± 2.50	6.89	89 ± 1.98
10	6.80	93 ± 3.1	6.45	92 ± 3.10	6.84	92 ± 2.20	6.92	90 ± 2.40
12	6.89	94 ± 1.5	6.46	92 ± 3.30	6.87	93 ± 2.30	6.93	91 ± 2.30
24	6.95	95 ± 2.4	6.68	95 ± 2.70	7.15	96 ± 1.60	7.06	92 ± 2.10
48	7.00	96 ± 2.5	6.98	99.8 ± 3.3	7.23	97 ± 2.40	7.37	95 ± 2.50

CPR : Cumulative Percentage Release

Figure 3.36 Comparative in-vitro release profile of batches CC_1 to CC_4



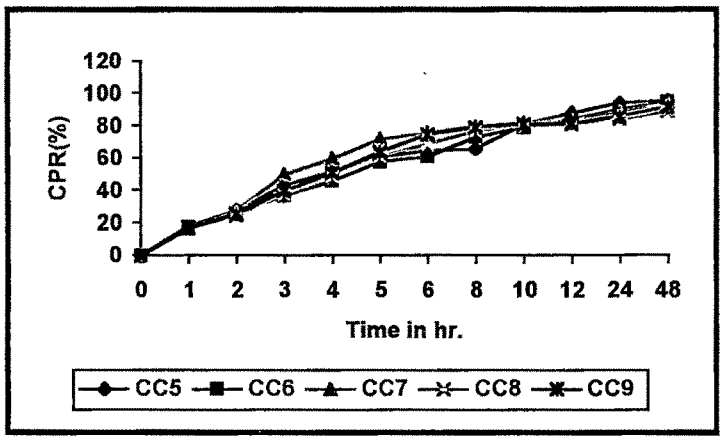
CPR : Cumulative Percentage Release

Table 3.26 The effect of Drug : Polymer ratio on in-vitro release profile of chitosan coated CQP microspheres.

Time in hr.	CC_5		CC_6		CC_7		CC_8		CC_9	
	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD
1	1.53	18 ± 0.648	0.975	18 ± 0.712	0.81	17 ± 0.586	0.59	17 ± 0.412	0.47	17 ± 0.713
2	2.34	28 ± 1.18	1.38	26 ± 1.2	1.32	28 ± 0.98	0.86	25 ± 0.82	0.72	26 ± 1.03
3	3.55	43 ± 1.68	1.96	37 ± 1.48	2.29	50 ± 1.75	1.32	38 ± 1.2	1.13	40 ± 1.4
4	4.26	52 ± 1.4	2.38	46 ± 1.23	2.78	60 ± 2.5	1.69	49 ± 2.3	1.44	51 ± 2.1
5	5.06	61 ± 2.48	3.04	58 ± 2.69	3.32	72 ± 3.1	2.07	61 ± 3.3	1.80	64 ± 3.4
6	5.34	65 ± 3.3	3.19	61 ± 2.8	3.50	76 ± 3.6	2.36	69 ± 2.9	2.11	75 ± 4.2
8	5.41	66 ± 3.6	3.77	73 ± 4.2	3.68	80 ± 4.2	2.63	77 ± 4.1	2.24	79 ± 4.7
10	6.64	81 ± 5.5	4.15	79 ± 4.39	3.79	82 ± 4.4	2.74	80 ± 3.2	2.29	81 ± 2.6
12	7.21	88 ± 4.1	4.38	84 ± 4.2	3.88	84 ± 4.6	2.77	81 ± 3.8	2.30	82 ± 4.4
24	7.72	94.4 ± 2.2	4.67	90 ± 3.2	4.12	89 ± 1.9	2.88	84 ± 2.4	2.43	86 ± 3.3
48	7.80	96 ± 3.2	4.93	95.05 ± 1.8	4.32	94 ± 1.5	3.05	89 ± 2.4	2.59	92 ± 1.6

CPR : Cumulative Percentage Release

Figure 3.37 Comparative in-vitro release profile of batches CC_5 to CC_9



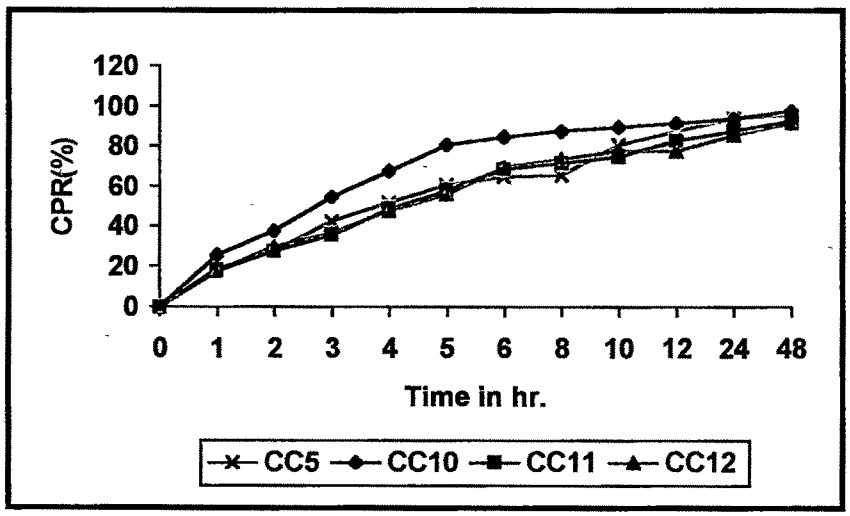
CPR : Cumulative Percentage Release

Table 3.27 The effect of polymer concentration on in-vitro release profile of chitosan coated CQP microspheres.

Time in hr.	CC_5		CC_{10}		CC_{11}		CC_{12}	
	Mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD
1	1.53	18 ± 0.648	2.10	26 ± 0.94	1.42	19 ± 0.81	1.38	18 ± 0.78
2	2.34	28 ± 1.18	3.14	38 ± 1.01	2.50	28 ± 1.05	2.22	30 ± 1.05
3	3.55	43 ± 1.68	4.50	55 ± 1.50	2.61	36 ± 1.20	2.74	37 ± 2.34
4	4.26	52 ± 1.40	5.59	68 ± 2.05	3.55	49 ± 1.35	3.57	48 ± 3.30
5	5.06	61 ± 2.48	6.66	81 ± 2.10	4.19	58 ± 2.10	4.16	56 ± 3.23
6	5.34	65 ± 3.30	6.99	85 ± 3.20	4.97	69 ± 2.40	5.16	70 ± 3.43
8	5.41	66 ± 3.60	7.25	88 ± 3.30	5.18	72 ± 2.85	5.47	74 ± 2.85
10	6.64	81 ± 5.50	7.39	90 ± 3.40	5.38	75 ± 3.10	5.77	78 ± 2.98
12	7.21	88 ± 4.10	7.54	92 ± 3.50	5.94	83 ± 3.23	5.98	78 ± 3.30
24	7.72	94.4 ± 2.20	7.74	94 ± 2.80	6.28	88 ± 3.40	6.34	86 ± 3.24
48	7.80	96 ± 3.20	8.08	98 ± 3.20	6.64	93 ± 3.60	6.72	**91.92±3.60**

CPR : Cumulative Percentage Release

Figure 3.38 Comparative in-vitro release profile of batches CC_5 & CC_{10} to CC_{12}



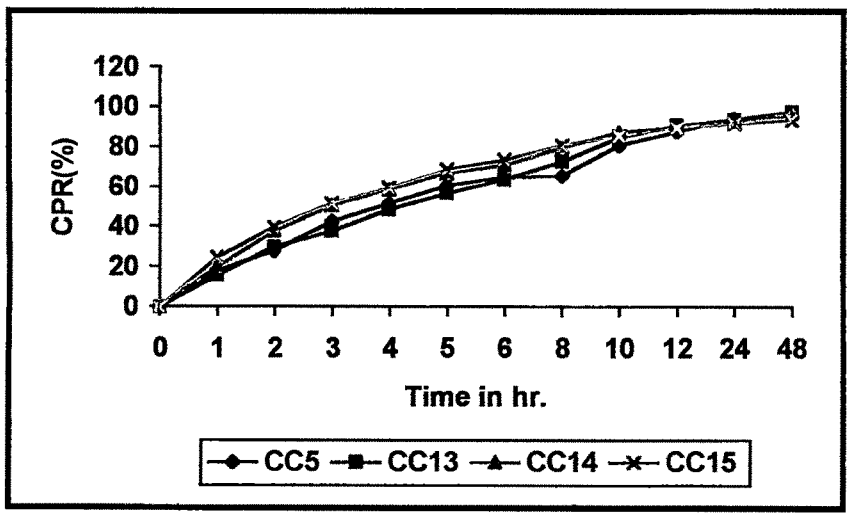
CPR : Cumulative Percentage Release

Table 3.28 The effect of stirring speed on in-vitro release profile of chitosan coated CQP microspheres.

Time in hr.	CC_5		CC_{13}		CC_{14}		CC_{15}	
	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD
1	1.53	18 ± 0.648	1.27	16 ± 0.76	1.35	20 ± 0.68	2.07	25 ± 1.20
2	2.34	28 ± 1.18	2.31	30 ± 0.98	2.60	38 ± 0.88	3.32	40 ± 1.30
3	3.55	43 ± 1.68	2.95	38 ± 1.15	3.45	51 ± 1.88	4.28	52 ± 1.38
4	4.26	52 ± 1.40	3.77	49 ± 1.20	4.02	59 ± 1.25	4.99	60 ± 2.05
5	5.06	61 ± 2.48	4.35	57 ± 1.35	4.54	67 ± 2.85	5.71	69 ± 2.20
6	5.34	65 ± 3.30	4.91	64 ± 2.50	4.79	71 ± 2.78	6.12	74 ± 2.40
8	5.41	66 ± 3.60	5.59	73 ± 2.62	5.35	80 ± 3.30	6.72	81 ± 3.10
10	6.64	81 ± 5.50	6.54	85 ± 2.88	5.94	88 ± 3.10	7.13	86 ± 2.91
12	7.21	88 ± 4.10	7.00	91 ± 3.10	6.06	90 ± 2.80	7.40	90 ± 2.89
24	7.72	94.4 ± 2.20	7.20	94 ± 3.40	6.25	93 ± 2.75	7.58	92 ± 3.12
48	7.80	96 ± 3.20	7.53	98 ± 3.20	6.55	97 ± 2.98	7.65	94 ± 3.30

CPR : Cumulative Percentage Release

Figure 3.39 Comparative in-vitro release profile of batches CC_5 & CC_{13} to CC_{15}



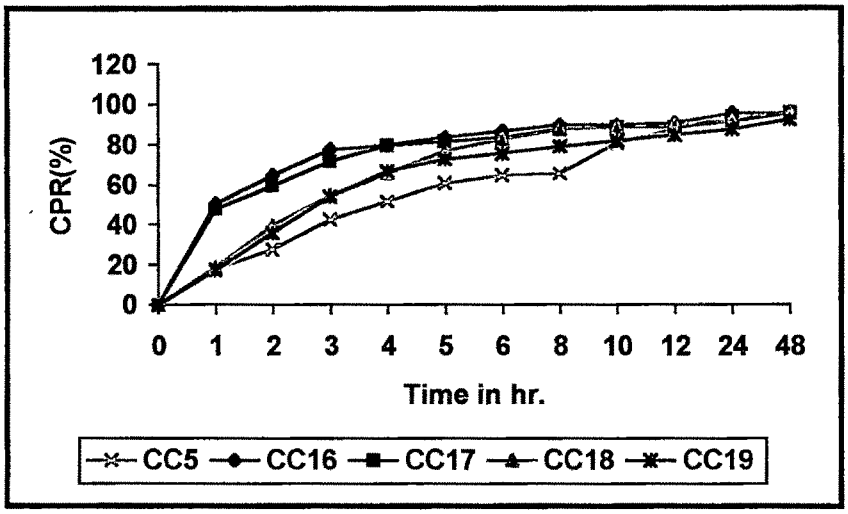
CPR : Cumulative Percentage Release

Table 3.29 The effect of hardening time on in-vitro release profile of chitosan coated CQP microsphere.

Time in hr.	CC_5		CC_{16}		CC_{17}		CC_{18}		CC_{19}	
	Mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD	Mg	CPR ± SD	mg	CPR ± SD
1	1.53	18 ± 0.64	3.40	51 ± 2.30	3.93	48 ± 1.10	1.58	19 ± 1.80	1.37	17.79 ±0.98
2	2.34	28 ± 1.18	4.39	65 ± 2.50	4.89	60 ± 1.40	3.35	40 ± 1.70	2.82	36.58 ±1.2
3	3.55	43 ± 1.68	5.29	78 ± 1.81	5.88	72 ± 1.38	4.67	55 ± 1.52	4.20	54.46 ±1.05
4	4.26	52 ± 1.40	5.40	80 ± 2.34	6.52	80 ± 2.30	5.59	66 ± 1.45	5.15	66.88 ±1.98
5	5.06	61 ± 2.48	5.64	84 ± 3.30	6.66	82 ± 3.10	6.45	77 ± 2.10	5.60	72.96 ±1.6
6	5.34	65 ± 3.30	5.84	87 ± 2.60	6.84	84 ± 2.45	6.95	83 ± 2.80	5.85	75.84 ±2.05
8	5.41	66 ± 3.60	6.08	90 ± 2.80	7.17	88 ± 3.30	7.39	88 ± 3.30	6.13	79.54 ±2.3
10	6.64	81 ± 5.50	6.08	90 ± 2.40	7.25	89 ± 3.20	7.48	89 ± 3.10	6.34	82.22 ±2.1
12	7.21	88 ± 4.10	6.17	91 ± 2.38	7.25	89 ± 1.20	7.56	90 ± 2.30	6.56	84.95 ±1.65
24	7.72	94.4 ± 2.20	6.46	96 ± 3.10	7.45	92 ± 2.20	7.74	92 ± 1.24	6.78	87.95 ±2.3
48	7.80	96 ± 3.20	6.48	96 ± 2.12	7.79	96 ± 2.30	8.11	96 ± 1.30	7.17	92.76 ±2.1

CPR : Cumulative Percentage Release

Figure 3.40 Comparative in-vitro release profile of batches CC_5 & CC_{16} to CC_{19}



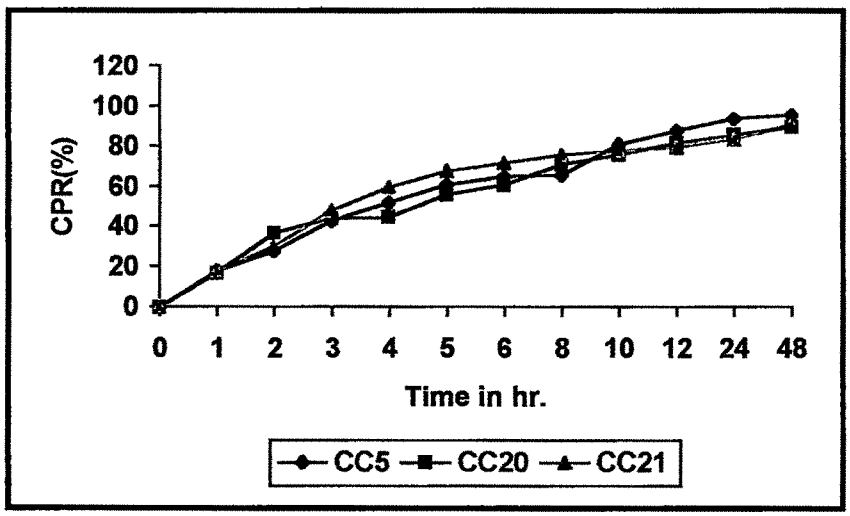
CPR : Cumulative Percentage Release

Table 3.30 The effect of concentration of glutraldehyde (cross-linking agent) on in-vitro release profile of chitosan coated CQP microspheres.

Time in hr.	CC_5		CC_{20}		CC_{21}	
	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD
1	1.53	18 ± 0.68	1.39	17 ± 0.58	1.387	18 ± 1.10
2	2.34	28 ± 1.09	2.30	37 ± 1.10	2.320	30 ± 1.28
3	3.55	43 ± 1.23	2.98	44 ± 1.24	3.660	48 ± 2.45
4	4.26	52 ± 1.45	3.73	45 ± 1.38	4.580	60 ± 2.98
5	5.06	61 ± 1.48	4.61	56 ± 1.66	5.240	68 ± 2.67
6	5.34	65 ± 2.35	5.02	61 ± 2.15	5.540	72 ± 2.76
8	5.42	66 ± 2.62	5.75	71 ± 2.23	5.790	76 ± 2.85
10	6.64	81 ± 2.78	6.39	76 ± 2.43	5.950	78 ± 3.20
12	7.21	88 ± 3.30	6.73	82 ± 3.25	6.100	80 ± 4.10
24	7.72	94 ± 3.24	7.02	86 ± 3.36	6.380	84 ± 3.88
48	7.89	96 ± 3.80	7.35	90 ± 3.80	6.920	91 ± 3.67

CPR : Cumulative Percentage Release

Figure 3.41 Comparative in-vitro release profile of batches CC_5 to CC_{21}



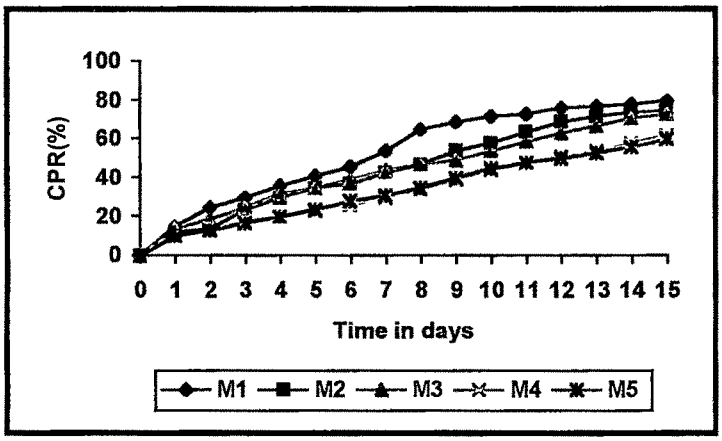
CPR : Cumulative Percentage Release

Table 3.31 The effect of Drug : Polymer ratio on in-vitro release profile of ethyl cellulose coated mefloquine hydrochloride microspheres.

