Determination of Percent Drug Release of Different Lipid-Based Formulations of Anti-Malarial Drugs, In Different Physiological Media

Nilesh Jaiswal [1], Prof, (Dr.) Mehta Parulben [2]

[1] Research Scholar, School of Pharmacy, LNCT University, Bhopal, Madhya Pradesh, India.

[2] Professor, School of Pharmacy, LNCT University, Bhopal, Madhya Pradesh, India.

Email: [1] nileshjaiswal2@gmail.com, [2] parulmehta1@rediffmail.com

Abstract

One of the most dreadful diseases in Asia, Africa, and Latin America is malaria, which over the decades has been highly fatal when not controlled at an early stage and when not managed properly. Even though there are multiple categories of anti-malarial drugs available on the market, one of the most common demerits of such drugs is that most of them face the major challenge of complete absorption via oral route. This is due to either the first-pass effect or the poor delivery system, which does not deliver the drugs completely until the site of action. The current study emphasizes the study of the preparation of different lipid-based formulations and comparative assessments of their percent drug release. Hence, for the research purpose, four anti-malarial drugs (i.e., artemisinin, amodiaquine, halofantrine, and chloroquine) are well known for being highly lipophilic in nature and having the least affinity for polar solvents like water, because of which they are least soluble in aqueous media and solvents. The oral absorption of such drugs is a challenge, and hence they face poor oral bioavailability due to their limited aqueous solubility. In the absorption process of such drugs, the solubility remains at its "rate-limiting step." The research study discusses the preparation of different lipid formulations as it was believed that such molecules, which have limited aqueous solubility and exhibit positive food effects (i.e., an increase in absorption post-intake of food), have a better chance of enhanced oral absorption due to the formation of in-situ micelles that get absorbed via the lymphatic circulatory system. The current research study primarily focuses on studying the percent drug release of such anti-malarial drugs across a wider physiological range of pH, i.e., in the acidic region pH ~1, ~2, and ~4.5, in the neutral range pH~6.8, and in the alkaline range pH ~7.4, including simulated fasted-state gastric fluid, simulated fasted-state intestinal, simulated fed-state gastric fluid, simulated fed-state intestinal fluid, lipolytic digestive medium, and purified water. The formulations wherein lipids were used exhibited the highest percent drug release in bio-relevant simulated gastric, intestinal, and lipolytic digestive media when compared to formulations wherein there were no lipids. The study indicated that such lipoidal formulations tend to solubilize and therefore exhibit a higher percentage of drug release because the drug undergoes solubilization in the presence of surfactants present in the medium, e.g., sodium taurocholate.

All lipoidal formulations selected in the study exhibited a similar trend and solubilization effect when compared to formulations where no lipids were used.

Keywords: Low aqueous solubility, Physiological pH percent drug release profile, Biorelevant media.

1. Introduction

Malaria is one of the deadliest in the world and has a high prevalence in subtropical and tropical areas. In 2021, the Department of Malaria and Tropical Diseases of the World Health Organization (WHO) recorded[1] that there were approximately 241 million malaria cases, with over 620 000 deaths, worldwide in 2020. Anti-malarial drugs, which are being used for quite some time by needy patients, have their own advantages and disadvantages. While many of us, know the advantages of such drugs, most of us are unaware of their demerits, which are predominantly associated with their pharmacokinetics, especially their poor oral absorption. Klayman DL et al., in their publication[2], expressed that artemisinin, which is a potent antimalarial drug, has been isolated from the traditional Chinese medicinal herb, *Artemisia annua. It* is a fast-acting blood schizonticide with a shorter parasite clearance time, leading to rapid symptomatic relief of malarial infections. Similarly, later White NJ et al., also in 2015 in their publication[3] emphasised the origin of Artemisinin (i.e., Qinghaosu) as an antimalarial drug from China. It is also effective against multidrug-resistant strains of *Plasmodium falciparum* and produces rapid recovery even in patients with cerebral malaria. However, artemisinin has low aqueous solubility, resulting in poor and erratic absorption upon oral administration. This, together with its short half-life and high first-pass metabolism, might lead to low oral bioavailability.

Anti-malarial drugs, which can be pretty at the same time as being used by needy patients, have their own benefits and drawbacks. At the same time, a lot of us know the benefits of such drugs; however, most of us are ignorant of their demerits, which might be predominantly related to their pharmacokinetics, particularly their oral limited absorption.

