Available online at www.ijpsdronline.com

International Journal of Pharmaceutical Sciences and Drug Research 2015; 7(1): 22-26

Research Article

ISSN: 0975-248X
CODEN (USA): IJPSPP

Development and Characterization of Hydroxyl Chloroquine Sulphate (HCQ) Nanoparticles

Roopkishora[1], Amit Singh[2], Chhote Lal Singh[2*]

[1]*Akums Drugs $ Pharmaceuticals Ltd. Haridwar, Uttarakhand, India*
[2]*R. V. Northland Institute (Pharmacy), Chithera, Dadri, Gautam Budh Nagar-203 207, Uttar Pradesh, India*

ABSTRACT

The objective of present study was to formulate and characterize the nanoparticles of hydroxyl chloroquine sulphate (HCQ). Nanoparticles were prepared by the w/o/w emulsion - solvent evaporation method using polymer Eudragit® RL-100, acetone, and surfactant poloxmer-188 solutions. Amount of polymer, organic solvent and surfactant was selected as formulation variable. Characterization of the nanoparticles was performed by measuring particle size, zeta potential, surface morphology, interactions, drug entrapment efficiency, in-vitro drug release, % yield and stability. The formulated nanoparticles were found to be spherical and uniform in particle size with less than one poly dispersity index. The zeta potential of nanoparticles was found -34 (mv) represent higher stability of colloidal dispersion. In another set of characterization the percentage yield and entrapment was found to be in the range of 55-68% and 63.14% respectively. The in-vitro release shows 87.93% at its maximum level in 24 hours of study. The suspension of formulation was found more stable in refrigerated environment. The release kinetics evaluation revealed the drug release was following Higuchi model, which ensures the possibility of long term release profile from the nanoparticles formulation so that its concentration maintained within therapeutic level for larger period of time so that safe and efficacious for malarial and rheumatoid patients.

Keywords: Hydroxy chloroquine sulphate (HCQ), Nanoparticles, Rheumatoid arthritis, Antimalarial.

INTRODUCTION

Malaria is the most prevalent parasitic disease in the world, with an estimated 500 million cases arising annually and with 1 million to 3 million deaths being attributed to this disease. [1] Furthermore, most victims of malaria are below 7 years of age. Of the four species of *Plasmodium* that can cause human malaria, *Plasmodium vivax*, the causative agent of vivax malaria, is the second most common species of malaria, with an estimated 35 million *P. Vivax*-transmitted malaria cases oyear. [2] Hydroxy chloroquine sulphate was first synthesized in 1946 by the addition of the hydroxy group to the parent compound, chloroquine (CQ), in an effort to reduce toxicity. Chloroquine (CQ) was found to be 2-3 fold more toxic than HCQ in experimental animal studies. [3] HCQ is one of a large series of 4-amino quinolines with antimalarial activity. It has been used in malaria therapy since 1955 as an alternative to or in combination with CQ, which only differs slightly in structure from HCQ. Although HCQ was developed primarily as an antimalarial agent, it possesses several

***Corresponding author: Mr. Chhote Lal Singh,**
R. V. Northland Institute (Pharmacy), Chithera, Dadri, Gautam Budh Nagar-203207, Uttar Pradesh, India; **Tel.:** +91-120-2666444, 2666445, +91-7838781211, 7836062102; **Fax:** +91-120-2666445; **E-mail:** chhotelal007@gmail.com
Received: 20 October, 2014; **Accepted:** 07 November, 2014