Time in days	M_1		M_2		M_3		M_4		M_5	
	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD
1	0.593	15 ± 0.64	0.324	12 ± 0.54	0.293	14 ± 0.58	0.169	10 ± 0.49	0.142	10 ± 0.567
2	0.988	25 ± 0.71	0.540	14 ± 0.69	0.406	19 ± 0.73	0.237	14 ± 0.62	0.185	13 ± 0.68
3	1.185	30 ± 0.77	0.648	24 ± 0.98	0.513	25 ± 0.88	0.304	18 ± 0.93	0.241	17 ± 0.98
4	1.420	36 ± 0.79	0.810	30 ± 1.2	0.656	32 ± 1.1	0.338	20 ± 1.15	0.284	20 ± 1.2
5	1.620	41 ± 1.1	0.945	35 ± 1.38	0.718	35 ± 1.24	0.389	23 ± 1.31	0.341	24 ± 1.5
6	1.820	46 ± 1.23	0.999	37 ± 1.46	0.799	39 ± 1.36	0.439	26 ± 1.48	0.397	28 ± 1.62
7	2.130	54 ± 1.24	1.161	43 ± 1.54	0.902	44 ± 1.42	0.507	30 ± 1.53	0.439	31 ± 1.71
8	2.570	65 ± 1.4	1.269	47 ± 1.76	0.964	47 ± 1.48	0.575	34 ± 1.67	0.497	35 ± 1.79
9	2.730	69 ± 1.48	1.458	54 ± 1.88	1.005	49 ± 1.56	0.659	39 ± 1.81	0.566	40 ± 1.81
10	2.840	72 ± 1.56	1.566	58 ± 1.98	1.090	54 ± 1.62	0.747	44 ± 1.89	0.639	45 ± 1.86
11	2.880	73 ± 1.71	1.728	64 ± 2.13	1.210	59 ± 1.71	0.814	48 ± 2.1	0.682	48 ± 2.21
12	2.960	76 ± 1.8	1.863	69 ± 2.38	1.290	63 ± 1.74	0.865	51 ± 2.34	0.709	50 ± 2.34
13	3.030	76.8 ± 2.1	1.944	72 ± 2.48	1.380	67 ± 1.9	0.916	54 ± 2.48	0.763	53 ± 2.45
14	3.080	78 ± 2.23	1.998	74 ± 2.48	1.456	71 ± 2.1	0.983	58 ± 2.5	0.804	56 ± 2.6
15	3.160	80 ± 2.5	2.025	75 ± 2.68	1.499	73 ± 2.49	1.050	62 ± 2.6	0.852	60 ± 3.1

CPR : Cumulative Percentage Release

Figure 3.42 Comparative in-vitro release profile of batches M_1 to M_5



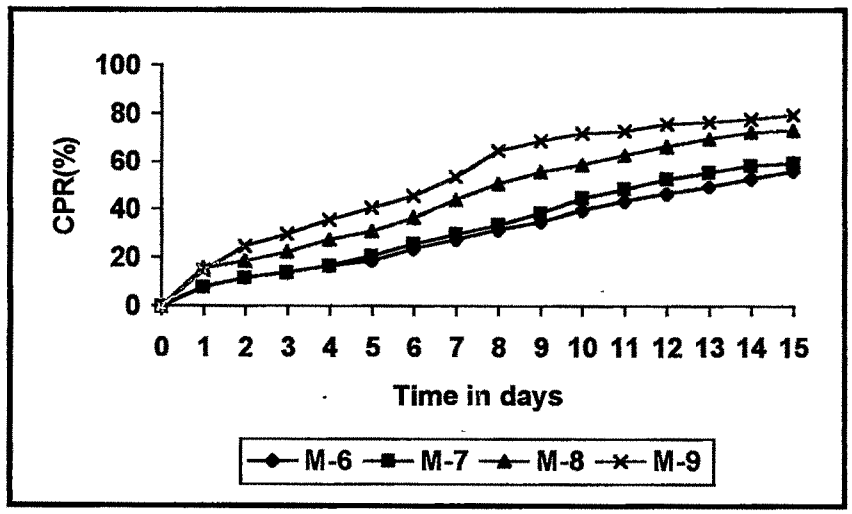
CPR : Cumulative Percentage Release

Table 3.32 The Effect of solvent ratio on in-vitro release profile of ethyl cellulose coated mefloquine hydrochloride microsphere

Time In days	M_6		M_7		M_8		M_9	
	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD
1	0.313	08 ± 0.42	0.305	08 ± 0.38	0.605	16.00 ± 0.63	0.593	15 ± 0.64
2	0.469	12 ± 0.64	0.457	12 ± 0.58	0.711	18.80 ± 0.63	0.988	25 ± 0.83
3	0.547	14 ± 0.71	0.533	14 ± 0.62	0.855	22.60 ± 0.83	1.185	30 ± 0.86
4	0.665	17 ± 0.81	0.648	17 ± 0.84	1.055	27.90 ± 0.86	1.422	36 ± 1.10
5	0.743	19 ± 0.83	0.800	21 ± 0.88	1.184	31.33 ± 1.20	1.620	41± 1.15
6	0.938	24 ± 1.20	0.990	26 ± 0.98	1.403	37.10 ± 1.24	1.820	46 ± 1.32
7	1.090	28 ± 1.24	1.143	30 ± 1.20	1.682	44.50 ± 1.26	2.130	54 ± 1.36
8	1.250	32 ± 1.32	1.298	34 ± 1.32	1.934	51.15 ± 1.29	2.570	65 ± 1.42
9	1.370	35 ± 1.36	1.486	39 ± 1.42	2.120	56.00 ± 1.31	2.730	69 ± 1.39
10	1.560	40 ± 1.46	1.720	45 ± 1.51	2.230	59.00 ± 1.33	2.840	72 ± 1.56
11	1.720	44 ± 1.48	1.870	49 ± 1.52	2.380	63.00 ± 1.36	2.880	73 ± 1.58
12	1.840	47 ± 1.50	2.010	53 ± 1.54	2.520	66.50 ± 1.38	2.960	76 ± 2.10
13	1.950	49.9±1.52	2.130	55.9±1.48	2.640	69.90 ± 1.39	3.034	76.8±2.40
14	2.080	53.3±1.62	2.240	58.8±1.62	2.710	72.50 ± 1.45	3.060	78 ± 2.50
15	2.210	56.5±1.63	2.290	60 ± 2.10	2.710	73.40 ± 1.62	3.160	80 ± 2.45

CPR : Cumulative Percentage Release

Figure 3.43 Comparative in-vitro release profile of batches M_6 to M_9



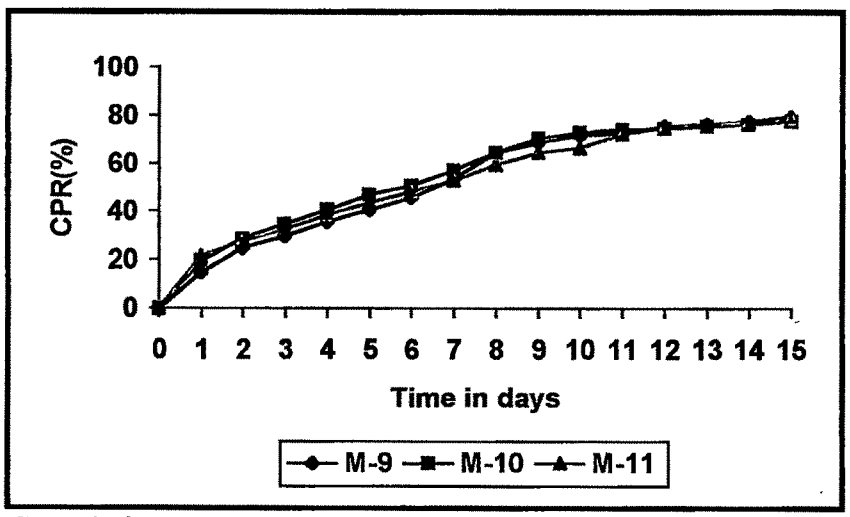
CPR : Cumulative Percentage Release

Table 3.33 The Effect of volume of disperse phase on in-vitro release profile of Ethyl Cellulose coated mefloquine hydrochloride microspheres.

Time In days	M_9		M_{10}		M_{11}	
	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD
1	0.593	15 ± 0.64	0.689	20.00 ± 0.712	0.858	22.00 ± 0.51
2	0.988	25 ± 0.83	1.005	28.80 ± 0.823	1.070	28.00 ± 0.67
3	1.185	30 ± 0.86	1.225	35.10 ± 0.880	1.264	33.00 ±0.80
4	1.422	36 ± 1.10	1.430	41.00 ± 0.980	1.496	39.00 ± 0.89
5	1.620	41 ± 1.15	1.647	47.18 ± 1.100	1.682	43.90 ± 1.10
6	1.820	46 ± 1.32	1.786	51.18 ± 1.240	1.869	48.80 ± 1.28
7	2.130	54 ± 1.36	2.003	57.40 ± 1.280	2.036	53.18 ± 1.24
8	2.570	65 ± 1.42	2.260	64.80 ± 1.320	2.244	59.90 ± 1.33
9	2.730	69 ± 1.39	2.478	71.00 ± 1.330	2.482	64.80 ± 1.48
10	2.840	72 ± 1.56	2.558	73.30 ± 1.360	2.558	66.80 ± 1.56
11	2.880	73 ± 1.58	2.596	74.40 ± 1.420	2.758	72.60 ± 1.67
12	2.960	76 ± 2.10	2.640	75.00 ± 1.440	2.892	75.50 ± 1.71
13	3.034	76.8 ± 2.40	2.660	76.00 ± 1.620	2.940	76.80 ± 1.78
14	3.060	78 ± 2.50	2.680	76.80 ± 1.780	2.984	77.90 ± 1.98
15	3.160	80 ± 2.45	2.720	78.00 ± 1.860	3.026	79.50 ± 2.10

CPR : Cumulative Percentage Release

Figure 3.44 Comparative in-vitro release profile of batches M_9 to M_{11}



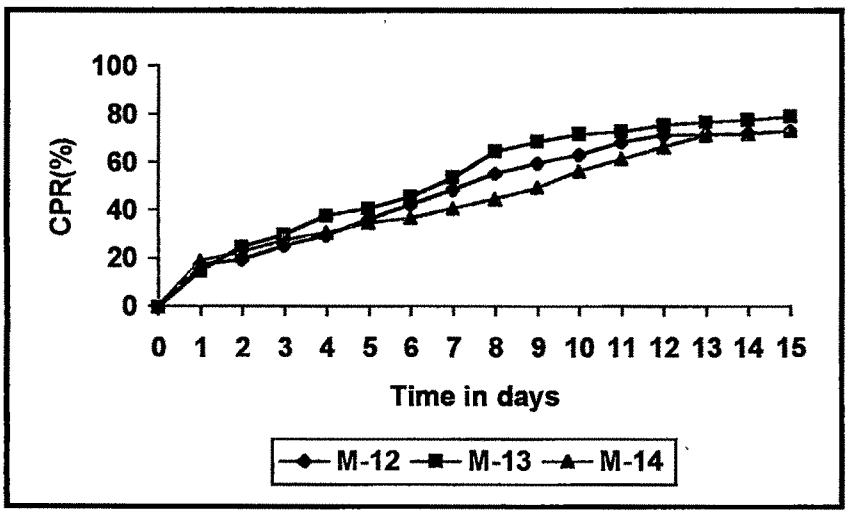
CPR : Cumulative Percentage Release

Table 3.34 The Effect of PVA concentration (continuous) phase on in-vitro release profile of Ethyl Cellulose coated mefloquine hydrochloride microspheres.

Time In days	M_{12}		M_{13}		M_{14}	
	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD
1	0.712	17.60 ± 0.82	0.617	15 ± 0.68	0.762	19 ± 0.71
2	0.805	19.90 ± 0.71	1.028	25 ± 0.71	0.922	23 ± 0.81
3	1.030	25.50 ± 0.81	1.233	30 ± 0.80	1.123	28 ± 0.86
4	1.212	29.95 ± 0.87	1.562	38 ± 1.24	1.240	31 ± 1.10
5	1.476	36.50 ± 1.05	1.685	41 ± 1.28	1.410	35 ± 1.25
6	1.730	42.80 ± 1.10	1.891	46 ± 1.39	1.484	37 ± 1.28
7	1.982	49.00 ± 1.15	2.212	54 ± 1.44	1.645	41 ± 1.39
8	2.248	55.60 ± 1.23	2.672	65 ± 1.58	1.805	45 ± 1.44
9	2.418	59.80 ± 1.28	2.836	69 ± 1.62	2.000	49.9 ± 1.58
10	2.560	63.38 ± 1.80	2.954	72 ± 1.71	2.266	56.5 ± 1.62
11	2.780	68.85 ± 1.85	3.000	73 ± 1.74	2.478	61.8 ± 1.71
12	2.893	71.52 ± 1.89	3.124	76 ± 1.50	2.683	66.9 ± 1.74
13	2.910	72.00 ± 1.90	3.165	77 ± 1.55	2.867	71.5 ± 1.76
14	2.932	72.50 ±2.10	3.210	78 ± 1.60	2.900	72.4 ± 1.78
15	2.977	73.60 ± 2.20	3.268	79.5 ± 1.80	2.950	73.5 ± 1.88

CPR : Cumulative Percentage Release

Figure 3.45 Comparative in-vitro release profile of batches M_{12} to M_{14}



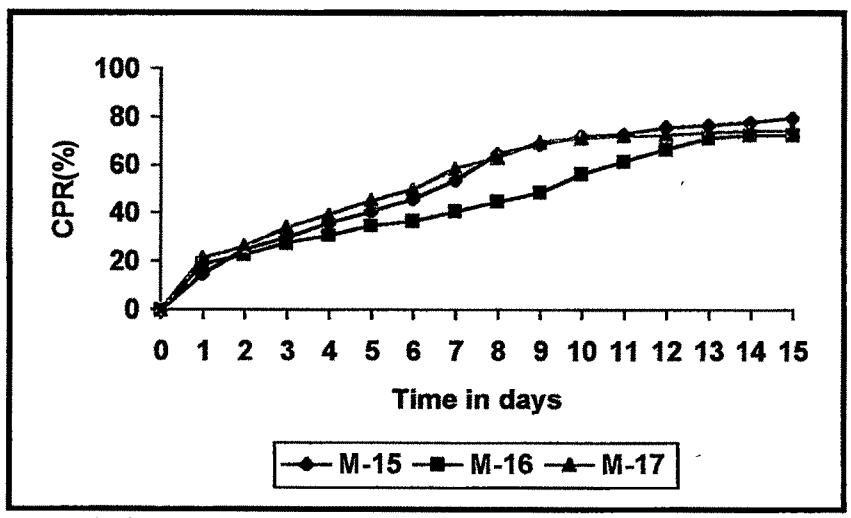
CPR : Cumulative Percentage Release

Table 3.35 The Effect of volume of continuous phase on in-vitro release profile of Ethyl Cellulose coated mefloquine hydrochloride microspheres.

Time In days	M_{15}		M_{16}		M_{17}	
	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD
1	0.593	15 ± 0.50	0.739	19 ± 0.80	0.752	21.80 ± 0.60
2	0.988	25 ± 0.80	0.895	23 ± 0.65	0.918	26.60 ± 0.90
3	1.185	30 ± 1.10	1.084	28 ± 0.95	1.164	34.25 ± 1.20
4	1.422	36 ± 1.15	1.210	31 ± 0.98	1.368	39.65 ± 1.20
5	1.620	41 ± 1.56	1.362	35 ± 1.10	1.570	45.58 ± 1.60
6	1.820	46 ± 1.50	1.440	37 ± 1.20	1.730	50.15 ± 1.40
7	2.130	54 ± 1.42	1.595	41 ± 1.25	2.030	58.85 ± 1.20
8	2.570	65 ± 1.58	1.750	45 ± 1.23	2.190	63.38 ± 1.50
9	2.730	69 ± 1.62	1.910	49 ± 1.24	2.390	69.90 ± 1.60
10	2.840	72 ± 1.71	2.198	56.5 ± 1.5	2.415	71.50 ± 1.70
11	2.880	73 ± 1.58	2.400	61.8 ± 1.8	2.490	72.50 ± 1.58
12	2.960	76 ± 1.90	2.610	66.9 ± 1.7	2.510	72.80 ± 1.50
13	3.030	76.8 ± 2.1	2.782	71.5 ± 1.8	2.550	73.80 ± 1.60
14	3.080	78 ± 2.20	2.840	72.8 ± 2.6	2.580	74.80 ± 2.80
15	3.160	80 ± 2.30	2.880	73 ± 2.40	2.585	75.00 ± 2.35

CPR : Cumulative Percentage Release

Figure 3.46 Comparative in-vitro release profile of batches M_{15} to M_{17}



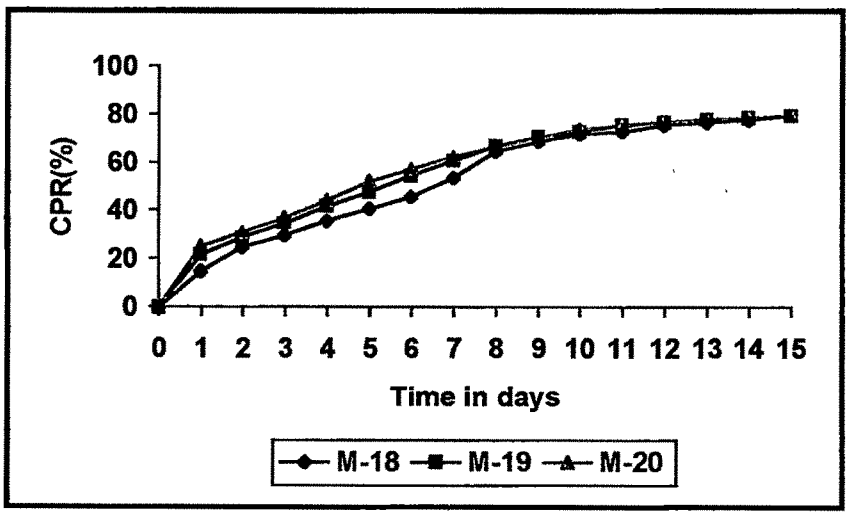
CPR : Cumulative Percentage Release

Table 3.36 The Effect of stirring speed on in-vitro release profile of Ethyl Cellulose coated mefloquine hydrochloride microspheres.

Time In days	M_{18}		M_{19}		M_{20}	
	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD
1	0.593	15 ± 1.10	0.832	22 ± 0.62	0.859	25.50 ± 1.30
2	0.988	25 ± 1.50	1.085	29 ± 0.86	1.056	31.33 ± 1.35
3	1.185	30 ± 1.60	1.310	35 ± 1.10	1.272	37.35 ± 1.42
4	1.422	36 ± 2.10	1.570	42 ± 1.23	1.500	44.52 ± 1.52
5	1.620	41 ± 2.00	1.790	48 ± 1.28	1.764	52.50 ± 1.48
6	1.820	46 ± 2.05	2.050	55 ± 1.34	1.938	57.50 ± 1.45
7	2.130	54 ± 2.30	2.280	61 ± 1.36	2.106	62.50 ± 1.62
8	2.570	65 ± 2.45	2.490	67 ± 1.42	2.240	66.50 ± 2.12
9	2.730	69 ± 2.38	2.650	71 ± 2.18	2.376	70.50 ± 2.15
10	2.840	72 ± 2.45	2.730	73 ± 2.17	2.494	74.00 ± 2.23
11	2.880	73 ± 2.36	2.810	76 ± 3.32	2.544	75.50 ± 2.40
12	2.960	76 ± 2.45	2.890	77 ± 3.36	2.545	77.00 ± 2.48
13	3.030	76.8 ± 2.60	2.980	78 ± 3.39	2.645	78.40 ± 2.46
14	3.080	78 ± 2.56	2.985	79 ± 3.41	2.660	79.00 ± 2.30
15	3.140	80 ± 2.80	2.990	80 ± 2.60	2.696	80.00 ± 1.90

CPR : Cumulative Percentage Release

Figure 3.47 Comparative in-vitro release profile of batches M_{18} to M_{20}



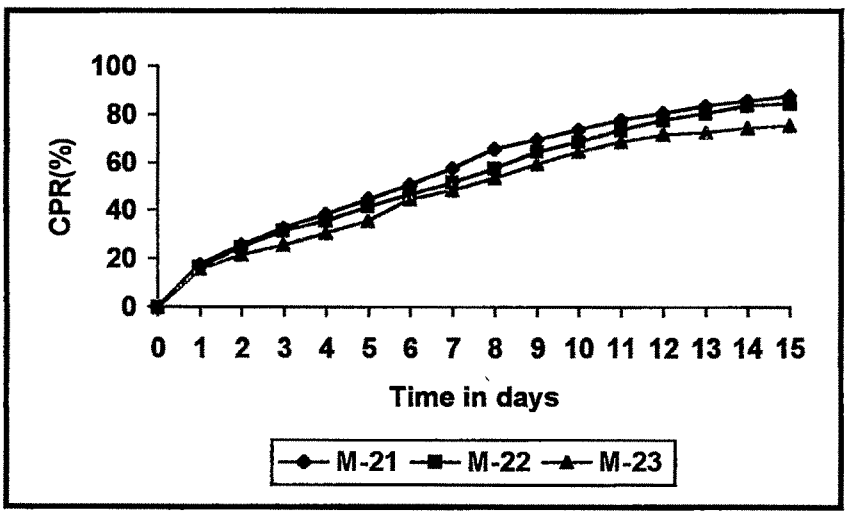
CPR : Cumulative Percentage Release

Table 3.37 The Effect of different molecular weight of PEG on in-vitro release profile of Ethyl Cellulose coated mefloquine hydrochloride microspheres.