Li J et. al., in their work[4], concluded that Artemisinin, which is an effective antimalarial drug, is a quick-appearing blood schizonticide with a shorter parasite clearance time and results in symptomatic relief from malarial infections. It is also effective against multidrug-resistant strains of Plasmodium falciparum and produces speedy healing even in patients with cerebral malaria. However, artemisinin, which has been known for its low aqueous solubility, results in poor and erratic absorption upon oral ingestion. The drug's short elimination half-life and excessive first-pass metabolism would possibly lead to low oral bioavailability.

Further, in 1987, Winstanley et al. referred[5] to the fact that amodiaquine, after oral administration, is swiftly absorbed, but additionally undergoes a fast and enormous metabolism to desethylamodiaquine, which concentrates in blood cells. It is particularly likely that desethylamodiaquine, not amodiaquine, is accountable for the maximum pharmacological action of antimalarial interest, and the adverse effects of amodiaquine after oral management may additionally in element be, because of desethylamodiaquine. The hepatic bio-transformation to desethylamodiaquine (the primary biologically active metabolite) is the important reason behind amodiaquine clearance which leads to a

completely little orally administered amodiaquine, which escapes untransformed into the systemic stream.

Karbwang et al., in 1994 studied[6] that Halofantrine, which was also exceptionally lipophilic drug and available in tablet and suspension oral dosage forms, the oral absorption of such is erratic and in addition found to be decreased in patients suffering from malaria, however can tremendously enhance three folds, while given with meals. Moreover, the drug clearance turned out to be reduced. The C_{max} was attained 6 hours after oral administration, indicative of the large obvious extent of distribution (~50 L/kg), with an elimination half-life of 1–5 days in patients with malaria.

In addition, in 1982, Tulpule et al. mentioned in their research study[7] that a standard breakfast was provided to seven male subjects, and as a control group, the same subjects were also deprived of the breakfast; the results indicated an increase in the oral bioavailability of chloroquine. The chloroquine drug was administered orally, and blood samples that were withdrawn for determination of drug concentration in serum indicated that the C_{max} and AUC were higher in the group of subjects that were given food. This indicated that there is a positive postprandial food effect on chloroquine absorption. As a result, the administration of chloroquine, collectively with food, was indicated to be a beneficial clinical exercise.

In 2016, Rezhodo et al. mentioned in their publication[8] that orally administered drugs that demonstrate highly limited water solubility and are dosed to subjects with certain classes of lipid excipients have been exhibiting an increase in bioavailability. Lipids have been known to influence the transport and absorption of certain categories of drugs and their metabolism in the GUT. This includes an increase in their in-situ solubility and/or even an alteration of the coefficient of permeability across intestinal epithelial cells. This influence of lipid excipients can be highly challenging to understand because of the involvement of complicated digestion processes within the lumen of the GIT. Lipid excipients have been widely used these days to alter the solubility and/or permeability of the needy drug molecules. Herein, we provide a critical review on the comparative examination of the percent drug release of lipoidal formulations of Artemisinin, Amodiaquine, Halofantrine, and Chloroquine across a wider physiological dissolution medium, i.e., on the acidic side, like pH ~1, ~2, and ~4.5, on the neutral side, at pH 6.8, and on the alkaline side, pH 7.4, including simulated fasted-state gastric fluids, simulated fasted-state intestinal fluids, simulated fed-state gastric fluids, simulated fed-state intestinal fluids, simulated lipolytic-digestive fluids, and purified water, compared to formulations wherein there are no lipids.

2. Material and Methods

Artemisinin was sourced from M/s Rhyme Organics and Chemicals Limited, Gandhi Nagar, Hyderabad. Amodiaquine was sourced from M/s. Merix Laboratories Pvt. Ltd., 5th Floor, Merix Square, Bajaj Electronics, Pet Bas, Bashirabad, Jeedimetla, Hyderabad, Telangana 500010, India. Halofantrine was sourced from Sigma Aldrich, 3rd Floor, F Block Brij Tarang, Greenlands, Begumpet, Hyderabad, Telangana 500018. Chloroquine was sourced from M/s. Dev-Life Corp., Vikhroli, Mumbai-79. 50 g of each drug received as a gift sample was utilized for research purposes.