other pharmacological properties as well. Its anti-inflammatory properties are well known and it has been useful in the treatment of rheumatoid arthritis and in systemic lupus erythematosus. Its applicability in the treatment of photo-allergic reactions is also established. Particulate systems like nanoparticles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. They have been used *in-vivo* to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. The late 1960's and early 1970's saw the advent of polymer nanoparticles based on acrylamide micelle polymerization. [4] Since then, along with different polymerization methods, use of preformed polymers have also been developed and studied. [4-6] The majority of studies on nanoparticles reported to date have dealt with nanoparticles of poly (D, L lactide), poly (lactic acid) PLA, poly (D, L glycolide) [PLG], poly (lactide-co-glycolide) [PLGA] and Poly-cyanoacrylate [PCA]. The therapeutic activity of ampicillin [7], ciprofloxacin [8-9], sparfloxacin [10] was shown to be dramatically enhanced after its binding to nanoparticles. It act by formation of chloroquine - heme complex because CQ having a weekly basic nature , it raises ph and thereby interfere with degradation of hemoglobin by parasitic lysosomes and the development of resistance decreased by inhibiting the hemoglobin degradation and also deliver the drug at a controlled and sustained rate to the site of action. Thus, the aim of the present work was to investigate different formulation composition to obtaining a statically optimized nanoparticle formulation with an adequate HCQ loading and release properties. The entrapment – efficiency, the *in-vivo* drug release profile and drug-polymer interaction was also investigated.

MATERIALS AND METHODS

Drug (Hydroxy chloroquine sulphate), Polymer Eudragit® RL100, Surfactant Poloxamer-188 (Sigma), Acetone (Renkem), Double distilled water. Methanol (HPLC) grade were purchased from (Fisher Scientific (Qualigen), India), Sodium 1-pentanesulfonate of HPLC grade was purchased from (Glaxo India Pvt. Ltd. Mumbai, India), Acetonitrile and phosphoric acid were purchased from (RFCL Limited, New Delhi, India). The filters with pore size of 0.22µm were arranged from Pall Life Sciences, Mumbai used for filtration of mobile phase and sample solutions, Hydrochloric acid, Disodium hydrogen phosphate, and Potassium dihydrogen phosphate, Dialysis membrane of 12000 Daltons cut off (Himedia, Mumbai, India) provided from R. V. Northland Dadri, Greater Noida, G. B. Nagar.

Formulation development

Hydroxyl choloroquine sulphate nanoparticles were prepared by the solvent displacement method. Briefly, a 5 mg portion of drug and various proportion of polymer (5-50 mg) dissolved in acetone (Table 1-3). This organic phase was poured drop wise into solution of poloxamer – 188 with moderate magnetic stirring at room temperature. Nanoparticles were spontaneously formed and turned the solution slightly turbid then; acetone was removed by continuous stirring at 35-40°C. The prepared suspension was centrifuged, supernatant was removed and the sediment was freeze dried for further analysis. [11]

Table 1: Constant parameter for formulation

S. No	Constant Parameter	Value
1	Diameter of needle nozzle	0.8 mm
2	Height of needle tip from solution surface	5 cm
3	Dropping rate	2 mL/min
4	Distilled water	10 mL
5	Drug	5 mg

Table 2: Variable parameters used in the preparation of nanoparticles

S. No	Variable parameters	Range investigated
1	Polymer	5-50 mg
2	Organic solvent	2-5 mL
3	Surfactant	10-50 mg

Table 3: Composition of different formulation

S. No	Formulation Code	Drug (mg)	Polymer (mg)	Acetone (mL)	Water (mL)	Surfactant (mg)
1	F1	5	5	2	2	10
2	F2	5	10	2	1	20
3	F3	5	15	3	1	30
4	F4	5	20	4	1	40
5	F5	5	50	5	2	50
6	F6	5	10	2	4	10

Physio-chemical characterization

Particle size and zeta potential

Nanoparticles size distribution was determined using photon correlation spectroscopy (PCS) with Zetasizer 3000 (Malvern instrument Ltd., Malvern, Worcestershire United Kingdom). The size distribution analysis was performed at a scattering angle of 90° and at a temperature of 25°C using samples appropriately diluted with filtered water. The mean particle size Z_{avg} of each sample was determined three times and the average values were calculated.