Time In days	M_{21}		M_{22}		M_{23}	
	Mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD
1	0.67	18 ± 0.62	0.66	17 ± 0.58	0.620	16 ± 0.63
2	0.97	26 ± 0.73	0.98	25 ± 0.64	0.770	22 ± 0.71
3	1.24	33 ± 0.98	1.25	32 ± 0.79	0.995	26 ± 1.10
4	1.45	39 ± 1.40	1.41	36 ± 0.96	1.190	31 ± 2.10
5	1.68	45 ± 1.20	1.64	42 ± 1.05	1.380	36 ± 2.42
6	1.89	51 ± 1.80	1.83	47 ± 1.40	1.720	45 ± 1.81
7	2.13	58 ± 1.30	2.03	52 ± 1.32	1.880	49 ± 2.35
8	2.46	66 ± 2.10	2.26	58 ± 2.30	2.070	54 ± 1.42
9	2.61	70 ± 1.90	2.53	65 ± 1.62	2.290	60 ± 1.38
10	2.76	74 ± 2.30	2.69	69 ± 1.49	2.490	65 ± 2.10
11	2.91	78 ± 2.50	2.88	74 ± 2.20	2.640	69 ± 3.20
12	3.02	81 ± 0.90	3.04	78 ± 2.40	2.760	72 ± 3.10
13	3.13	84 ± 1.25	3.15	81 ± 2.38	2.790	73 ± 2.60
14	3.20	86 ± 0.88	3.27	84 ± 1.71	2.870	75 ± 2.49
15	3.28	88 ± 1.35	3.31	85 ± 1.49	2.910	76 ± 2.10

CPR : Cumulative Percentage Release

Figure 3.48 Comparative in-vitro release profile of batches M_{21} to M_{23}



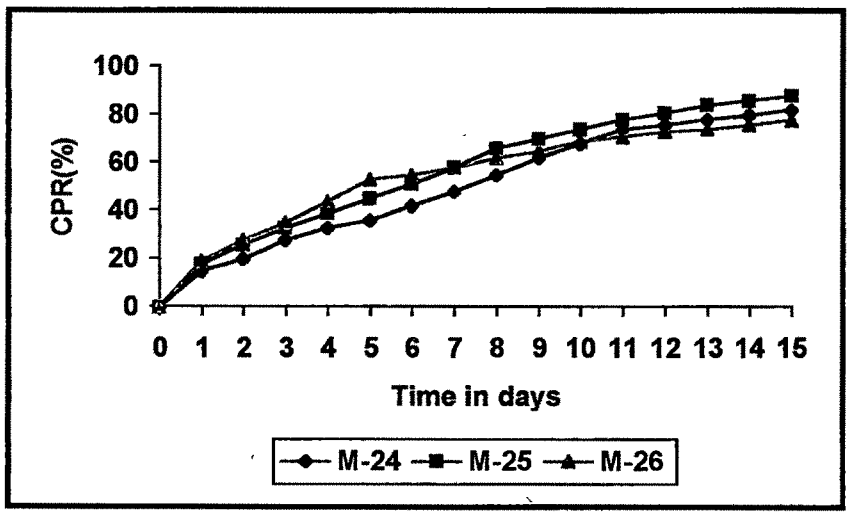
CPR : Cumulative Percentage Release

Table 3.38 The Effect of PEG concentration on in-vitro release profile of Ethyl Cellulose coated mefloquine hydrochloride microspheres.

Time In days	M_{24}		M_{25}		M_{26}	
	mg	CPR ± SD	mg	CPR ± SD	mg	CPR ± SD
1	0.59	15 ± 0.62	0.670	18 ± 0.71	0.73	19 ± 0.63
2	0.78	20 ± 0.73	0.970	26 ± 1.10	1.08	28 ± 0.88
3	1.09	28 ± 0.98	1.239	33 ± 1.30	1.34	35 ± 1.10
4	1.29	33 ± 1.40	1.453	39 ± 1.25	1.69	44 ± 1.30
5	1.41	36 ± 1.20	1.680	45 ± 2.10	2.03	53 ± 1.39
6	1.64	42 ± 1.80	1.890	51 ± 0.90	2.11	55 ± 1.54
7	1.88	48 ± 1.30	2.160	58 ± 1.21	2.23	58 ± 0.98
8	2.15	55 ± 2.10	2.460	66 ± 1.48	2.38	62 ± 0.78
9	2.43	62 ± 1.90	2.610	70 ± 1.70	2.49	65 ± 0.88
10	2.66	68 ± 2.30	2.760	74 ± 1.50	2.65	69 ± 1.25
11	2.89	74 ± 2.50	2.910	78 ± 2.10	2.73	71 ± 3.10
12	2.97	76 ± 0.90	3.020	81 ± 0.90	2.81	73 ± 2.60
13	3.05	78 ± 1.25	3.130	84 ± 0.88	2.84	74 ± 2.40
14	3.13	80 ± 0.88	3.200	86 ± 1.90	2.92	76 ± 1.60
15	3.21	82 ± 1.35	3.280	88 ± 2.10	2.99	78 ± 0.80

CPR : Cumulative Percentage Release

Figure 3.49 Comparative in-vitro release profile of batches M_{24} to M_{26}



CPR : Cumulative Percentage Release

3.3.4.2 Statistical Analysis

Table 3.39 Factorial Transformed Values for Chitosan Coated Chloroquine Phosphate Microspheres

Real values		Transformed values		Particle size	Drug content
X_1	X_2	X_1	X_2	(μ)	(%)
Stirring speed (rpm)	polymer conc. (%w/v)			Y_1	Y_1
500	1.5	-1	1	38.48	73.38
1000	1.5	0	1	26.63	71.10
1500	1.5	1	1	22.34	81.00
500	1.0	-1	0	26.20	76.20
1000	1.0	0	0	18.18	81.80
1500	1.0	1	0	23.33	67.08
500	0.5	-1	-1	20.10	82.80
1000	0.5	0	-1	16.30	81.68
1500	0.5	1	-1	14.38	78.83

Table 3.40

Results of multiple regression analysis and ANOVA for drug content of chitosan coated chloroquine phosphate microsphere
(Stirring speed (X_1), polymer conc. (X_2), drug content (Y_1)

X_1	X_2	X_1X_2	X_1^2	X_2^2	Drug Content (%)
-1	1	-1	1	1	73.38
0	1	0	0	1	71.10
1	1	1	1	1	81.00
-1	0	0	1	0	76.20
0	0	0	0	0	81.80
1	0	0	1	0	67.08
-1	-1	1	1	1	82.80
0	-1	0	0	1	81.68
1	-1	-1	1	1	78.83
Average					77.10

Regression Output :

	Full Model $X_1, X_2, X_1X_2, X_1^2, X_2^2$					Reduced Model X_1, X_2 and Y	
Constant [b_0]	76.123					77.097	
Std. Err of Y Est	6.546					5.58	
R Squared	0.474					0.236	
Multiple R	0.689					0.486	
No. of Observations	9					9	
Degrees of Freedom	3					6	
	b_1	b_2	b_{12}	b_{11}	B_{22}	b_1	b_2
X Coefficients	-0.912	-2.972	2.898	-1.645	3.105	-0.912	-2.972
Std. Err of Coeff.	2.672	2.672	3.273	4.629	4.629	2.278	2.278
Calc. t Value	-0.341	-1.112	0.885	-0.355	0.671	-0.400	-1.304
P value	0.756	0.347	0.441	0.746	0.550	0.702	0.239

Results of ANOVA

		SS	DF	MS	F
Source	FM	116.25	5	23.24	0.54
	RM	57.97	2	28.98	0.93
Total	FM	244.8	8	42.85	
	RM	244.8	8	31.13	

Table 3.41

Results of multiple regression analysis and ANOVA for particle size of chitosan coated chloroquine phosphate microsphere
(Stirring speed (X_1), polymer conc. (X_2), particle size (Y_1)

X_1	X_2	X_1X_2	X_1^2	X_2^2	Particle size(μ)
-1	1	-1	1	1	38.48
0	1	0	0	1	36.62
1	1	1	1	1	22.34
-1	0	0	1	0	26.20
0	0	0	0	0	18.18
1	0	0	1	0	23.43
-1	-1	1	1	1	20.10
0	-1	0	0	1	16.30
1	-1	-1	1	1	14.38
Average					24.00

Regression Output :

	Full Model $X_1,X_2, X_1X_2,X_1^2,X_2^2$					Reduced Model X_1, X_2 and Y	
Constant [b_0]	22.27					23.99	
Std. Err of Y Est	5.04					4.31	
R Squared	0.867					0.739	
Multiple R	0.931					0.896	
No. of Observations	9					9	
Degrees of Freedom	3					6	
	b_1	b_2	b_{12}	b_{11}	b_{22}	b_1	b_2
X Coefficients	-4.122	7.777	-2.605	0.438	2.133	-4.122	7.777
Std. Err of Coeff.	2.061	2.061	2.524	3.570	3.570	1.773	1.773
Calc. t Value	-2.000	3.773	-1.032	0.123	0.598	-2.325	4.387
P value	0.139	0.033	0.378	0.910	0.592	0.059	0.004

Results of ANOVA

		SS	DF	MS	F
Source	FM	501.41	5	100.28	3.93
	RM	464.78	2	232.39	12.32
Total	FM	577.89	8		
	RM	577.89	8		

Table 3.42 Factorial Transformed Values of Chitosan Coated Chloroquine Phosphate Microspheres

Real values		Transformed values		Particle size
X_1	X_2	X_1	X_2	(μ)
Stirring speed (rpm)	Conc. of c.a. (% w/w)			Y_1
500	15	-1	1	71.80
1000	15	0	1	57.64
1500	15	1	1	40.32
500	10	-1	0	51.80
1000	10	0	0	49.95
1500	10	1	0	44.30
500	5	-1	-1	22.50
1000	5	0	-1	18.80
1500	5	1	-1	16.20

c.a. = crosslinking agent (% w/w of Glutaraldehyde)

Table 3.43

Results of multiple regression analysis and ANOVA for particle size of chitosan coated chloroquine phosphate microsphere (Stirring speed (X_1), conc. of cross-linking agent (X_2), particle size (Y_1)					
X_1	X_2	X_1X_2	X_1^2	X_2^2	Particle size(μ)
-1	1	-1	1	1	71.80
0	1	0	0	1	57.64
1	1	1	1	1	40.32
-1	0	0	1	0	51.80
0	0	0	0	0	49.25
1	0	0	1	0	84.30
-1	-1	1	1	1	22.50
0	-1	0	0	1	18.80
1	-1	-1	1	1	16.20
Average					45.85

Regression Output :

	Full Model $X_1, X_2, X_1X_2, X_1^2, X_2^2$					Reduced Model X_1, X_2 and Y	
Constant [b_0]	57.83					45.84	
Std. Err of Y Est	18.687					20.08	
R Squared	0.768					0.465	
Multiple R	0.876					0.682	
No. of Observations	9					9	
Degrees of Freedom	3					6	
	b_1	b_2	b_{12}	b_{11}	b_{22}	b_1	b_2
X Coefficients	-0.880	18.710	-6.295	5.923	-23.907	-0.880	18.710
Std. Err of Coeff.	7.629	7.629	9.344	13.214	13.214	8.198	8.198
Calc. t Value	-0.115	2.452	-0.674	0.448	-1.809	-0.107	2.282
P value	0.915	0.091	0.549	0.684	0.168	0.918	0.063

Results of ANOVA

		SS	DF	MS	F
Source	FM	3476.76	5	695.35	1.99
	RM	2105.03	2	1052.51	2.6
Total	FM	452.39	8		
	RM	452.39	8		

Table 3.44 Factorial Transformed Values of Ethyl Cellulose Coated Mefloquine Hydrochloride Microspheres

Real values		Transformed values		Particle size	Drug content
X_1	X_2	X_1	X_2	(μ)	(%)
Volume of DCM (ml)	Volume of PVA (ml)			Y_1	Y_1
5	200	-1	1	10.50	76.50
10	200	0	1	6.82	68.83
20	200	1	1	4.20	72.00
5	100	-1	0	12.35	76.63
10	100	0	0	9.80	79.00
20	100	1	0	9.60	69.95
5	50	-1	-1	18.50	81.00
10	50	0	-1	15.85	77.80
20	50	1	-1	12.30	82.00

Table 3.45

Results of multiple regression analysis and ANOVA for drug content of ethyl cellulose coated mefloquine hydrochloride microsphere (Volume of DCM(ml) (X_1), volume of PVA(ml) (X_2), drug content (Y_1)					
X_1	X_2	X_1X_2	X_1^2	X_2^2	Drug content (%)
-1	1	-1	1	1	76.50
0	1	0	0	1	68.83
1	1	1	1	1	72.00
-1	0	0	1	0	76.63
0	0	0	0	0	79.00
1	0	0	1	0	69.95
-1	-1	1	1	1	81.00
0	-1	0	0	1	77.80
1	-1	-1	1	1	82.00
Average					75.97

Regression Output :

	Full Model $X_1,X_2, X_1X_2,X_1^2,X_2^2$					Reduced Model X_1, X_2 and Y	
Constant [b_0]	74.43					75.96	
Std. Err of Y Est	4.32					3.38	
R Squared	0.685					0.613	
Multiple R	0.827					0.783	
No. of Observations	9					9	
Degrees of Freedom	3					6	
	b_1	b_2	b_{12}	b_{11}	b_{22}	b_1	b_2
X Coefficients	-1.697	-3.912	-1.375	1.137	1.162	-1.697	-3.912
Std. Err of Coeff.	1.763	1.763	2.160	3.054	3.054	1.383	1.383
Calc. t Value	-0.962	-2.218	-0.637	0.372	0.380	-1.227	-2.829
P value	0.407	0.113	0.570	0.734	0.729	0.266	0.030

Results of ANOVA

		SS	DF	MS	F
Source	FM	121.92	5	24.38	1.3
	RM	109.07	2	54.53	4.76
Total	FM	177.88	8		
	RM	177.88	8		

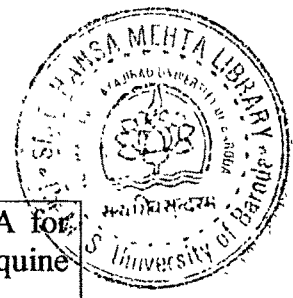


Table 3.46

Results of multiple regression analysis and ANOVA for particle size of ethyl cellulose coated mefloquine hydrochloride microsphere (Volume of DCM(ml) (X_1), volume of PVA(ml) (X_2), particle size (Y_1)					
X_1	X_2	X_1X_2	X_1^2	X_2^2	Particle size (μ)
-1	1	-1	1	1	10.50
0	1	0	0	1	6.82
1	1	1	1	1	4.20
-1	0	0	1	0	12.35
0	0	0	0	0	9.80
1	0	0	1	0	9.60
-1	-1	1	1	1	18.50
0	-1	0	0	1	15.85
1	-1	-1	1	1	12.30
Average					11.10

Regression Output :

	Full Model $X_1, X_2, X_1X_2, X_1^2, X_2^2$					Reduced Model X_1, X_2 and Y	
Constant [b_0]	10.304					11.102	
Std. Err of Y Est	1.287					1.044	
R Squared	0.966					0.956	
Multiple R	0.983					0.978	
No. of Observations	9					9	
Degrees of Freedom	3					6	
	b_1	b_2	b_{12}	b_{11}	b_{22}	b_1	b_2
X Coefficients	-2.542	-4.188	-0.025	0.418	0.778	-2.542	-4.188
Std. Err of Coeff.	0.526	0.526	0.644	0.911	0.911	0.426	0.426
Calc. t Value	-4.834	-7.966	-0.039	0.459	0.855	-5.963	-9.827
P value	0.017	0.004	0.971	0.677	0.456	0.00099618	6.3993 E-05

Results of ANOVA

		SS	DF	MS	F
Source	FM	145.57	5	29.11	17.55
	RM	144.01	2	72.06	66.05
Total	FM	150.55	8		
	RM	150.55	8		

Table 3.47 Summary results of Regression for measured response of chitosan coated chloroquine phosphate microspheres

Response	variable	b_0	b_1	b_2	b_{12}	b_{11}	B_{22}	R^2	F
Particle Size μ	X_1 – stirring speed X_2 – conc. of cross-linking agent								
Full model		57.834	-0.889	18.710	-6.295	5.923	-23.907	0.768	1.991
Reduced model		45.846	-0.880	18.710	-	-	-	0.465	2.61
Drug content %	X_1– polymer concentration X_2 –stirring speed								
Full model		76.123	-0.912	-2.972	2.898	-1.645	3.105	0.474	0.543
Reduced model		77.097	-0.912	-2.972	-	-	-	0.236	0.930

Table 3.48 Summary results of Regression for measured response of ethyl cellulose coated mefloquine hydrochloride microspheres

Response	variable	b_0	b_1	b_2	b_{12}	b_{11}	B_{22}	R^2	F
Particle Size μ	X_1 – volume of DCM X_2 – volume of PVA solution								
Full model		10.304	-2.542	-4.188	-0.025	0.418	0.778	0.967	17.553
Reduced model		11.102	-2.542	-4.188	-	-	-	0.956	66.05
Drug content	X_1 – volume of DCM X_2 – volume of PVA solution								
Full model		74.436	-1.697	-3.912	-1.375	1.137	1.162	0.685	1.307
Reduced model		75.968	-1.697	-3.912	-	-	-	0.613	4.756