3. Preparation of different buffers and biorelevant dissolution media for the study of percent drug release:

0.1 N HCL (~pH 1)

In a clean volumetric flask of 1000 ml, 8.5 mL of hydrochloric acid (37% v/v) was added, and the volume was made up using purified water or deionized water. mixed well and sonicated to degas. Labelled accordingly.

0.01 N HCL (~pH 2)

In a clean volumetric flask of 1000 ml, 0.85 mL of hydrochloric acid (37% v/v) was added, and the volume was made up using purified water or deionized water. mixed well and sonicated to degas. Labelled accordingly.

pH 4.5 sodium acetate buffer

In a 1000-ml volumetric flask glassware, add approximately 800 ml of deionized or purified water. I separately weighed accurately sodium acetate (27.2 g) using a precision weighing balance and added the salt to the volumetric flask. mixed well until no visible particles are observed. Further to this solution, add acetic acid (glacial grade). made volume up to the 1000 ml mark, followed by sonication and degassing, and labeled accordingly.

pH 6.8: potassium phosphate buffer

In a 1000-ml volumetric flask glassware, add approximately 800 ml of deionized or purified water. Separately weigh potassium dihydrogen phosphate (11.45 g) using a precision weighing balance and add the salt to the volumetric flask. mixed well until no visible particles are observed. Further to this solution, orthophosphoric acid and caustic soda (analytical pharma grade) were added in suitable amounts, which attained a pH of ~4.5. made volume up to the 1000 ml mark, sonicated and degassed, and labelled accordingly.

pH 7.4: potassium phosphate buffer

In a 1000-ml volumetric flask glassware, add approximately 800 ml of deionized or purified water. Separately weighed accurately Sodium Phosphate Monobasic hydrate (3.394 g) and 20.214 g of neutral sodium hydrogen phosphate using a precision weighing balance and added the salt(s) to the volumetric flask. mixed well until no visible particles are observed. Further to this solution, orthophosphoric acid and caustic soda (analytical pharma grade only) were added suitably in amounts, which attained a pH of ~7.4. made volume up to the 1000 ml mark, sonicated and degassed, and labelled accordingly.

Simulated fasting gastric fluid media

In a 1000-ml volumetric flask glassware, pepsin solution (3.2 ml) and a neutral calcium chloride solution of 0.3 molarity (0.01 ml) were added and mixed thoroughly. The volume was adjusted using distilled water. Through a 1/10th-molar hydrochloric acid solution, the pH was adjusted to ~1.5. The range for the pH was observed to be from 1.45 to 1.55.

Simulated fed-state gastric fluid media

In a 1000-ml volumetric flask glassware, the above solution (15.0 ml) of common salt (analytical pharma grade) of 237.02 milli-molarity was added and mixed thoroughly. To this solution, ethanoic acid (17.12 mM) and sodium acetate (29.05 mM) were also added, and the volume was adjusted using distilled water. Through a 1/10th-molar hydrochloric acid solution, the pH was adjusted to 5.0. The pH range was observed to be from 4.95 to 5.05.

Simulated fasting intestinal media

In a 500-ml volumetric flask glassware, accurately weighed caustic soda (analytical pharma grade) at 1.74 g and 19.77 g of monobasic sodium phosphate, common salt (analytical pharma grade) at 30.93 g. adjusted the pH to ~6.5 using hydrochloric acid and caustic soda. Nomenclated this solution as a Fa-SSIF solution. To this was added sodium taurocholate (3.3 g), followed by lecithin solution *(*11.8 ml*; concentration 100 mg per ml), with dichloromethane as solvent, forming an emulsion. The methylene chloride was eliminated under vacuum at about 40 °C. The vacuum pressure was set at 250 mbar for 15 minutes and 100 mbar for another 15 minutes* .This resulted in micellar solutions. The solution was left undisturbed at room temperature for 1 hour and then adjusted to 2000 ml using the Fa-SSIF solution. Finally, the media was sonicated, degassed, and labeled accordingly as Fa-SSIF media.