Zeta potential values were determined by electrophoretic light scattering (ELS) using the same instrument. Nanoparticles were suspended in filtered water and diluted with water. For each preparation three samples were injected in the capillary cell of the Zetasizer 3000. Then the average values of three replicates were calculated.

Morphology

Morphology of nanoparticles was observed by transmittance electron microscopy (TEM). The shape and surface of particles were also important in terms of drug release therefore morphology was evaluated. Particle morphology was analyzed using a transmission electron microscope (Morgagni- 268-D, FEI Netherland) using an acceleration voltage of 120kv. Specimens were prepared by dropping the sample

solution on to an upper grid, and then a drop of 2% uranyl acetate was added to give negative stain. The grid was then allowed to stand for 1 minute before the excess staining solution was removed by draining. The specimens were air-dried and examined using TEM.

Nanoparticle recovery

The recovery of nanoparticles suspension was analyzed by centrifugation method, where 10 mL suspension was centrifuged at 15000 rpm at 4°C. The sediment nanoparticles were collected, freeze dried and calculated for % yield.

$$\% \text{ Yield} = \frac{\text{Weight of recovered particle}}{\text{Weight of drug and polymer used}} \times 100$$

All experiments were conducted in triplicate.

Drug entrapment

Take 15 mg of freeze dried nano-particles in a volumetric flask filled with distilled water for extraction of drug and kept for 24 hours. The mixture was sonicated for 20 min. Then filtered by using vacuum filter to obtain complete clear solution and sample will be assayed by UV-spectrophotometer at λ_{max} 343 nm. The percentage drug entrapment efficiency can be calculated by using following equation

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Weight of drug in nanoparticles}}{\text{Weight of drug used}} \times 100$$

All experiments were conducted in triplicate.

***In-vitro* drug release studies**

In-vitro drug release study was performing using dialysis membrane (12000 mol. wt. cut off) in which 10 ml of the nanosuspension was taken in dialysis membrane and it was sealed both sides by dialysis membrane closure clips, that was and attached with paddles of dissolution apparatus. Then it was dipped in to the phosphate buffer of pH 7.4. The dissolution was performed at 50 rpm. The samples were collected in different intervals up to 24 hours. The sampling volume was kept 5 mL & same quantity (5 mL) of phosphate buffer was added after each and every sample collection. The samples were analyzed by UV spectrophotometer to understand the release pattern.

Release kinetics evaluation

Release data were fitted to different mathematical models to reveal the release patterns at nanoparticles. Zero order, first order and Higuchi models were used for this purpose. The zero order plots was constructed by plotting cumulative percentage release versus time, first order plot was constructed by plotting log (cumulative percentage release) versus time and higuchi plot was constructed by plotting cumulative percentage release versus square root of time. All curves fitting, simulation and plotting were performed using commercially available Microsoft excel solver (Microsoft Corporation, USA).

Stability studies

Optimized formulation, F1 was selected for short term stability studies. The nanoparticulate suspension was placed at accelerated and stress conditioned environment for 6 months. The samples were collected at 0, 3, and 6 month intervals according to "accelerated" scheme and at 0, 1, 2 and 3 months according to "stress condition" study. Stability studies were performed according to ICH guidelines [ICH Q1A (R2), ICH Q1B, and ICH Q1C]. [12-13]

Table 4: Nanoparticle recovery of different formulations.

S. No	Formulation	% yield
1.	F1	68.48
2.	F2	58.13
3.	F3	58.89
4.	F4	55.48
5.	F5	65.39
6.	F6	66.38

Table 5: Drug entrapment efficiency of different Nanoparticle formulations.