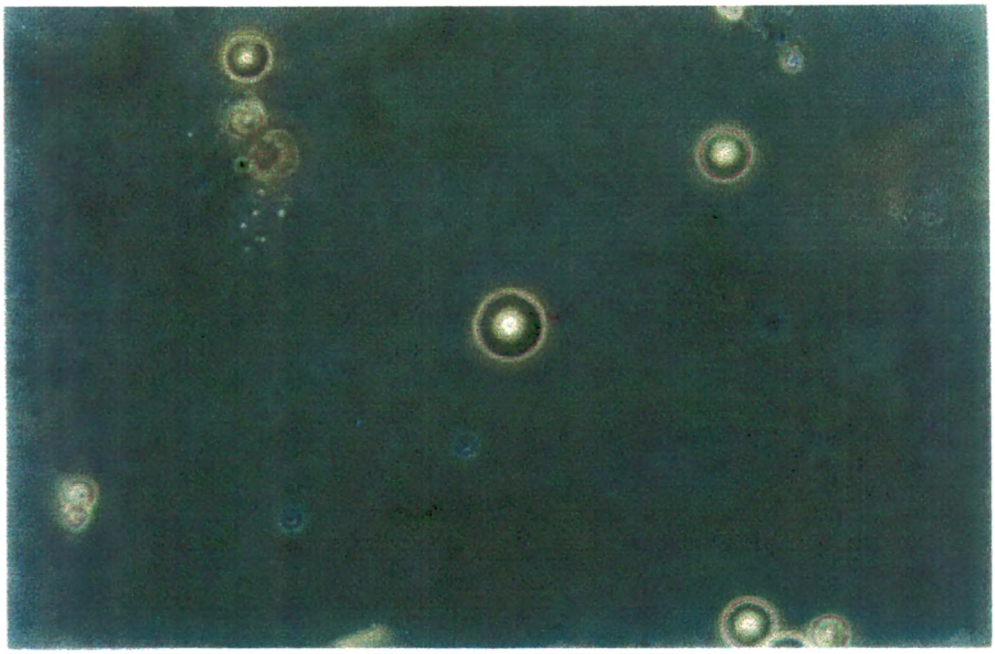


Plate : 3.1 Photomicrograph of Batch EC1

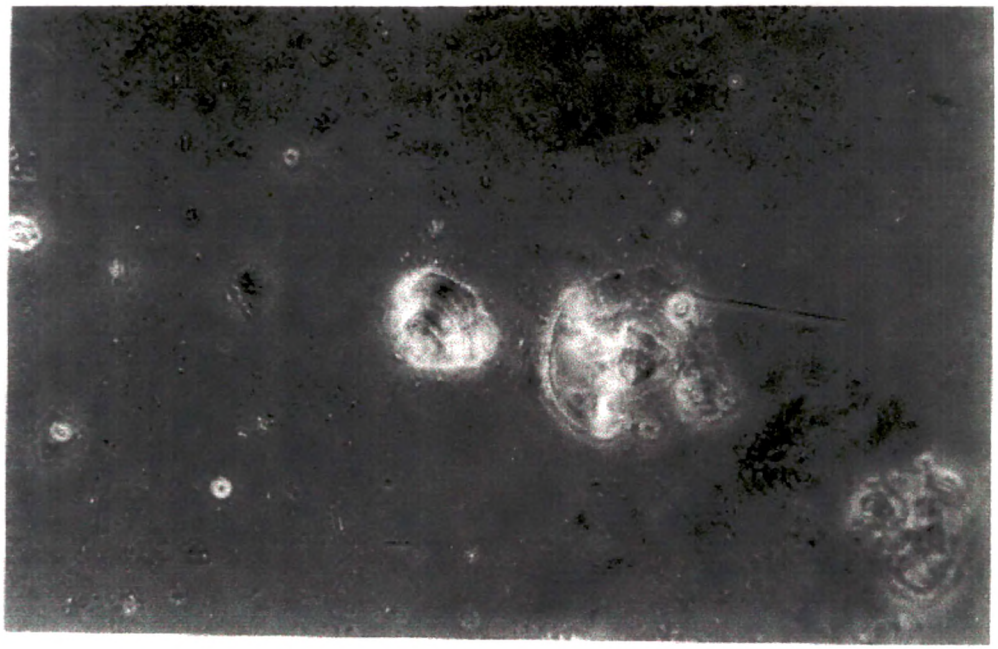


Plate : 3.2 Photomicrograph of Batch EC2

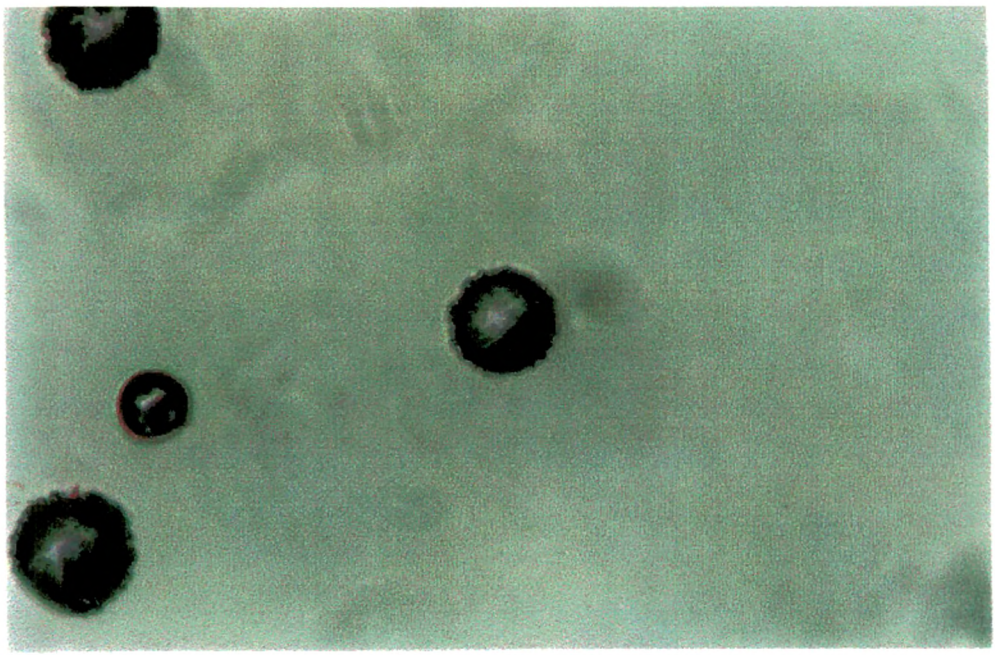


Plate : 3.3 Photomicrograph of Batch EC5

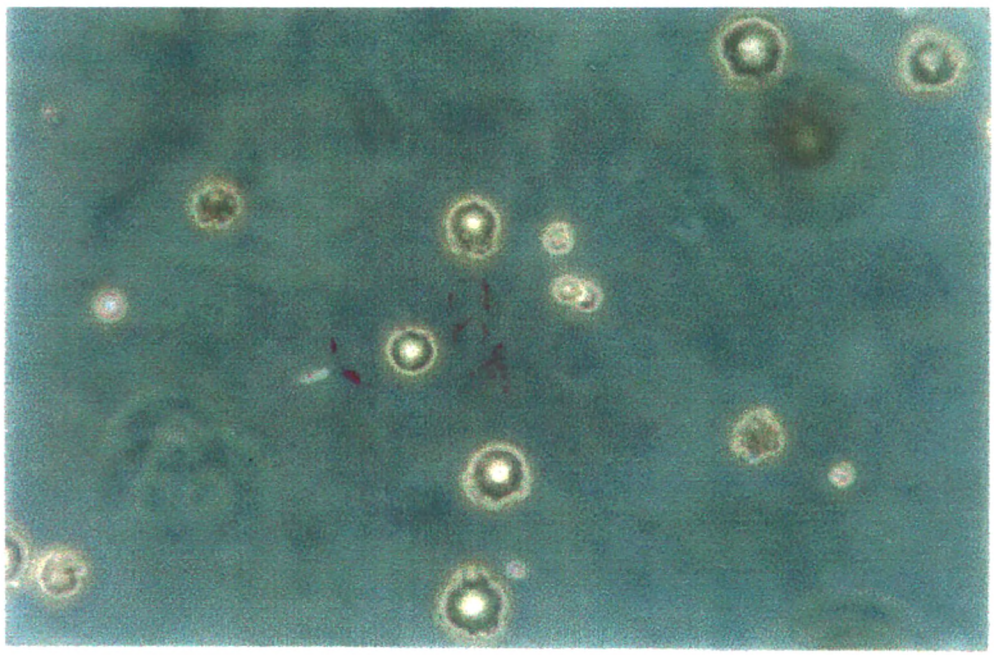


Plate : 3.4 Photomicrograph of Batch CC5

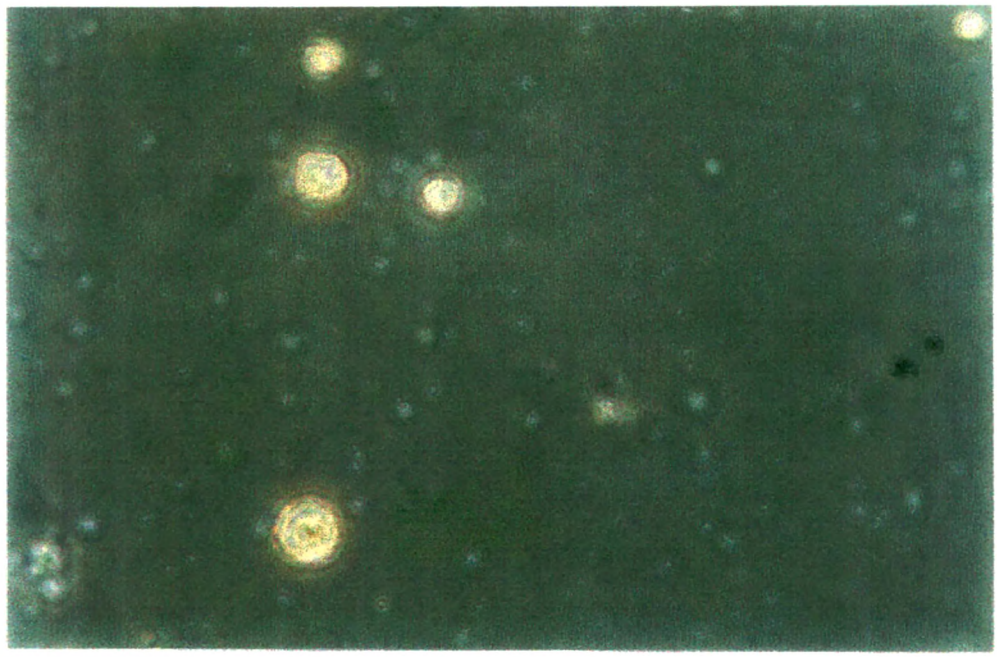


Plate : 3.5 Photomicrograph of Batch CC11

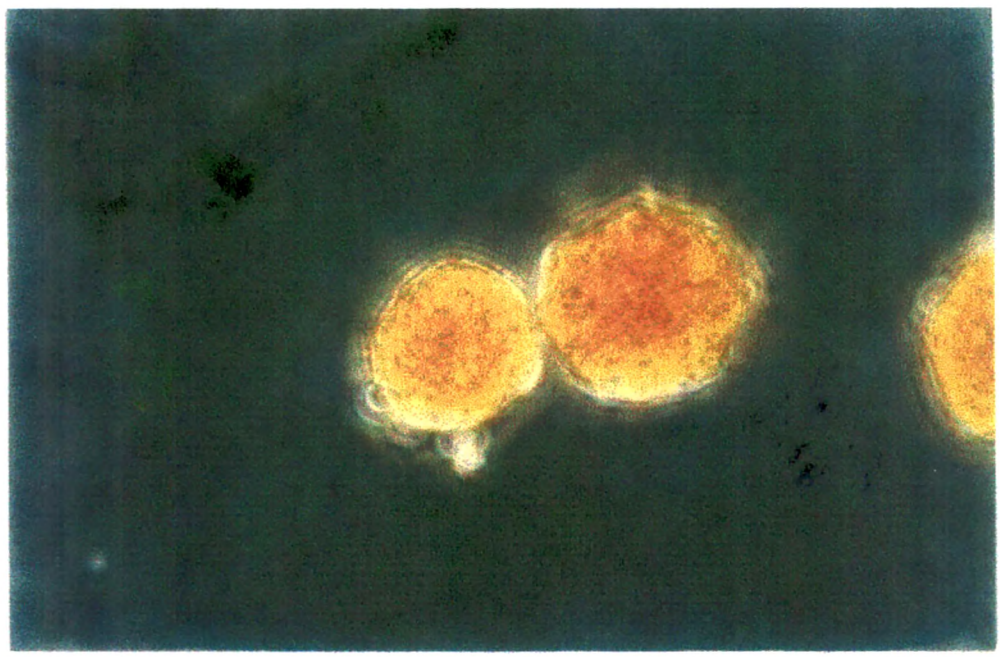


Plate : 3.6 Photomicrograph of Batch CC20

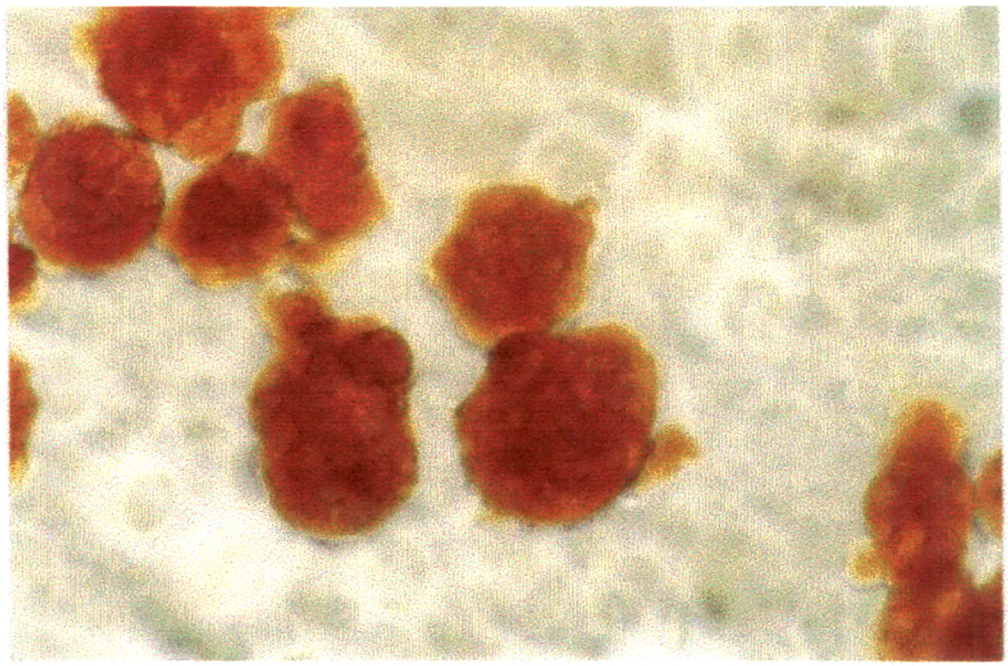


Plate : 3.7 Photomicrograph of Batch CC21

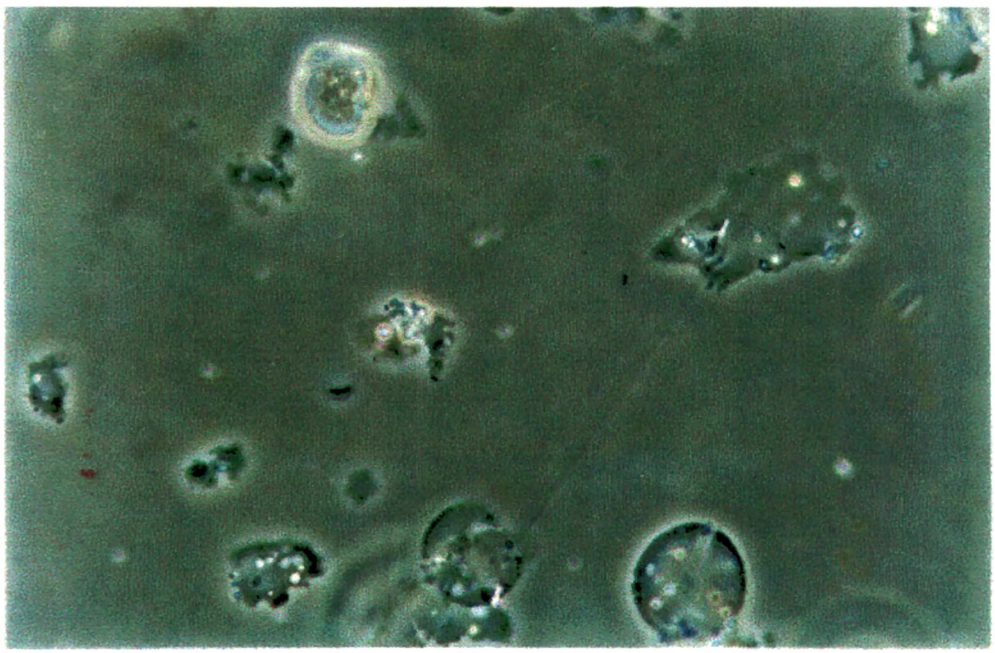


Plate : 3.8 Photomicrograph of Batch M6

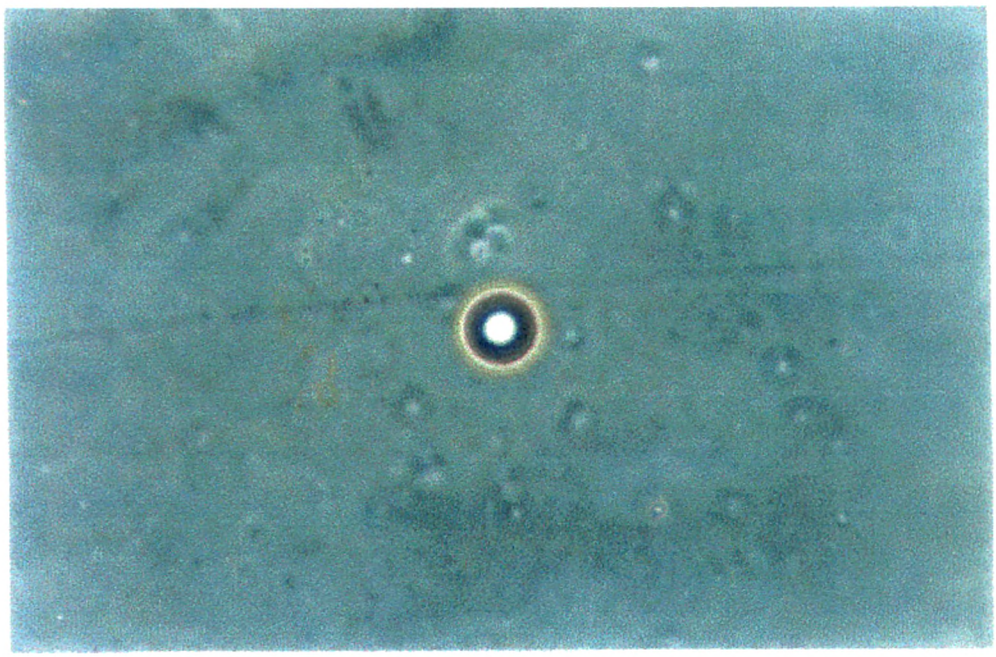


Plate : 3.9 Photomicrograph of Batch M18

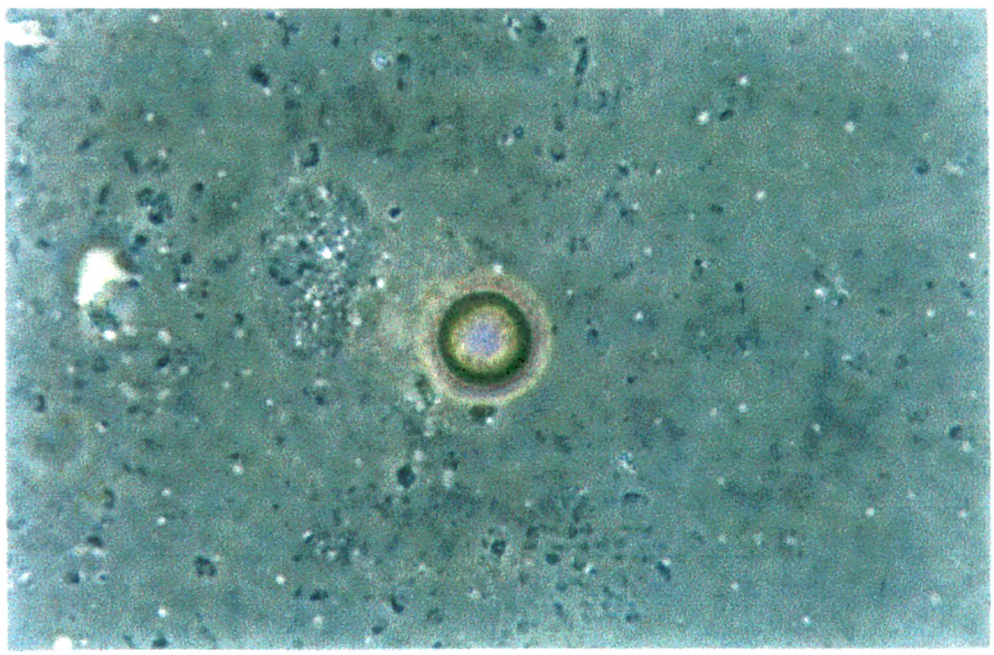


Plate : 3.10 Photomicrograph of Batch M26

3.3.4.3 Discussion

Preparation of ethyl cellulose coated chloroquine phosphate microspheres.