S. No	Formulation	% yield
1.	F1	63.14
2.	F2	66.49
3.	F3	65.79
4.	F4	69.38
5.	F5	58.40
6.	F6	65.27

Table 6: *In-vitro* drug release profile of HCQ Nanoparticles

Time	Cumulative % Drug release						
(Hrs)	F1	F2	F3	F4	F5	F6	Control
1	19.43	34.64	19.44	39.28	13.20	23.23	21.222
2	28.11	37.90	35.27	47.79	30.29	47.98	26.34
3	32.95	49.75	44.42	50.63	40.58	48.54	38.69
4	43.08	57.22	45.08	52.40	42.19	62.33	40.78
5	43.16	60.64	57.13	56.28	46.61	63.89	42.77
6	45.96	70.26	58.75	60.07	51.52	61.09	42.66
7	50.99	68.51	63.82	65.83	52.15	63.11	51.83
8	52.40	80.12	63.79	90.37	55.58	78.75	52.77
23	85.25	98.62	98.51	98.17	90.28	99.89	87.44
24	87.93	99.38	99.12	98.34	92.21	99.83	90.11

Table 7: Correlation coefficient of different mathematical model.

Formulation	Correlation Coefficient (r^2)		
	Zero order kinetics	First order kinetics	Higuchi kinetics
F1	0.957	0.855	0.995
F2	0.888	0.926	0.959
F3	0.890	0.884	0.972
F4	0.893	0.879	0.991
F5	0.931	0.881	0.996
F6	0.814	0.964	0.992
Control	0.959	0.918	0.989

Table 8: Stability data at accelerated and stress condition of formulation F1

S. No	Storage condition	Sampling periods (months)	Remarks/Result	
			% drug retained	Physical appearance of formulation
1.	Accelerated 40°C, 75% RH ± 5% RH	0	65.21	Milky white
		3	54.86	Milky white
		6	45.63	Milky white
2.	Stress (50±2° C)	0	63.20	Milky white
		1	42.66	Milky white
		2	30.34	Milky white
		3	22.42	Milky white
3.	Refrigerated (5°C± 3°C)	0	67.40	Milky white
		1	66.09	Milky white
		2	54.20	Milky white
		3	50.79	Milky white

RESULT AND DISCUSSION

Formulation development and optimization of formulation

On the basis of entrapment, drug loading and drug release the formulation F1 has been selected for further study.

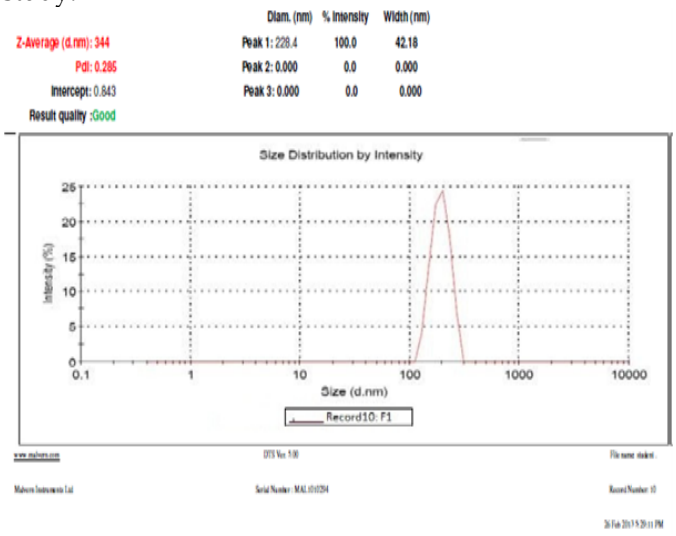


Fig. 1: Particle size distribution report of formulation f1.