Microspheres of Chloroquine Phosphate in ethyl cellulose base were first prepared by emulsifying aqueous solution of Chloroquine Phosphate with DCM containing ethyl cellulose and evaporating the organic phase in 100ml aqueous media containing 0.5% w/v PVA (Batch EC_1). The microspheres recovered were subjected to % drug entrapment, particle size & drug release profile and are tabulated in Table 3.19.

The percentage yield of microsphere was around 55.6 ± 1.5 % while drug entrapment in it was very low (12.0 ± 2.3 %). The low percentage entrapment was obvious due to high water solubility of the drug which gets leaked out to the aqueous media during particle formation. Hence, the preparation method was modified by changing the media for particle recovery to liquid paraffin.

The use of oily media in the recovery of microspheres containing water soluble drug has been reported by several workers (Lee et al 2000, Kumbar et al 2002) for effective recovery of microcapsules with high drug loading capacity. The batch EC_2 prepared using liquid paraffin as recovery media gave better yield (68.0 ± 2.3 %) of microspheres with significantly high % of drug loading (58 ± 1.5). Microspheres obtained were washed with n-hexane to remove residual liquid paraffin from microsphere as both the drug and the polymer are insoluble in n-hexane.

The batch EC_2 though showed better yield, % drug entrapment and expected release rate (t_{50} = 60 minutes, t_{70} = 240 minutes) the particle size obtained was in higher range (33.4 ± 0.48 to 68 ± 1.5 μm). This may be due to formation of coarse emulsion with low stability and hence possibility of aggregation during particle formation & rigidigation.

Therefore further batches (EC_3 to EC_8) were prepared by using emulsifying agents to prepare emulsion with low globule size and improved stability.

The microspheres obtained by using liquid paraffin as continuous phase (EC_2) released 70% drug within 3-4 hours. This may be due to break-down of primary emulsion between drug aqueous solution & polymer solution. To improve stability of this emulsion till solidification of stable microspheres; emulsifying agents were employed.

To form stable emulsion droplets, a certain amount of time was required before the organic solvents diffused into liquid paraffin phase & the ethyl cellulose polymer. DCM was chosen as a primary organic phase, since it was miscible with mineral oil & diffused out as soon as the w/o emulsion was poured into the mineral oil phase.

Effect of emulsifying agent (EA) & its concentration

Two emulsifying agents Span-80 & gelatin were selected based on the reports of Lee at al (2000). When concentration of Span-80 increased over a range of 1% w/v to 3% w/v the percentage yield was 69.95 ± 1.56 to 81 ± 3.6 & particle size decreased 69.95 ± 1.2 μ to 33.4 ± 0.48 μ respectively, & spherical shape was found.

In the literature, it was reported that increasing the concentration of emulsifying agent, resulted in reduction in the size of microspheres (Jalil & Nixon 1990)

When gelatin was used as an emulsifying agent drug content & particle size were significantly affected. Particles formed were granular & with rough surface. Release behavior was nearly the same as microspheres prepared with span-80.

Preparation of chitosan coated CQP microspheres

Microspheres were prepared by w/o emulsion method. To select the optimum preparation conditions the effect of various experimental parameters such as types of hardening agent, drug : polymer ratio, concentration of hardening / cross linking agent, chitosan concentration, stirring speed & hardening times on the particle size, morphology, percentage yield, drug content and in-vitro drug release were investigated.

Drug polymer ratio

Different Drug : polymer ratios were studied i.e. 1:1, 1:2, 1:3, 1:4 & 1:5 for the batches CC_5 to CC_9 respectively. The percentage yield was found in the range of 67.5 ± 3.3 to 92 ± 3.6 %. Drug content was found in the range of 78.05 ± 3.9 to 91.8 ± 5.1 % .With an increase in the Drug : Polymer ratio the particle size increases (15.8 ± 0.79μ to 62.85 ± 3.36μ), which may be due to increase in the polymer concentration.

From these results 1:1 drug : polymer ratio was selected for further investigation. D:P ratio played insignificant role on drug loading and particle size was increased by increasing D:P ratio. However, increase in particle size was found to have negligible influence on in-vitro release profile. Looking into the advantage of lower particle size, D:P ratio was fixed as 1:1.

Hardening agents

Both formaldehyde and glutaraldehyde were tried as hardening agents Batches CC_1 to CC_4 were prepared by using formaldehyde in which cross-linking was not efficient and the microspheres obtained were rough, irregular in shape with wrinkle surface in the form of flakes. Drug content & the percentage of yield was found satisfactory, but it was less compared to batch CC_5 which was prepared using Glutaraldehyde (GA) as crosslinking agent. The effect of cross-linking agents on the properties of microspheres was

studied by 't' test, which was significant at the level of $p \leq 0.05$. (Kumbar et al., 2002) It also gave smooth spherical microspheres with good reproducibility at lower concentration that is 5% w/w compared to 33% w/w of formaldehyde (1ml). So, Glutaraldehyde was selected as a cross-linking agent for further study.

Polymer concentration

Polymer concentration in the range 0.5, 1, 1.5 & 2 % w/v was employed to prepare batches CC_{10}, CC_5, CC_{11} & CC_{12} respectively. This parameter has significantly affected on particle size. With increasing polymer concentration the particle size was increased. When the polymer concentration increased particle size was increased, which leads to reduced release rate.

1% w/v chitosan solution in 1.5% w/v glacial acetic acid solution was used in the preparation as it yielded smooth & spherical particles with less aggregation. (Table 3.20 & batch CC_5) in comparison to other concentrations.

Effect of Stirring speed

The speed of rotation of the stirrer usually controls the size of microspheres. However, it does not change the shape of microspheres significantly. Batches CC_5, CC_{13}, CC_{14} & CC_{15} were prepared with different stirring speed 1000, 500, 1500 & 2000 rpm respectively. The particle size was reduced by increasing stirring speed which resulted into increase in in-vitro release rate. Results are depicted in table 3.20, 3.28 & Figure 3.39.

Effect of hardening time

Hardening of microcapsules renders slow release of entrapped drug. Batches CC_{16}, CC_{17}, CC_{18}, CC_{19} & CC_5 were fabricated with increasing hardening

time, from 1 hour to 5 hours. From the results shown in Table 3.20, 3.29 & Figure 3.40, three hours hardening time was found optimum.

Effect of concentration of cross linking agent

Microspheres of chitosan are crosslinked to retard release rate. Glutaraldehyde was used as cross-linking agent in the range of 5 to15% w/w with respect to polymer. Batches CC_5, CC_{20} & CC_{21} formulated with different concentrations to study the extent of cross-linking. With higher concentration of cross-linking agent the particles formed were found to be granular, rough, hard & irregular in shape. (Table 3.20, 3.30 & Figure3.41; Plate 3.4 to 3.7).

Preparation of Ethyl Cellulose coated Mefloquine Hydrochloride microspheres

Effect of solvent composition

The effect of various ratios of solvents used in preparation of primary emulsion & for the formation of microspheres were investigated. The varying volume ratios of Methanol :DCM were tested at 1:0,1:1,1:1.5& 0:1 for batches M_6 to M_9 respectively at fixed amount of total volume (10ml). This affected the formation of microspheres during solidification time as well as the size & shape of microspheres .With the increase in methanol concentration in the solvent mixture larger microspheres with irregular shape were obtained. With only methanol; particles obtained were crystalline in shape with particle size of 55.58±1.1μ (Batch M_6) and with only DCM (Batch M_9). Spherical smooth particles were obtained having smaller particle size (9.8 ± 0.16μ). These results are shown in Table 3.21(b), 3.32 & Figure 3.43.

Effect of disperse phase to continuous phase ratio

The ratio of disperse phase to continuous phase was found to have significant influence on particle size and its morphology. When the ratio of disperse phase decreased, formation of microspheres was faster with bigger size (12.35 ± 0.25μ) rough spherical particles formed. When the ratio of disperse phase increased smaller particles (9.6 ± 0.17μ) were obtained [Table 3.21(c & e) & 3.33, 3.35& Figure3.44, 3.46.]. This may be due to that , the volume of continuous phase was reduced microspheres had more opportunities to collide with each other & fuse together to form larger microspheres.

Effect of PVA concentration

It was observed that at least one stabilizer was needed for microspheres formation & suspension stabilization. In preliminary trials , 2%w/v PVA aqueous solution as continuous phase tried as stabilizer where difficulties in recovery of microspheres was experienced probably due to high viscosity of the continuous phase. The role of stabilizer in the recovery of microspheres is well documented by Aeshady, et al, (Aeshady et al 1990). Different concentration of PVA (0.1%, 0.5%, 1 %) were employed for batches M_{12}, M_{13} & M_{14} respectively. Microsphere obtained using 100ml 0.5% w/v PVA aqueous solution (M_{13}) gave particles of size 9.8±0.16μ. With the increase in concentration of PVA increase in particle size(14.35±.32μ) was observed [Table 3.21 (d)]

Effect of stirring speed

The speed of rotation of the stirrer usually controls the size of microspheres. However, it does not change the shape of microspheres significantly. Batches M_{18}, M_{19}, M_{20} were prepared with different stirring speed 1000, 2000, 3000rpm respectively. The particle size was reduced by increasing

stirring speed which resulted into increase in in-vitro release rate. Results are depicted in table 3.19 (f) &3.36 & Figure3.47.

Effect of PEG

To enhance release rate of MQH different grades of PEG (400,2000,&4000) were employed for the Batches M_{21}, M_{22}, M_{23} respectively. Addition of PEG in organic phase, enlarged the size of microparticles (12.12 ± 0.41μ to 18.5 ± 0.82μ). The in-vitro release was faster in comparison with plain microspheres because of the increase in the solubility of MQH in release medium containing PEG. Dissolution and leaching of PEG from the ethyl cellulose microsphere matrix into the dissolution medium may render the microspheres porous with numerous microchannels. This phenomenan is also reported by A similar enhancing effect for PEG was reported by PongPaibul et. al., (1988) for enhancing the release rate of theophylline from polymethyl methacrylate microspheres and by Nokhodchi & Farid (1992) for enhancing the release of acetyl salicylic acid from cellulose acetate phthalate microspheres. Khidr et. al.(1998), also reported that polyethylene glycol can be used as a channeling agent to improve the release properties of mefenenic acid at 1:2:1 drug : polymer : PEG ratio. This may facilitate high rate of diffusion & dissolution of drug into the dissolution medium.

Influence of process variable on characterization of CQP and MQH microspheres

Percentage Yield

Percentage yield of microspheres is of concerned for scaling of production in practical sense. Experiments conducted with different conditions of preparation of microspheres keeping drug : polymer ratio constant (1:1) resulted in different percentage yield (Table 3.19). Overall two conditions were tried viz. (i) composition of continuous phase (ii) composition of disperse phase.

Out of two continuous phase used (0.5% aqueous solution of PVA and liquid paraffin) 0.5% aqueous PVA solution (batchEC_1) resulted in lower yield (55.6±1.5%) of microspheres with very low drug entrapment efficiency (12 ± 2.3 %) in comparison to those prepared in liquid paraffin media (batch EC_2). Hence, liquid paraffin was used to prepare the batches EC_2 to EC_8 which gave percentage yield in the range of 58±1.5 to 86.0 ± 3.6.

The dispersed phase selected were

(i) Aqueous drug solution dispersed in polymer in DCM (EC_2).

(ii) Disperse medium containing Span-80 (EC_3 to EC_5).

(iii) Disperse medium containing gelatin. (EC_6 to EC_8).

Percentage yield implies our concern for material balance. The yield values was found to be 62.5 ± 2.95 to 92 ±3.6 % for chitosan coated CQP microspheres. Percentage yield was low in ethyl cellulose coated CQP microsphere (55.6 ± 1.5 to 85.0 ± 4.2) in comparison to chitosan coated CQP microspheres . This may be owing to when 0.5% w/v PVA aqueous solution was used as continuous phase , PVA aqueous solution gives higher solubility of CQP (hydrophilic drug with low molecular weight). The yield was increased when 0.5% PVA aqueous solution was replaced by mineral oil (liquid paraffin) as a continuous phase which was in between 75.8 ± 2.3 to 85 ± 4.2. Here the primary emulsion droplets were not stable, this may cause that the drug has gone into continuous phase. To stabilize the primary emulsion emulsifying agents (span-80& gelatin) were employed.

Percentage yield was appreciable in chitosan CQP microsphere (62.5 ± 2.95 to 92 ± 3.36 %). Ethyl cellulose coated mefloquine hydrochloride microspheres yield was in the range of 68.83 ± 2.1 to 82.15 ± 3.32.

Selection of solvent played an important role in microsphere formulation. The results are summarized in Table 3.19 (ethyl cellulose coated CQP microsphere), Table 3.20 (chitosan coated CQP microsphere), Table 3.21

(Ethyl cellulose coated mefloquine hydrochloride microspheres). All batches were prepared in triplicate & statistically analysed by its standard deviation for its characters.

Drug content

Drug entrapment depends on solubility of the drug in the continuous phase and on the physicochemical properties of polymer. Drug content was very low in EC_1 batch (12.0 ± 2.3) which was improved when continuous phase was replaced by mineral oil / arachis oil (58.26 ± 1.5 % EC_2). The drug content of microsphere prepared by chitosan coating on CQP with different process variables obtained in the range of 60.2 ± 2.35 to 91.8 ± 5.1%. Results are included in Table 3.19, 3.20 & 3.21.

Except the continuous phase other process variables were not affected significantly. This might be due to solubility of drug & polymer in selected solvent system.

Particle size

Particle size plays a vital role in parenteral formulation for its physical stability of pharmaceutical suspension especially in the range of fine particles suspension range (1-20 μm). The bioavailibility of drugs is often improved by reducing the size of suspension particles. Drug particles below 20μm produce less pain & tissue irritation, when injected parenterally. It has been reported that the production of fine particles may have a deleterious effect on chemical stability and show increased dissolution rate of drug from small particles (Michael, 1996)

In case of EC2 the dispersed phase obtained for mixing aqueous drug solution with polymer in organic media (DCM) gave coarse emulsion. This on processing in liquid paraffin media yielded microparticles with higher particle size. In order to decrease the particle size it was felt necessary to

control globule size of the emulsion (disperse phase). Hence, emulsifying agents Span-80 & gelatin were used in various concentrations to prepare batches EC_3 to EC_5 showed tendency of decrease in particle size. When 2% or more % of Span-80 (EC_4 & EC_5) with good percentage yield (81 ± 3.6 to 85 ± 4.1 %) and drug entrapment (71 ± 2.8 to 76 ± 3.8 %).

Particle size of all formulations are tabulated in Table 3.19, 3.20 & 3.21. The effect of different process variables / parameters was discussed previously.

Morphology

Typical photomicrographs of the microspheres are shown in plate 3.1-3.10 Chitosan microspheres were smooth & spherical in shape. The concentration of cross-linking agent had significant effect on its porous & smooth surface. Glutaraldehyde concentration of 5%w/w with respect to polymer concentration played important role to give smooth surface which gave satisfactory effect at concentration of 15% GA lumps formed due to agglomeration. The surface was convoluted and sphericity was not well defined. (Plate 3.4 to 3.7)

Mefloquine containing ethyl cellulose coated microspheres were smooth spherical free from wrinkle & looked like coated reservoir. Microspheres prepared with PEG have porous surface. (Plate 3.8 to 3.10)

In-vitro release profile of CQP/MQH from microspheres

Ethyl cellulose & chitosan have been used as biocompatible & biodegradable respectively. Implants & injectable sustained release produces were developed for parenteral administration. It was also reported that biocompatible chitosan coated PLA / PLGA microspheres provided near zero-order, in-vitro release of 5-FU for its therapeutic applications (Chandy et. al. 2000)

The effect of different process variables on in-vitro drug release profile were studied.

Ethyl cellulose coated chloroquine phosphate microspheres shows faster initial release. The drug was diffused out into PBS 7.4 pH almost 80% within first 5 hour for batch EC_2. When span 80 / gelatin was introduced to improve stability of primary emulsion. This could help in reproducing the microsphere with its characters but not significantly affected its release pattern. The results are shown in Table 3.23, 3.24 Figure 3.34, 3.35.

The biphasic pattern of release of CQP from ethyl cellulose microsphere was very much evident and one can observe a high percentage of drug release during initial hours of dissolution by burst effect. The extent of burst effect of these preparations calculated as hypothetical percentage of drug release at zero time intercept by extrapolation of the second phase curve as recorded in Table 3.48a.

Table 3.48a Hypothetical % release of CQP from ethyl cellulose microsphere at zero hour. (% burst)

Batch no.	% Burst
EC_1	50
EC_2	28
EC_3	27
EC_4	19
EC_5	20
EC_6	31
EC_7	28
EC_8	28

Burst range : 19 to 31 %,for batches EC_2 to EC_8

In-vitro release of CQP from chitosan microspheres has also followed biphasic pattern with higher burst effect as compared to ethyl cellulose microsphere. The percentage burst of individual batch of chitosan microsphere containing CQP as given in table 3.48b.

Table 3.48b Hypothetical % release of CQP from chitosan microsphere at zero hour. (% burst)

Batch No.	% Burst	Batch No.	% Burst	Batch No.	% Burst
CC_1	47	CC_8	25	CC_{15}	44
CC_2	39	CC_9	25	CC_{16}	56
CC_3	41	CC_{10}	50	CC_{17}	46
CC_4	33	CC_{11}	28	CC_{18}	40
CC_5	25	CC_{12}	28	CC_{19}	52
CC_6	25	CC_{13}	41	CC_{20}	41
CC_7	21	CC_{14}	42	CC_{21}	42

Burst range : 21 to 56 %, for batches CC_1 to CC_{21}

Release of MQH from ethyl cellulose coated microsphere was also found to be biphasic but with low burst effect as compared to release of CQP from ethyl cellulose and chitosan microsphere. The percentage burst of individual batch of ethylcellulose microsphere containing MQH as given in table 3.48c.

Table 3.48c Hypothetical % release of MQH from ethylcellulose microsphere at zero hour. (% burst)

Batch No.	% Burst	Batch No.	% Burst
M1	12	M14	10
M2	07	M15	11
M3	07	M16	14
M4	08	M17	13
M5	10	M18	09
M6	04	M19	15
M7	04	M20	15
M8	06	M21	14
M9	15	M22	10
M10	14	M23	09
M11	13	M24	11
M12	11	M25	10
M13	09	M26	09

Burst range : 4 to 15 %

Percentage of burst calculated by extrapolation of the second phase release profile for ethylcellulose 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 shows 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 (4 to 15 %) observed in these batches. In fact some batches of MQH (M5,M6,M7) release was almost monophasic with negligible burst effect.