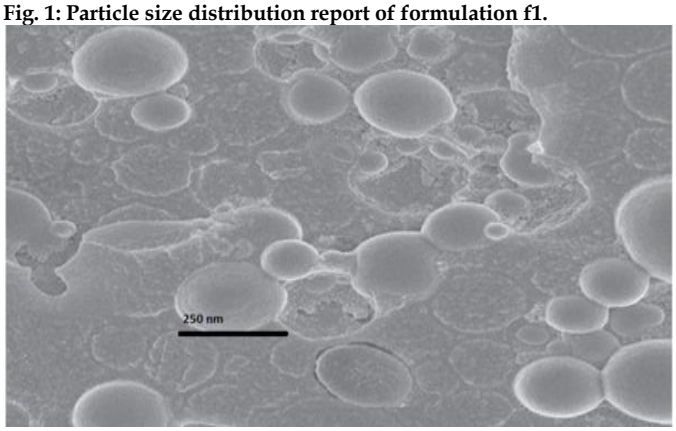


Fig. 2: TEM image of nanoparticles suspension

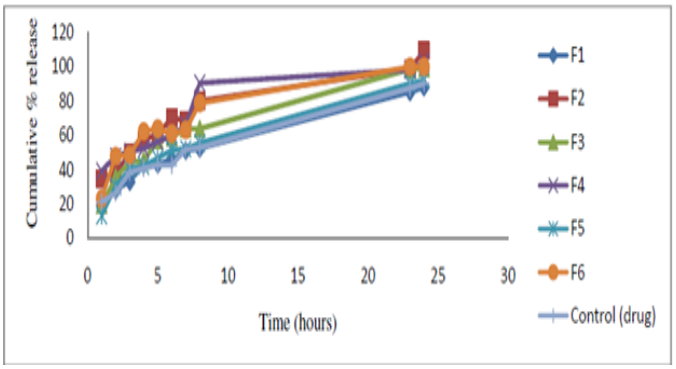


Fig. 3: Zero order release kinetics

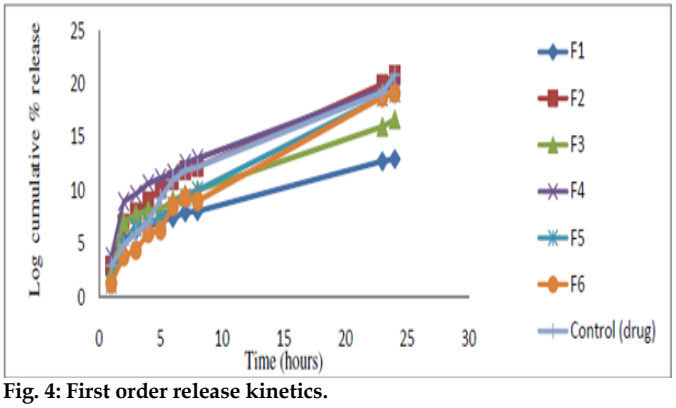


Fig. 4: First order release kinetics.

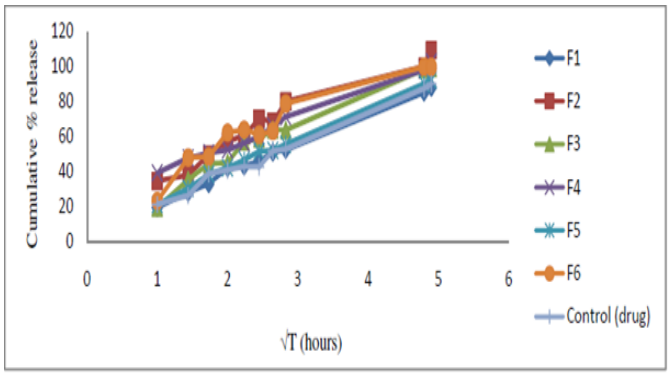
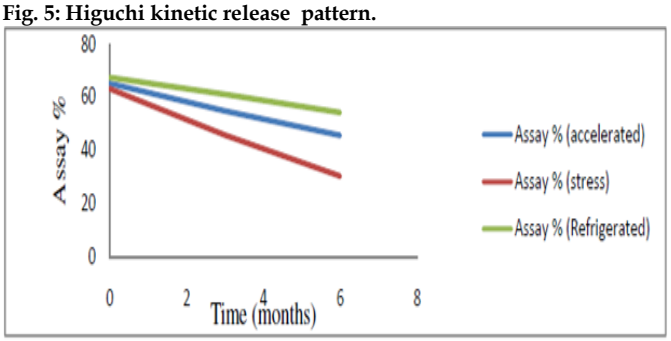


Fig. 5: Higuchi kinetic release pattern.



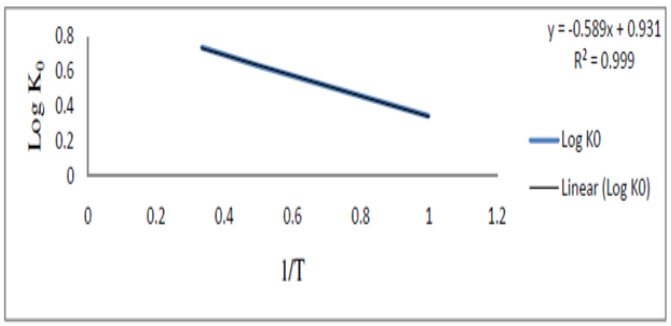
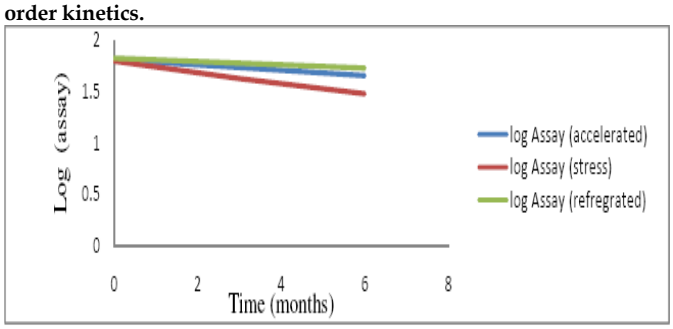


Fig. 6: Graphical representation of degradation data treated as zero order kinetics.



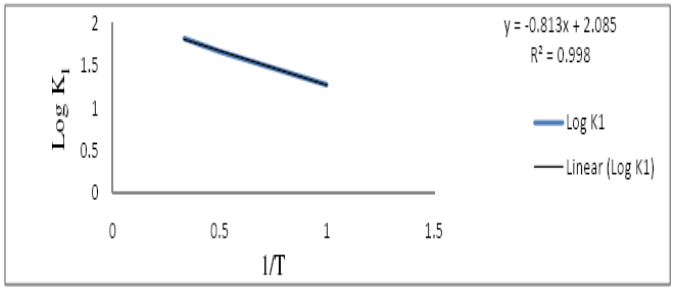


Fig. 7: Graphical representation of degradation data treated as first order kinetics.

Physicochemical characterizations

Particle size analysis

Among the different formulations, selected F1 and F2 were checked for particle size and particle size

distribution. The obtained sizes were 344 and 338 nm respectively, whereas polydispersity index was 0.285 and 0.646 respectively (Figure 1). The sizes are in nano-range and less than one polydispersity proves less fluctuation in sizes.

Zeta potential

Since the zeta potential of formulation F1 and F2 was found to be -34 mV and -32 mV. Therefore the formulation can be said to be stable, because theoretically it was proven that zeta potential ± 30 mV represents higher stability of colloidal dispersion.

Morphology

TEM photograph of formulation F1 is shown in (Figure 2). The particles were found nearly spherical shape which may provide uniformity in drug release.

Nanoparticle recovery

The recovery of nanosuspensions was found less than 100 %, recovery was found to be at the range 55.48% - 68.48 % in all formulations. The highest % yield was found in case of F4 among the six formulations. The high yield in case of F4, F5 and F6 because of high amount of polymer and surfactant used (Table 4).

Drug entrapment efficiency

There is no such significant difference in drug entrapment efficiency between the different formulations. However, a slight increment in drug entrapment efficiency was found when amount of polymer is less and also less amount of organic solvent, drug entrapment efficiency was at the range of 58.40% - 69.38% (Table 5).