The release pattern of microsphere prepared with different drug : polymer ratio were very less effective which is depicted in Table 3.26 & Figure 3.37. So 1:1 drug:polymer ratio was selected for further investigation which contain low polymer compared to other to avoid extra excipient introduced to body which can produce side effects.

Same way in case of ethyl cellulose coated mefloquine hydrochloride have similar effect as above which is shown in Table 3.31 & Figure 3.42.

When the concentration of polymer / chitosan increased which leads to faster release of chloroquine. These results are shown in Table 3.27 & Figure 3.38.

When the stirring speed was increased the particle size was reduced. The reduction in particle size cause increase exposed surface area which tends to faster dissolution of the drug in media.

Table 3.28, Figure 3.39 & Table 3.36 Figure 3.47 depicted release data obtained for the effect of stirring speed on CQP coated chitosan & ethyl cellulose coated mefloquine hydrochloride microspheres respectively.

The effect of crosslinking time & concentration on chitosan coated CQP microspheres are revealed in Table 3.29 & Table 3.30 Figure 3.40, 3.41 respectively.

These parameters were not significantly affected on release pattern but it has valuable effect on morphology & particle size of microsphere. The higher concentration of crosslinking agent leads to rough hard particles in aggregate form. One hour & two hour time period have efficient cross-linking but here the formation of emulsion time was less compared with 3 hours. Here the formation of emulsion was not stabilize due to lack of time. The hardening agent was added before the uniform distribution of particle which resulted into faster dissolution. The bond between drug-polymer may be poor which form weak matrix has faster dissolution of drug into release medium.

The solvent ratio has significant effect on drug release. When the 1:0 methanol : DCM ratio was applied the formed microspheres were fluffy & bulky. Due to hydrophobicity of microsphere the release was slower which indicated in Table 3.32 Figure 3.43.

The volume of disperse phase & continuous phase have affected in same manner. When the volume increased the particle size reduced & this increased release rate which are shown in Table 3.33 & 3.35 Figure 3.44, 3.46 respectively.

The effect of PVA concentration in continuous phase was not significantly affected on release behaviour which depicted in Table 3.34 & Figure 3.45.

Incorporating PEG in the formula improved the rate of mefloquine hydrochloride release significantly (Table 3.37, 3.38 Figure 3.48, 3.49) A similar enhancing effect for PEG was reported by PongPaibul et. al., (1988) for enhancing the release rate of theophylline from polymethyl methacrylate microspheres and by Nokhodchi & Farid (1992) for enhancing the release of acetyl salicylic acid from cellulose acetate phthalate microspheres. Khidr et. al.(1998), also reported that polyethylene glycol can be used as a channeling agent to improve the release properties of mefenenic acid at 1:2:1 drug : polymer : PEG ratio.

Result for multiple linear regression analysis

A 3^2 factorial design was used to optimize the drug loaded microspheres with respect to the independent variables of two different solvent ratios.

Different batches f1 to f9 were prepared to determine the effect of the different variables on the drug loading, particle size, & % yield. The average response for the nine runs was considered for different variables. The main effect represents the average result of changing one factor at a time from low to high value. The interaction show how the dependent variables changes when two factor are simultaneously changed.

The results are expressed as second order polynomial equation of the following form.

$$Y_i = b_o b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2 + b_{11} x_1^2 + b_{22} x_2^2$$

Where b_1 is the estimated co-efficient corresponding linear effects (b_1 and b_2), interaction (b_{12}) and quadratic effects (b_{11} & b_{12}) were determined from the results of the experiment.

The drug content (Y_1) for chitosan coated CQP microspheres from the nine experiments were used to generate predictor equations for batches prepared with different stirring speed (X_1) & polymer concentration (X_2)

Full model & reduced model equations are as under :

$$Y_1 = 76.123 - 0.912X_1 - 2.972X_2 + 2.898X_1X_2 - 1.645X_1^2 + 3.105X_2^2$$

$$Y_1 = 77.097 - 0.912X_1 - 2.972X_2$$

The percentage drug content in chitosan coated CQP microspheres showed R^2 values for full & reduced model 0.867 & 0.804 respectively indicating a good fit. The calculated F values were found to be smaller than critical value of F for full model & reduced model both, hence it may be concluded that these variables not have significant effect on drug content.

The particle size (Y_1) from nine experiments were used to generate predictor polynomial equations

Variables stirring speed (X_1)

Polymer concentration (X_2)

Particle size (Y_1)

Full model

$$Y_1 = 22.278 - 4.122X_1 + 7.777X_2 - 2.605X_1Y_2 + 0.438X_1^2 + 2.133X_2^2$$

$R^2 = 0.867$ $F = 3.93$

Reduced model

$$Y_1 = 23.992 - 4.122X_1 + 7.777X_2$$

$R^2 = 0.804$ $F = 12.32$

Y_1 - Particle size

X_1 - stirring speed

X_2 – Concentration of crosslinking agent

Full model

$$Y_1 = 57.834 - 0.880X_1 + 18.71X_2 - 6.295X_1X_2 + 5.923X_1^2 - 23.907X_2^2$$

$R^2 = 0.768$ $F = 1.99$

Reduced model

$Y_1 = 45.846 - 0.880X_1 + 18.71X_2$

$R^2 = 0.465$ $F = 2.61$

Particle size was significantly affected by polymer concentration &/or stirring speed calculated F value is greater than critical value in reduced model so any one of the variables has significant effect on particle size.

The particle size ethyl cellulose coated MQH microspheres (Y_1) from the nine batches were used to produce predictor equations with two variables Volume of DCM – X_1 Volume of PVA solution X_2

Full model

$Y_1 = 10.304 - 2.542X_1 - 4.188X_2 - 0.025X_1X_2 + 0.418X_1^2 - 0.778X_2^2$

$F = 17.553$ $R^2 = 0.966$

Reduced model

$Y_1 = 11.102 - 2.542X_1 - 4.188X_2$

$F = 66.05$ $R^2 = 0.956$

The particle size in ethyl cellulose coated MQH microspheres is significantly effected by volume of disperse phase DCN &/or volume of PVA solution continuous phase. It showed that the calculated F value is greater than the critical value which indicate the process variable may play significant role on particle size.

The particle size in ethyl cellulose coated MQH microspheres is significantly effected by volume of disperse phase DCM &/or volume of PVA solution continuous phase. It showed that the calculated F value is greater than the critical value which indicate the process variable may play significant role on particle size.

The drug content (Y_1) of ethyl cellulose coated MQH microspheres was investigated by batches prepared using volume of disperse phase (DCM) X_1

Volume of continuous phase (PVA) X_2

Full model

$$Y_1 = 74.436 - 1.697X_1 - 3.912X_2 - 1.375X_1X_2 + 1.137X_1^2 + 1.62X_2^2$$

$R^2 = 0.685$ $F = 1.307$

Reduced model

$$Y_1 = 75.968 - 1.697X_1 - 3.912X_2$$

$R^2 = 0.613$ $F = 4.756$

3.3.5 References

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3.4 In-vivo study

3.4.1 Introduction

Critical to the successful development of microsphere preparation is the study of its behaviour in-vivo. Studies of the pharmacokinetics & biodistribution of the formulation in suitable animal model during the later stages of the development is important for attaining the desired product performance. For any new formulation, there should be in-vitro in-vivo correlation. The in-vitro study should be reproducible and mimic the in-vivo behaviour. So the in-vitro profile can be useful for forecasting its in-vivo effect. But one cannot confirm the performance without in-vivo investigation. The relationship should be established only after its pharmacokinetic and biodistribution analysis.

In-vitro characterization helps in the quality assurance programme of the product which can assure that each batch will meet the criteria required for successful in-vivo performance.

These studies are also decisive for the systems which are being investigated in-vivo distribution pattern which can indicate the pathway for further implication.

3.4.2 Materials

Phosphate buffered saline, Heparin solution, 1N sodium hydroxide, saturated sodium chloride solution, 5% w/v trichloroacetic acid solution 0.1N & 0.01N hydrochloric acid solution all these solutions were prepared as per procedure prescribed in chapter 3 section 3.1, 3.1.5. dichloromethane, alcohol 95% & chloroform.

Apparatus

Rat cages, glass syringe (2ml & 1ml capacity) with 18 gauge needle, centrifuge Remi Apparatus, Mumbai, Remi-mechanical tissue homogenizer

with Teflon pastel & glass homogenizer, Chemito UV-2600 spectrascan, Heating mental, hot plate, capillary

3.4.3 Selection of Animals

Spargue Dawley healthy female albino rats weighing between 225-250 g were selected for the study. The animals were kept in plastic cages & received standard food and water ad libitum. Two groups to be referred to as group I & group II each containing 4 randomly assigned subgroups of 6 rats, were formed for pharmacokinetic & biodistribution study.

3.4.4 Administration of microspheres & free drug

Rats were fasted overnight but water was freely available. After appropriate dilutions of the stock suspension of microsphere were immediately made before injection. It was injected intramuscularly into the gastrocnemius muscle of the right hind leg by 1ml syringe with 18 gauge needle. The point of insertion of the needle was then pressed with a cotton swab to stem the blood flow. Identification of the animals of specified group were made by picric acid stain to the specific body organ e.g. head, tail, back, head & tail, right ear, left ear etc. After administration of free drug & formulation, rats were replaced into the cages. Standard food & water were provided.

3.4.5 Collection of blood & tissue samples

At predetermined time intervals, blood was collected in a heparinized centrifuge tube from the retro-orbital plexus with aid of capillary tube. To avoid excessive bleeding rats were bleed according to two complementary schedules. Animals were sacrificed for biodistribution study. Rats were made unconscious by inhalation of an overdose of chloroform, then severing of carotid artery. The organs of interest lung, liver, heart, kidney & spleen were removed & divested of all extraneous tissues. Blood was also collected

for that specified time. The organs were then blotted thoroughly using filter paper, weighed & then minced with scissors. Each of the minced tissues was then homogenized in PBS using Remi mechanical homogenizer to obtain 10% w/v concentration of tissues.

Blood & tissue sample collection was done for chloroquine phosphate free drug, chloroquine phosphate formulation, mefloquine hydrochloride free drug & mefloquine hydrochloride formulation.

3.4.6 Analysis of Blood & Tissue samples

The samples obtained as above were then subjected to analysis using the procedure elaborated in chapter 3 section 3.1 (3.1.5 & 3.1.8) for chloroquine phosphate & mefloquine hydrochloride respectively.

3.4.7 Results and discussion

3.4.7.1 Results

Table 3.49 Concentration of chloroquine phosphate in various tissues of Sprague Dawley albino rats after intramuscular administration of free drug and formulation

Time	Blood (μg/ml)		Lung (μg/g)		Liver (μg/g)		Heart (μg/g)		Kidney (μg/g)		Spleen (μg/g)	
in hrs.	A	B	A	B	A	B	A	B	A	B	A	B
0.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0
0.5	05.2	03.3	02.1	01.8	03.4	05.5	04.9	00.6	00.5	00.9	00.8	00.5
1.0	15.3	05.5	06.4	01.9	05.7	07.5	08.0	01.8	09.9	03.2	02.4	01.5
2.0	18.3	07.9	08.8	02.4	15.5	09.8	18.3	04.8	13.3	04.8	02.5	01.9
3.0	17.8	08.8	11.5	02.8	16.8	08.8	16.3	05.4	12.9	05.5	03.4	02.2
6.0	05.3	07.6	07.1	3.01	09.8	08.1	09.8	04.3	11.8	06.4	03.8	02.1
12.0	03.2	06.8	05.2	02.1	04.6	06.9	4.98	04.1	03.8	05.8	04.2	02.3
24.0	01.4	06.5	02.3	01.9	03.8	06.5	01.9	03.4	02.4	04.6	01.9	1.85
48.0	00.4	05.5	00.8	01.5	02.9	04.8	00.9	02.8	00.8	02.8	01.6	00.9
72.0	-	02.3	00.5	00.8	01.5	04.1	00.6	01.5	00.3	02.4	00.8	00.9
144.0	-	00.4	-	-	-	00.9	-	-	-	-	-	0.75

A : Free drug B : Formulation

Table 3.50 Concentration of mefloquine hydrochloride in various tissues of Sprague Dawley albino rats after intramuscular administration of free drug and formulation

Time	Blood (μg/ml)		Lung (μg/g)		Liver (μg/g)		Heart (μg/g)		Kidney (μg/g)		Spleen (μg/g)	
in hrs.	A	B	A	B	A	B	A	B	A	B	A	B
0.0	00.0	00.0	0.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0	0.0	00.0
0.5	04.3	02.3	2.2	1.02	04.8	02.4	00.9	00.4	00.6	00.5	0.8	0.3
1.0	12.5	04.9	3.8	02.1	06.9	05.3	06.5	01.3	01.3	00.9	6.5	0.75
2.0	14.5	06.8	3.2	03.4	10.8	05.3	10.5	2.33	02.4	01.2	8.8	1.1
3.0	16.8	08.9	2.9	03.2	15.4	08.4	11.8	3.42	03.6	02.4	5.8	2.21
6.0	10.5	08.2	2.4	02.0	09.4	06.3	08.2	02.4	03.2	02.3	4.8	2.2
12.0	08.4	07.1	1.9	01.8	07.5	05.1	03.4	1.38	02.6	1.25	2.5	2.18
24.0	02.4	04.9	1.1	01.5	02.3	04.0	01.8	01.1	00.8	0.92	1.4	1.1
48.0	01.4	03.3	0.8	0.85	01.2	03.8	-	00.7	00.6	0.88	0.8	0.98
72.0	00.5	02.8	0.4	0.71	00.5	3.68	-	0.62	-	0.75	1.5	0.82
144.0	-	00.9	-	0.40	-	0.92	-	0.32	-	00.6	-	0.75
432.0	-	00.4	-	-	-	0.34	-	-	-	-	-	0.2

A : Free drug B : Formulation

Table 3.51 Comparison of the pharmacokinetic parameters in blood following intramuscular administration of CQP free drug, CQP formulation, MQH free drug and MQH formulation.

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
$AUC_{0-\alpha}$ µghr/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 ml	865.64	3127.59	889.07	2994.86

Figure 3.50 Comparison of free drug and microsphere of chloroquine phosphate concentration in blood

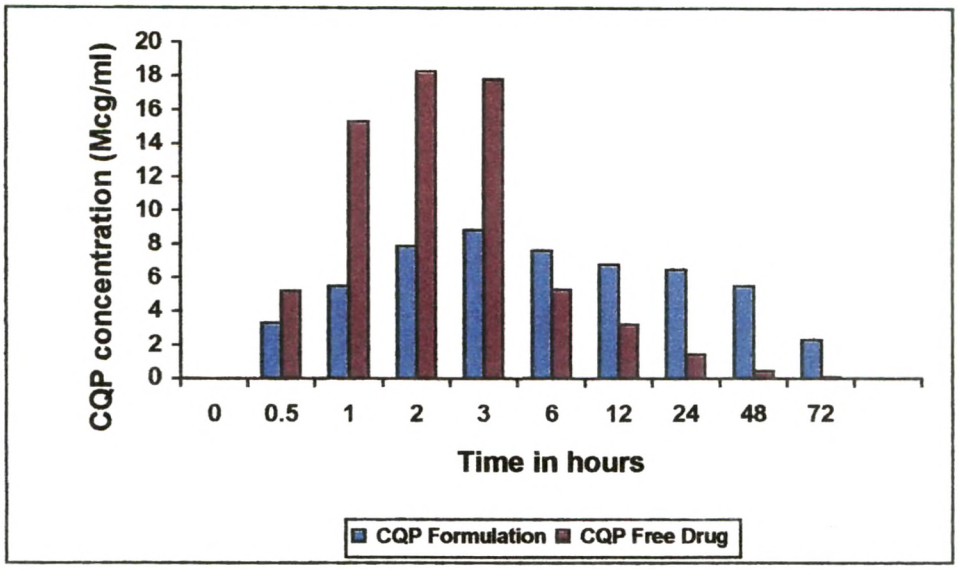


Figure 3.51 Comparison of free drug and microsphere of chloroquine phosphate concentration in blood

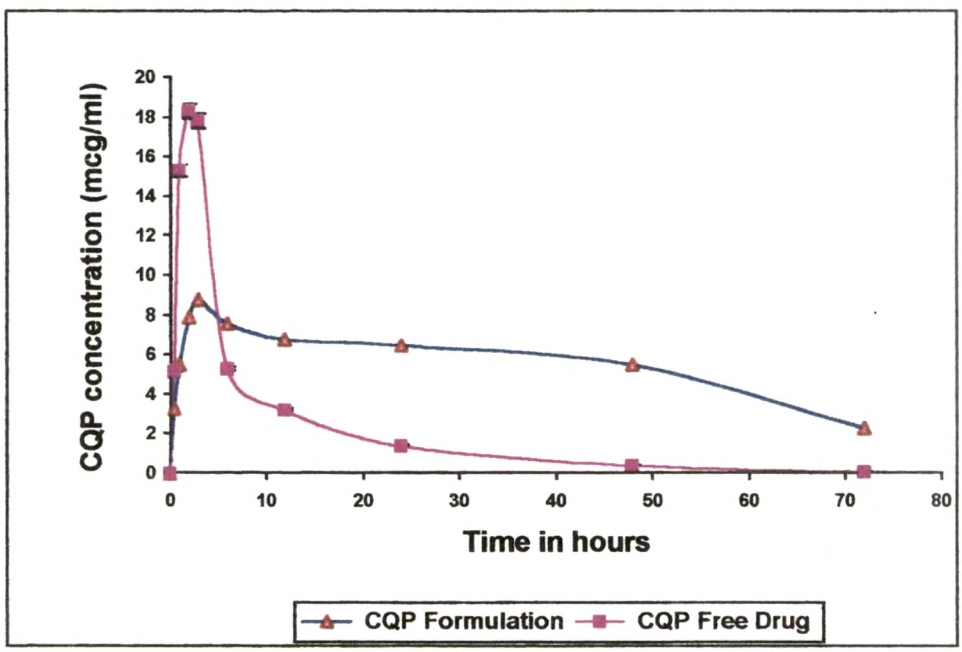


Figure 3.52 Comparison of free drug and microsphere of chloroquine phosphate concentration in heart

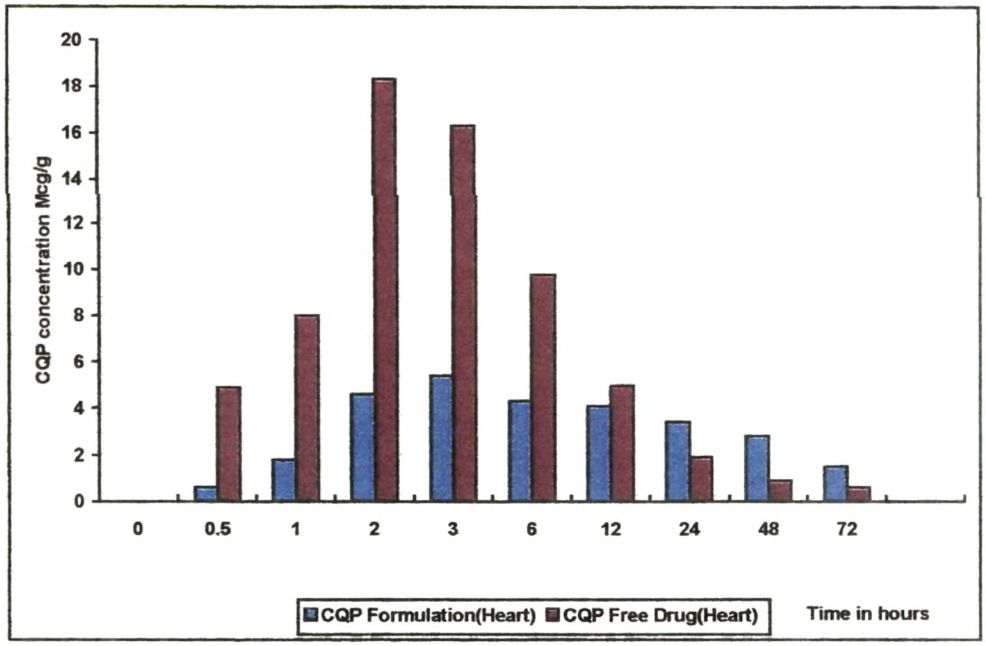


Figure 3.53 Comparison of free drug and microsphere of chloroquine phosphate concentration in heart

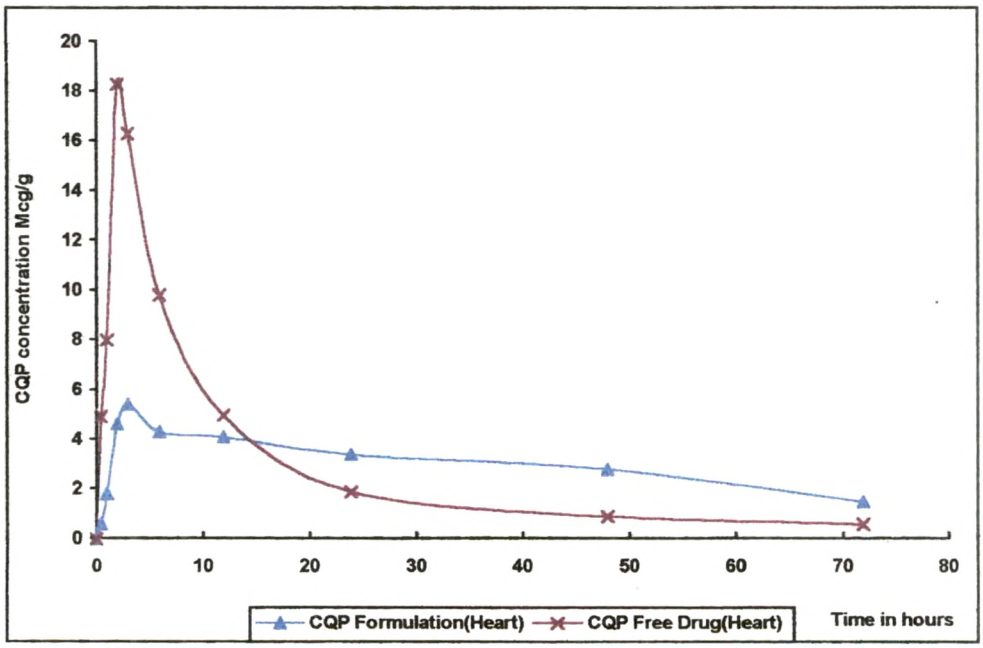


Figure 3.54 Comparison of Free Drug and microspheres of chloroquine phosphate concentration in kidney and lung.

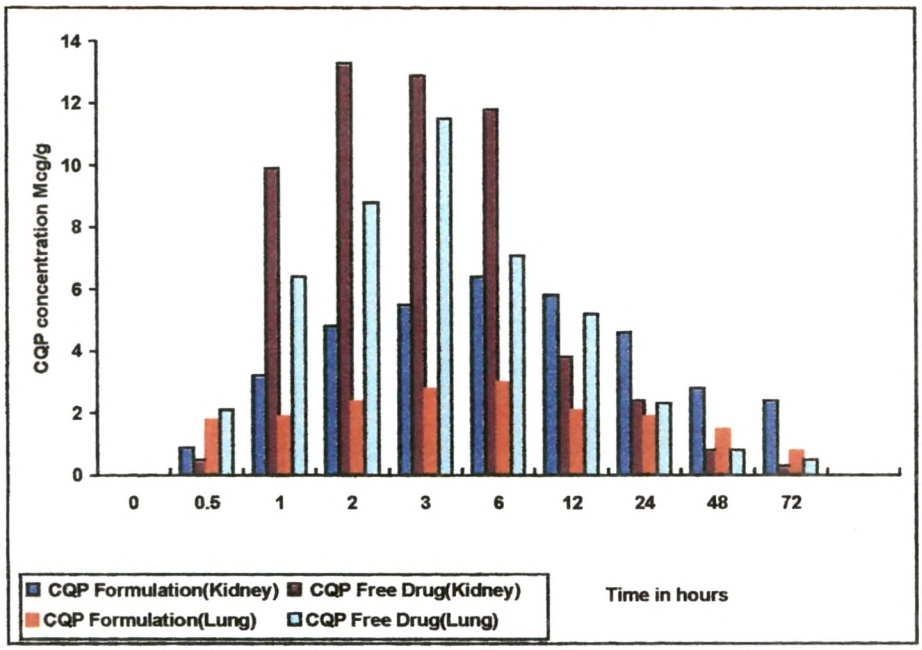


Figure 3.55 Comparison of Free Drug and microspheres of chloroquine phosphate concentration in kidney and lung.

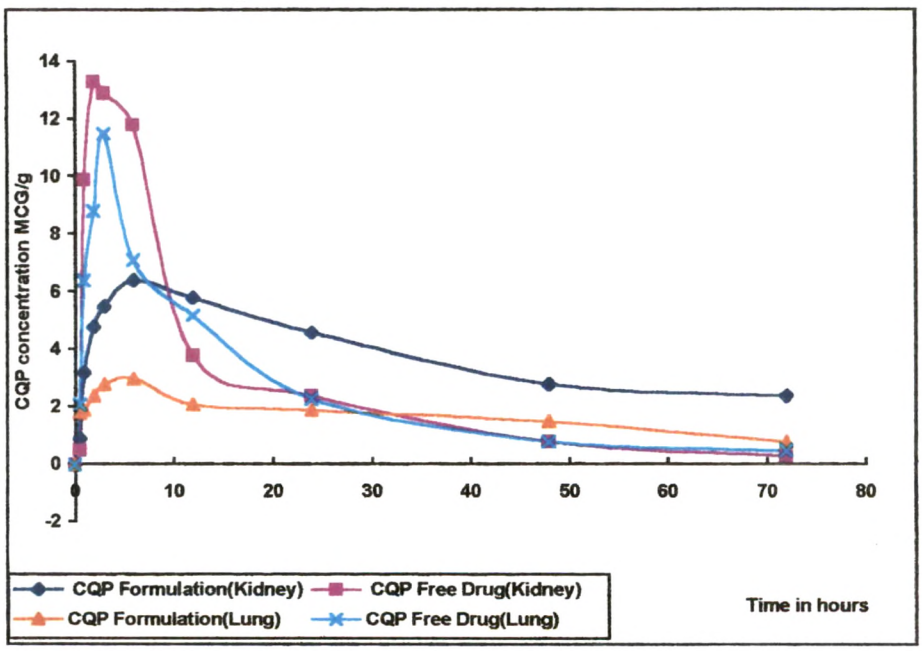


Figure 3.56 Comparison of Free Drug and microspheres of chloroquine phosphate concentration in liver and spleen.

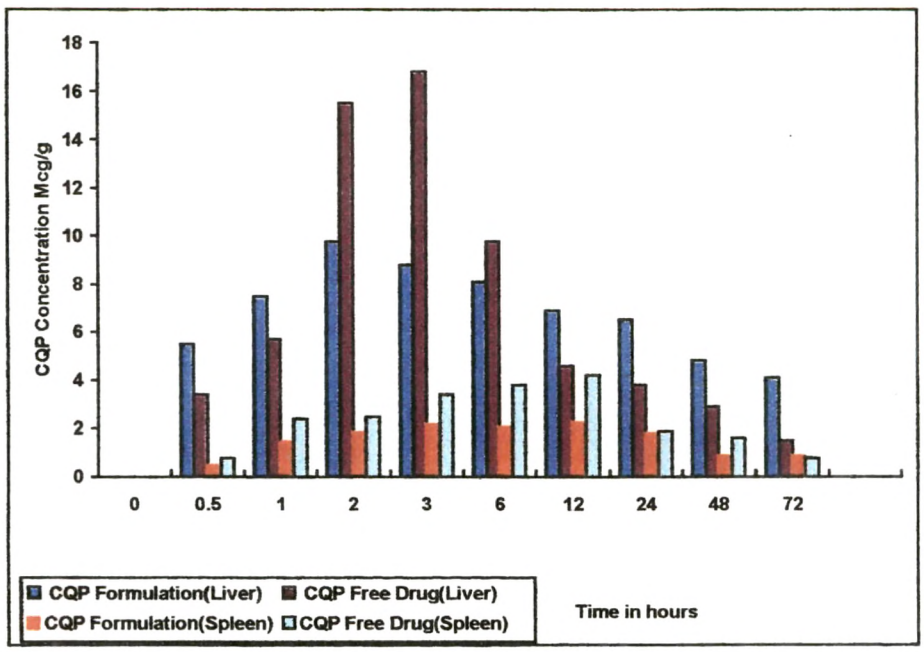


Figure 3.57 Comparison of Free Drug and microspheres of chloroquine phosphate concentration in liver and spleen.

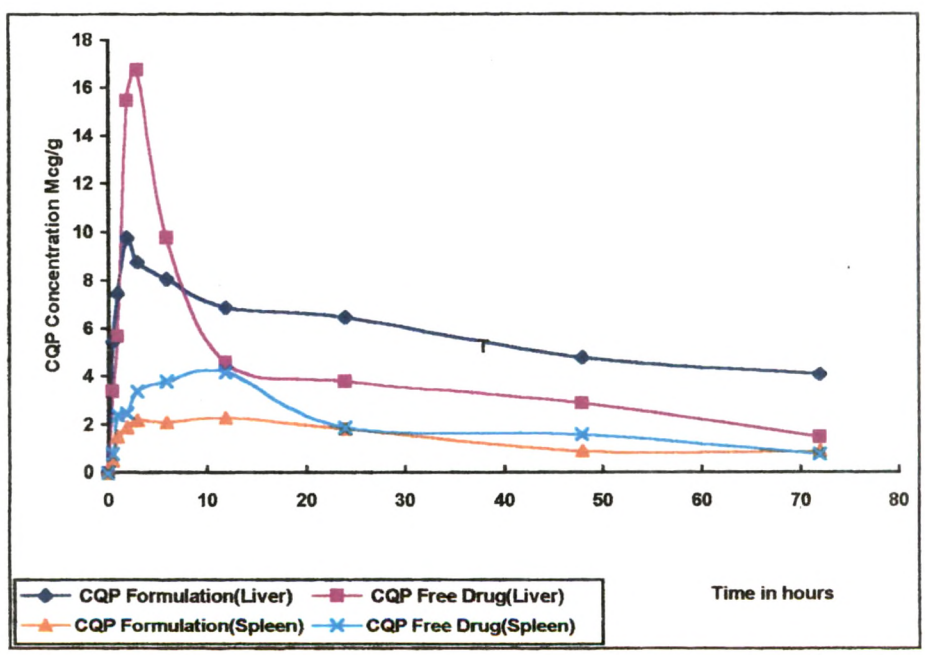


Figure 3.58 Comparison of free drug and microsphere of mefloquine hydrochloride concentration in blood

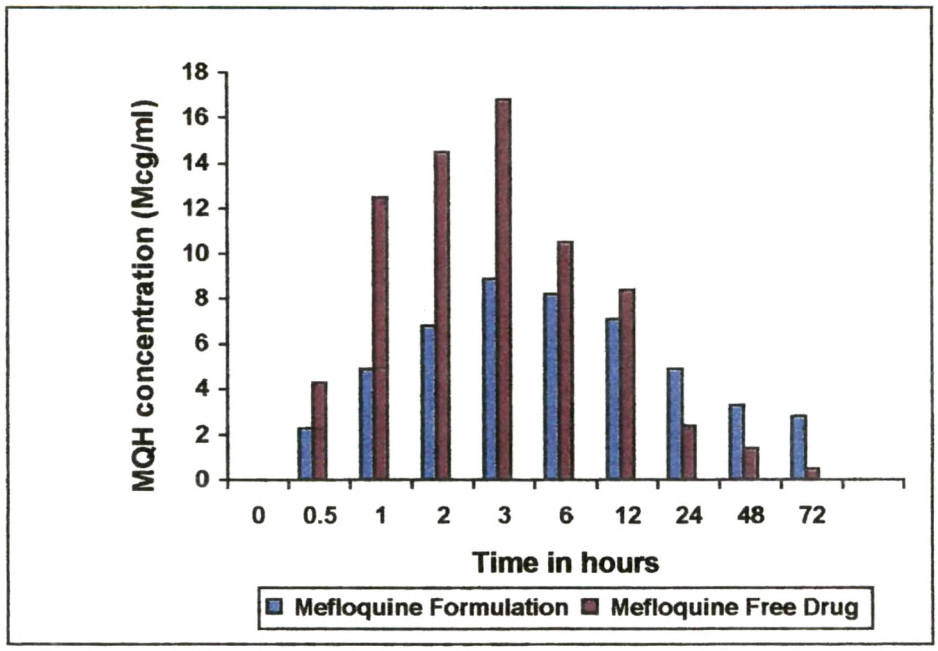


Figure 3.59 Comparison of free drug and microsphere of mefloquine hydrochloride concentration in blood

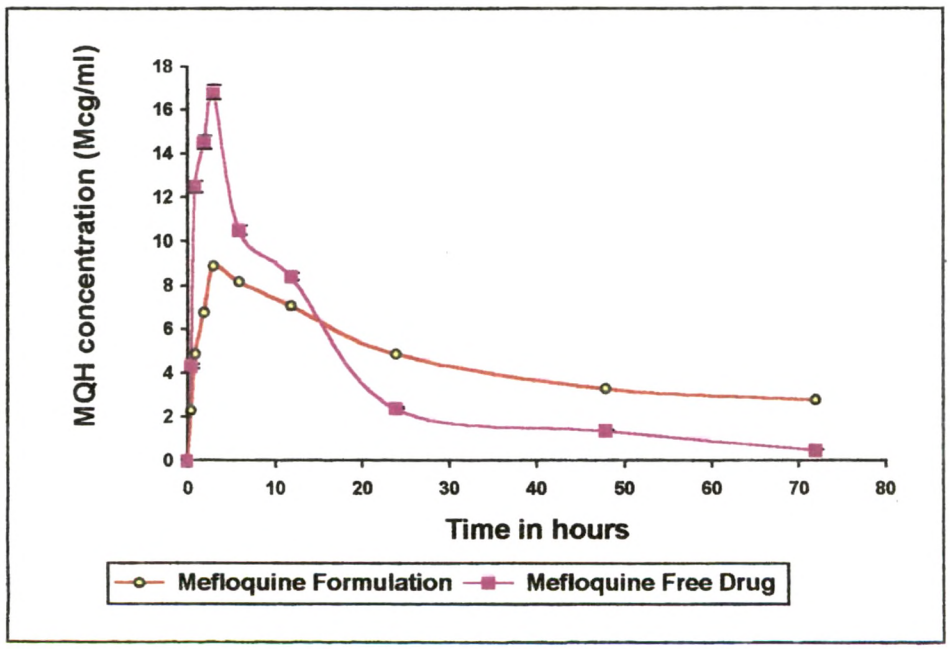


Figure 3.60 Comparison of free drug and microsphere of mefloquine hydrochloride concentration in heart

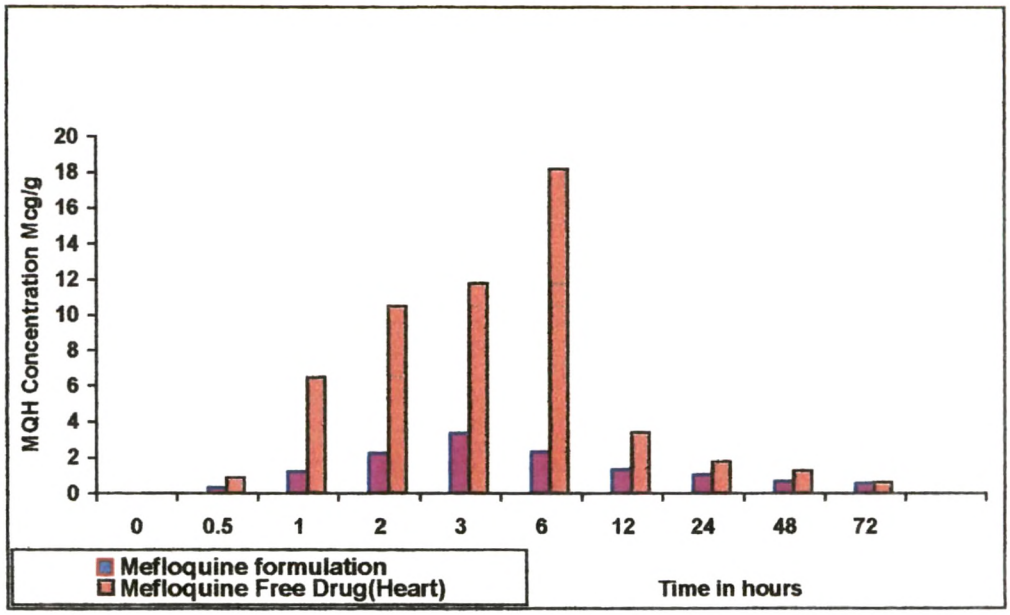


Figure 3.61 Comparison of free drug and microsphere of mefloquine hydrochloride concentration in heart

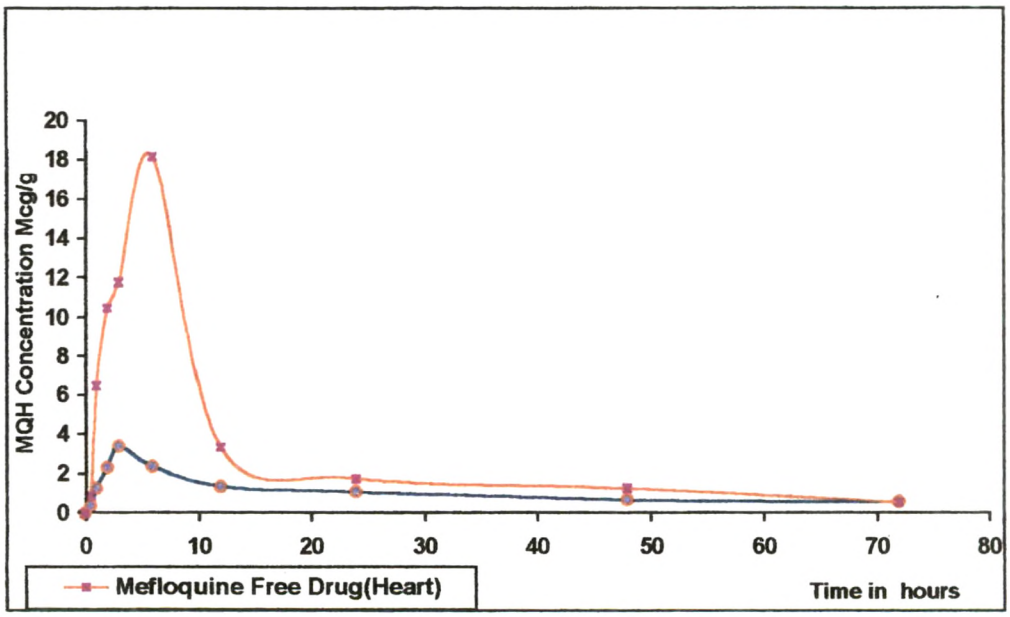


Figure 3.62 Comparison of free drug and microsphere of mefloquine hydrochloride concentration in kidney and lung