***In-vitro* drug release studies**

From (Table 6) it has been found that in case of F1, F3 and F5 the initial burst release was slower as compared to F2, and F4 which might be due to presence of dispersing agent in F2, and F4. Increased concentration of dispersant result in smaller particles, due to less particle size the effective surface area was increased which might induce to burst release. When the polymer content was increased gradually in different formulation keeping amount of drug constant, the release rate was increased, because having more entrapment due to high polymer content, drug release at 24 hours was found to be at the range of 13.20 - 99.89 % in all formulations.

Release kinetics evaluation

The release patterns using different mathematical model are shown in (Figure 3-5) and r^2 values of different model are shown in (Table 7). By analyzing release kinetics it was found that for all formulations, the correlation coefficient was highest in case of Higuchi kinetics. Therefore it can be said that the release follows Higuchi kinetics. Moreover the r^2 value of zero order kinetics is higher than that of first order kinetics and it was near to that of Higuchi kinetics. Therefore release following the mixed kinetics and the release was diffusion controlled. Hence, drug release can be prolonged from Eudragit® RL-100 nanoparticles in suitable combination.

Stability studies

The study was carried out according to ICH guidelines. The studies were framed in accelerated and stress condition by analyzing the results from (Table 8) the formulation was found to be unstable in all conditions for three months. The formulation was very quickly damaged in higher temperature and humidity (Figure 6-7). A better stability for refrigerated sample was found up to 45 days but that value may not be significant. Therefore, special technique shall be considered to improve the stability of the formulation such as freeze drying which can be conducted in future.

ACKNOWLEDGEMENT

The authors would like to grateful of the authorities of R.V. Northland Institute, Dadri, G. B. Nagar, India for providing required facilities to carry out the proposed work and also thankful to the KPS Clinical Services, Greater Noida, UP, India for providing the standard drug.

REFRENCES

1. Sachs J, Malaney P. The Economic and Social burden of Malaria. Nature 2002; 415: 680-685.
2. Galinski M, Barnwell JW. *Plasmodium Vivax*: Merozoite invasion of Reticulocytes and Considerations for Malaria Vaccine Development. Parasitol. Today 1996; 12:20-29.
3. Jordan P, Brookes JG, Nikolic G, Le Counteur D. Hydroxychloroquine overdose: toxicokinetics and management. J Toxicol Clin Toxicol. 1997; 37(7):861-864.
4. Kreuter. J. Nanoparticles, in Encyclopaedia of Pharmaceutical Technology, Swarbrick j, Marcel Dekker Inc. New York USA, 1994, pp. 165-90.
5. Barratt GM. Therapeutic Applications of Colloidal Drug Carriers. Pharm. Sci Technolo Today 2000; 3(5):163-171.
6. Pitt CG, Chasalow FI, Hibionada YM, Klimas DM, Schindler A. Aliphatic polyesters 1. The degradation of poly-caprolactone *in vivo*. J. Appl. Polym. Sci. 1983; 28:3779–87.
7. Fatal E, Youssef M, Couvreur P, Andremont A. Treatment of experimental salmonellosis in mice with amipicillin–bound nanoparticles. Antimicrobe Agents Chemother. 1989; 33(9): 1540-1543.
8. Kathleen D, Vandervoort J, Guy V, Annick L. Evaluation of Ciprofloxacin loaded Eudiragit® RS 100 or RL 100/PLGA Nanoparticles. International Journal of Pharmaceutics 2006; 314(1): 72-82.
9. PageClisson ME, PintoAlphandary H, Ourevitch M, Andremont A, Couvreur P. Development of ciprofloxacin-loaded nanoparticles: Physicochemical study of the drug carrier. J Contr Release 1998; 56:23–32.
10. Torchilin VP. Structure and design of polymeric surfactant based drug delivery systems. J Contr Release 2001; 73:137-172.
11. Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int. J. Pharm. 1989; 55: R1-R4.
12. ICH Q2A- Guidelines for Identify: Test on method validation of analytical procedure, March 1995.
13. ICH Q2B- Guidelines for industry validation of analytical procedure methodology, Nov. 1996.

Source of Support: Nil, Conflict of Interest: None declared.