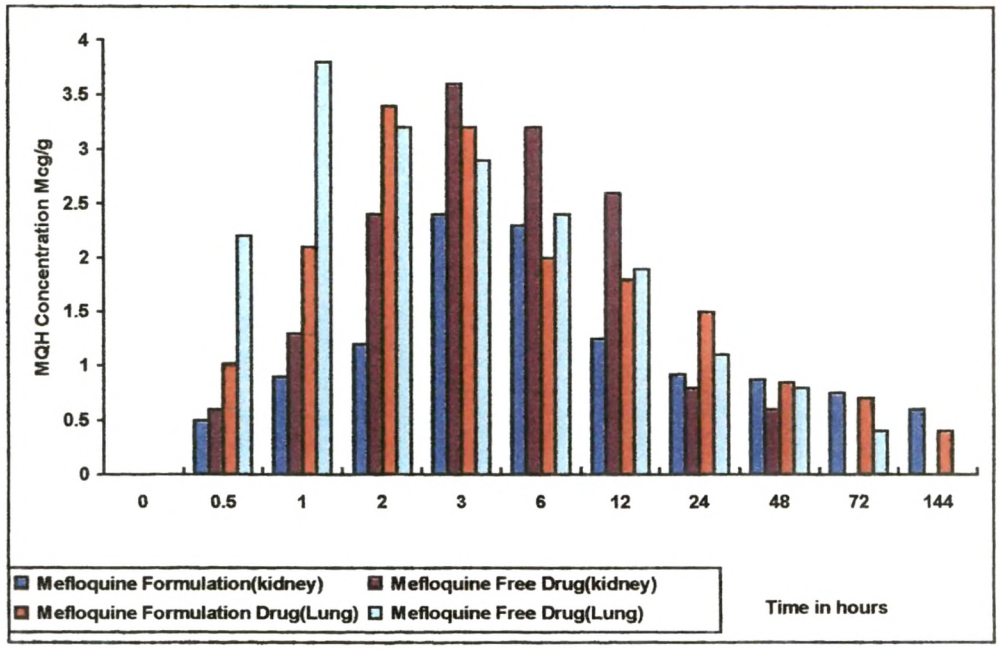


Figure 3.63 Comparison of free drug and microsphere of mefloquine hydrochloride concentration in kidney and lung

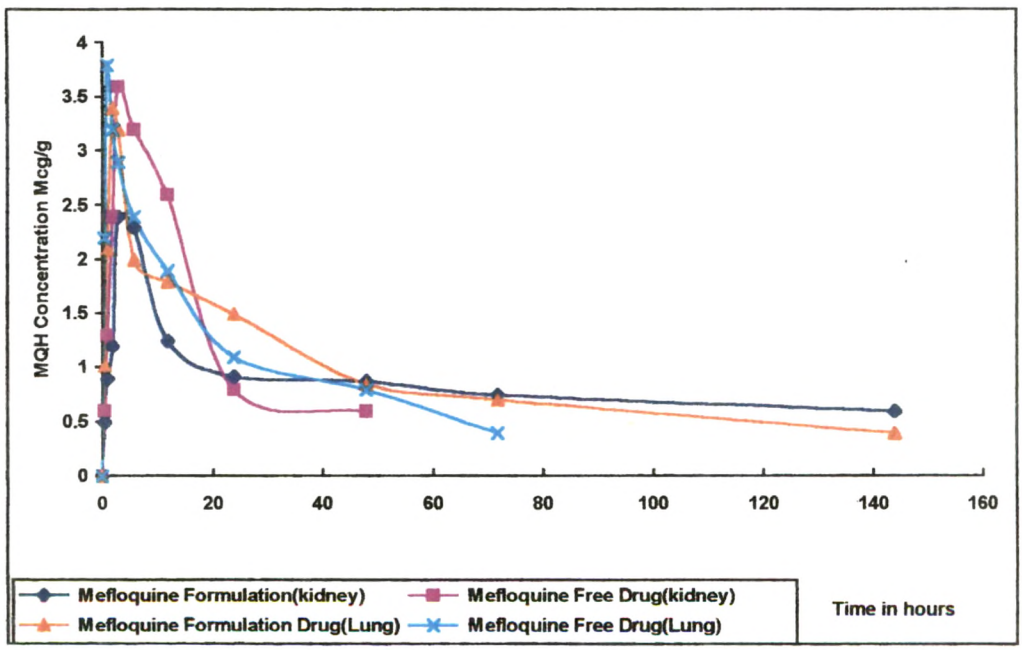


Figure 3.64 Comparison of free drug and microsphere of mefloquine hydrochloride concentration in liver and spleen

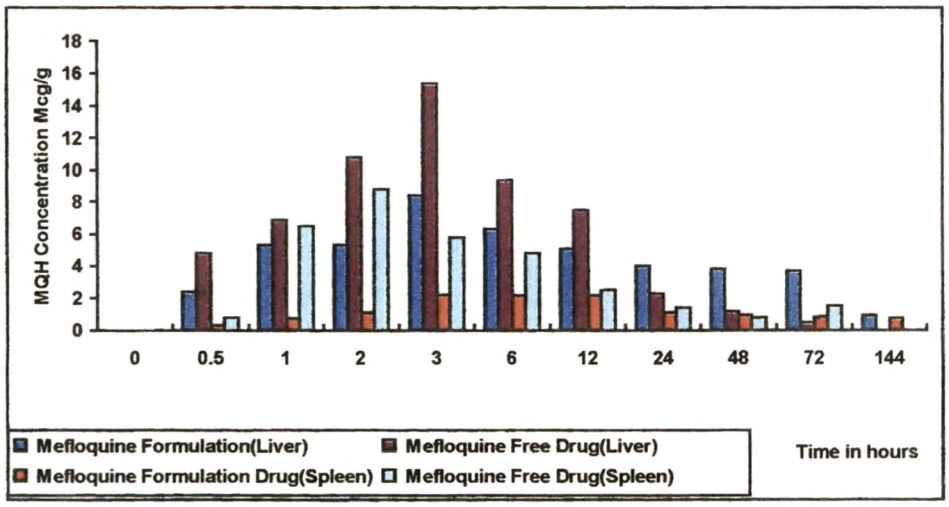
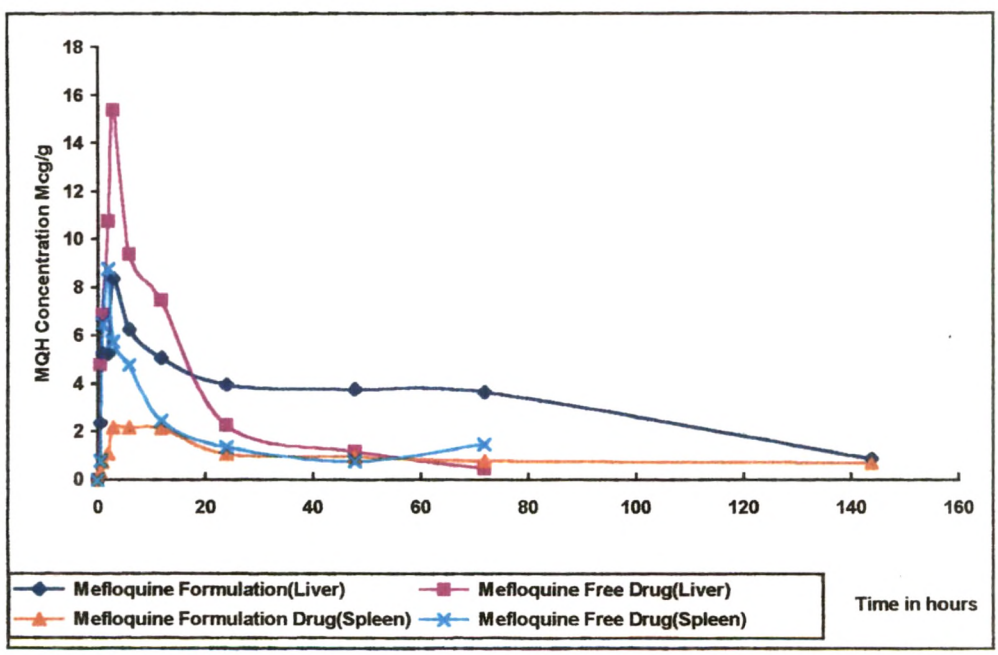


Figure 3.65 Comparison of free drug and microsphere of mefloquine hydrochloride concentration in liver and spleen



3.4.7.2 Discussion

Ethyl cellulose and chitosan coated chloroquine phosphate microsphere and ethyl cellulose coated mefloquine hydrochloride microsphere were prepared with the aim of increasing plasma residence time and limiting the wide distribution of these drugs. It was envisaged that this type of drug delivery would lead to drug release only at the sites required and would also lead to significantly reduced side effects. Thus, an increase in the therapeutic efficacy of these drugs would be achieved.

In order to ascertain whether these objectives have been achieved, comparative in-vivo studies between pure drug and a promising optimized microsphere batches of chloroquine phosphate and mefloquine hydrochloride (Batches CC_5 & M18) was performed by intramuscular administration of an appropriate volume of the microsphere suspension to the Sprague Dawley Albino rats of approximately equal body weights (225-250 g) and measurement of the blood levels of the drugs at predetermined time intervals. At different time intervals the rats were sacrificed and the accumulation of the drugs in different organs of interest such as the lung, liver, kidney, heart & spleen were studied. The data so obtained was subjected to statistical analysis to obtain the in-vivo pharmacokinetic profile of the microsphere.

Pharmacokinetics

The results of in-vivo study were analysed with methods of first order elimination. The pharmacokinetic parameters were generated by non compartmental mathematical expression from raw data (Gibaldi & Perier 1984) (Table 3.49).

The raw data for CQP and MQH free drugs and its microspheres formulation, for blood & different tissues concentrations were tabulated in

Table 3.49 & Table 3.50 respectively. Concentration versus time curves in whole blood & in different tissues e.g. lung, liver, heart, kidney & spleen are presented as mean plots.

Peak blood concentrations (maximum plasma concentration, C_{max}) were derived from concentration time plots. Time to reach maximum concentration (T_{max} or T_p) was directly determined from concentration versus time graph (Figure3.51 & Figure3.59). AUC, area under plasma concentration versus time curve for 0 to α hours. Area under first moment curves of plasma concentrations. time v/s time (AUMC), were calculated by trapezoidal rule. The mean residence time (MRT) was calculated using formula MRT(h) = AUMC/AUC. Mean absorption time (MAT) & absorption constant (K_a) were determined from time over which absorption or input takes place. The terminal elimination rate constant (β or K_{el}) was estimated by linear regression of the natural log transformed plasma concentrations in the apparent linear terminal phase using at least three time points. The data points determining the apparent linear phase were selected from visual inspection. The corresponding elimination half-life ($t_{1/2}$) was calculated as $\ln(2)/\beta$. Apparent volume of distribution was determined by the formula

$$V_d = \frac{\text{i.m.dose (F)}}{\text{AUC} \times \beta}$$

Average blood concentration µg/ml of CQP or MQH versus time profiles are shown in Table 3.49 & Table 3.50, Figure 3.50, 3.51, 3.58 & 3.59. The maximum concentration for free drug and formulation were 18.3 & 8.8, 16.8 & 8.9 µg/ml for chloroquine phosphate and mefloquine hydrochloride respectively. The C_{max} was much lower for the microspheres as compared to that of free drug. The lower C_{max} values which are above

MEC value implies that microsphere form may be safer than that of free drug injection. Elimination rate constant (K_{el}) & absorption rate constant (K_a) were found low following intramuscular administration of microspheres of CQP & MQH as compared to i.m. administration of free drugs (CQP & MQH). The apparent volume of distribution for microsphere formulations is much higher than the free drug. Mean residence time (MRT) and mean absorption time were much longer for the microsphere compared to that of free drug. Area under the curve and area under the first moment curve both were much greater for CQP & MQH microsphere formulation. There was not much difference in t_{max} .

These parameters AUC, AUMC, C_{max}, t_{max}, MAT, MRT, K_a and K_{el} can be considered as indicators to conclude that the prepared microsphere formulations of both the drug (CQP & MQH) has shown localized slow release character.

The MAT & MRT were prolonged following i.m. administration of microsphere formulation which indicates that microspheres acted as a local depot and provided gradual release of the drug. Finally it could be proposed that different pharmacokinetic parameters (viz. AUC, AUMC, C_{max}, t_{max}, MAT, MRT, K_a and K_{el} for i.m. administration) were useful to characterize these controllable sustained release dosage forms in compared to the free drugs (CQP & MQH).

The slow release of drug observed in case of microsphere may be due to particle agglomerates formed at the intramuscular injection site. In case of microspheres (CQP/MQH) dispersion a intramuscular depot might be formed as follows. Once injected, the microsphere dispersion is confined to the fibrous or membranes tissue fibers, while, the surrounding aqueous vehicle almost disappears by penetration into the adjacent tissue. Thus, the microspheres are kept together within the structure of the tissue and they

form a so called loose aggregates. This mechanism can be compared with the caking phenomenon of deflocculated suspensions under the influence of gravitation force. In the muscles this force is replaced an over shadowed by muscle tone. According to the hypothesis mentioned before there should be sustained release of CQP/ MQH. It was reported by Kadir et al (1992) that 25 μg/ml plasma concentration caused symptoms of severe toxicity in the mice. In our study highest peak level found was 8.8 μg/ml and 8.9 μg/ml for CQP & MQH microsphere formulation respectively, which is lower than the drug related toxicity. The phenomenon of lower C_{max} values are therefore very favourable for therapeutic applications.

Biodistribution study

The transfer of drug from blood to extra vascular fluids (i.e. extra cellular and intracellular water) and tissues is called as distribution. Drug distribution is usually rapid and a reversible process. Thus drug in the plasma exists in a distribution equilibrium with the drug in the erythrocytes, other body fluids and tissues. As a consequence of this equilibrium, changes in the concentration of the drug in the plasma are often indicative of changes in drug level in other tissues including sites of pharmacological effects.

The tissue distribution study was carried out by using same animal model used for pharmacokinetic study. Calibration curves of extracted CQP and MQH from different organs such as lung, liver, heart, kidney and spleen were developed. Obtained results for calibration curves are tabulated and graphically represented in chapter 3 (section 3.1.5 and 3.1.8).

The amount of drug (μg/g) present in different organs was estimated at specified time interval by the method described in chapter 3, section3.1.5 and 3.1.8 for the CQP and MQH respectively. Comparative tissue distribution studies for free drugs (CQP&MQH) and their optimized

formulations (CC5 & M18) were done and obtained results are tabulated in table 3.49 and 3.50.

The drug (CQP/MQH) concentration in whole blood versus time profiles (Figure3.51 and3.59) exhibits a drug release which could be monitored upto 6 and 18 days for CQP and MQH respectively. These figures also represents the concentrations of free drugs (CQP and MQH) as compared to their formulations (CC_5&M18) at different time intervals. The maximum blood concentrations were 18.3μg/ml after 2 hours and 8.8μg/ml after 3 hours for free drug CQP and its formulation (CC_5). Also maximum blood concentrations were observed 16.8μg/ml after 3 hours and 8.9μg/ml after 3 hours for free drug MQH and its formulation (M18). Initially, high concentrations of drugs (CQP & MQH) in blood were found which could be attributed to the burst effect due to non encapsulated free drug present in microsphere at the surface. The concentrations were maintained above 0.4μg/ml up to 6 days and 0.3μg/ml up to 18 days for chloroquine phosohate and mefloquine hydrochloride respectively. The blood level concentration of CQP observed was well above the minimum concentrations of 30ng/ml, as suggested by Hardman and Limbird for the antimalarial activity with any type of plasmodium parasite. Peak concentrations of both the drugs (CQP and MQH) were low and delayed. Formulations (CC_5 and M18) exhibits a prolonged and comparatively slower release of drugs (CQP and MQH).

The tissue distribution (different organs & blood) of free drug (CQP/MQH) and their formulations are tabulated in Table 3.49 & 3.50. Drug concentration in various organs and blood (μg/g or μg/ml) versus time plots are plotted (Figure 3.50 to 3.65). Figure 3.53 & 3.61 shows concentration of drug after administration of free drug and its formulation for chloroquine phosphate and mefloquine hydrochloride in heart. The maximum concentrations for formulations were 5.4 μg/g after 3 hours and 3.42 μg/g

after 3 hours for the drugs CQP and MQH respectively. These concentrations were lower than that of free drug concentrations, 18.3 μg/g after 3 hours and 11.8 μg/g after 3 hours of chloroquine phosphate and mefloquine hydrochloride respectively. Chesney et al (1967) studied in-vivo tissue distribution of muscle and liver are two main repositories organs for chloroquine in monkey. Anna and Culter (1986) investigated that the accumulation of CQP in muscle tissues is predominantly due to binding of cationic CQ species with anionic sites on tissue, particularly phospholipids. This accumulation is not saturable at therapeutic level. Due to this phenomenon, in free drug the toxic concentration can be achieved. Microspheres and colloidal drug delivery system can help to overcome from this toxicity by releasing the drug in controlled manner from depot from injection site.

From this one can conclude that the microsphere dosage form could lower the cardiac toxicity specifically for CQP. The lower concentration of mefloquine in blood and heart is due to release of drug from intact microsphere which is favourable to reduce drug related toxicity.

The CQP concentrations were much higher in blood and heart for free drug (CQP) as compared to microsphere formulation (CC_5) of the same drug. Kadir et al (1992) reported that low concentration of CQP in heart after intramuscular administration of chloroquine encapsulated liposomes which is favorable regarding cardiotoxicity of the drug. Phillips et al (1986) reported that CQP toxicity is related to its concentration in blood and high peak levels cause circulatory shock and death. Our results explain that after intramuscular administration of chitosan coated CQP microsphere lower the drug concentration in heart which helps in lowering cardiac toxicity. CQP cardiotoxicity is directly related to its concentration in heart muscle tissue

(Kadir et al, 1992) and so that our results favour the aspect of administration of encapsulated CQP in microspheres.

Figure 3.56, 3.57 & 3.64, 3.65 depicted the concentrations of CQP and MQH in liver and spleen. The drug concentration was increased with time initially and then it was decreased. This was ascribed to the tendency of drug to accumulate in red blood cells and the subsequent clearance of these cells by spleen.(Kadir et al 1991). Titular et al (1990) reported that the high chloroquine concentrations were observed in spleen and liver after intraperitoneal administration of liposomes containing chloroquine. Nassander et al (1990) reported that liposomes and other colloidal carrier are accumulated in organs like spleen and liver by phagocytosis through the action of the macrophages of the MPS–cells.

Figure 3.54, 3.55, 3.62 and 3.63 represents drug concentration at different time intervals in excretory organs, lung and kidney. It assumed that excretion of drug (CQP/MQH) may be slower because the low concentration with formulation was observed compared to free drug after intramuscular administration. This also described by elimination rate constant (K_{el}) which is low for formulation compared to free drug. The MQH concentration was higher in lung compared to kidney. This might be due to lipophilic nature of drug and polymer.

3.4.8 References

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