Simulated fed-state intestinal fluid media

In a 2000-ml volumetric flask, glassware transferred accurately weighed sodium taurocholate (natural bile salt; 16.50 g). To this flask, 500 ml of the above Fa-SSIF media were added as blank. I added a lecithin-dichloromethane solution (59.08 ml) to form a milky emulsion. concentration of *100 mg per ml with dichloromethane as solvent, forming an emulsion. The methylene chloride was eliminated under vacuum at about 40 °C. The vacuum pressure was set at 250 mbar for 15 minutes and 100 mbar for another 15 minutes.*

The emulsion was left undisturbed at room temperature for one hour, and the volume was adjusted up to 2000 ml using Fe-SSIF media. adjusted the pH to 5.0 (range ~4.98 to 5.02) with the help of the addition of suitable quantities of caustic soda and hydrochloric acid. Finally, the media was sonicated, degassed, and labeled accordingly as Fe-SSIF media.

Simulated digestive intra-gastric lipolytic media[9]

A hydrochloric acid solution of 1.83 molarity was taken in a 1000-ml empty volumetric flask. To this volumetric flask, pepsin (pancreatic) 9.375 g and pancreatic lipase 3.125 g were slowly added under continuous stirring. The stirring was conducted for 90 minutes until a clear, milky, translucent solution was observed. Finally, 0.5 ml of a 3.3 mM calcium chloride solution was added to the above solution and stirred for another 30 minutes. The media was sonicated, degassed, and labeled as "simulated digestive/lipolytic media." Note: The addition of calcium chloride solution is highly important in order to activate the lipase enzyme; however, if an activated grade of pancreatic lipase is used, then the use of calcium addition can be omitted.

Preparation of the Lipoidal Formulation of Artemisinin:

Procedure: Based on the above composition, beeswax was heated up to 40 °C until it melted, and added to it were lanolin, paraffin, and cetyl alcohol. When the mixture was translucent and warm, drugs were added to it and mixed vigorously in a mortar and pestle for approximately 5 minutes, until a thick cream was formed. The HLB value was calculated theoretically for the lipoidal formulation, which was found to be 10. This lipoidal formulation was nomenclated as batch no. A-1 (HLB~10). Refer to Table No. 1.

Table No. 1: Pharmaceutical Composition of HLB system of Artemisinin (A-1)

S. No.	Lipids Excipients / Drug	Individual HLB value	% w/w

1.	Artemisinin	-	6.0
2	Beeswax	9	24.0
3	Lanolin	12	20.0
4	Paraffin	10	40.0
5	Cetyl Alcohol	15	10.0
	Final HLB Value	**10.0**	100.0

Preparation of the Lipoidal Formulation of Amodiaquine:

Procedure: Based on the above composition, beeswax was heated up to 40 °C until it melted, and added to it were lanolin, paraffin, and cetyl alcohol. When the mixture was translucent and warm, the drug was added to it and mixed vigorously in a motor and pestle for approximately 5 minutes until a cream was formed. The HLB value was calculated theoretically for the lipoidal formulation, which was found to be 10. This lipoidal formulation was nomenclated as batch no. A-2 (HLB~10). Refer to table no. 2.

Table No. 2: Pharmaceutical Composition of HLB system of Amodiaquine (A-2)

S. No.	Lipids Excipients / Drug	Individual HLB value	% w/w
1.	Amodiaquine	-	6.0
2	Beeswax	9	24.0
3	Lanolin	12	20.0
4	Paraffin	10	40.0
5	Cetyl Alcohol	15	10.0
	Final HLB Value	**10.0**	100.0

Preparation of Lipoidal Formulation of Halofantrine:

Procedure: Based on the above composition, beeswax was heated up to 40 °C until it melted, and added to it were lanolin, paraffin, and cetyl alcohol. When the mixture was translucent and warm, the drug was added to it and mixed vigorously in a motor and pestle for approximately 5 minutes until a cream was formed. The HLB value was calculated theoretically for the lipoidal formulation, which was found to be 10. This lipoidal formulation was nomenclated as batch no. A-3 (HLB~10). Refer to table no. 3.

Table No. 3: Pharmaceutical Composition of HLB system of Halofantrine (A-3)

S. No.	Lipids Excipients / Drug	Individual HLB value	% w/w
1.	Halofantrine	-	6.0
2	Beeswax	9	24.0
3	Lanolin	12	20.0
4	Paraffin	10	40.0
5	Cetyl Alcohol	15	10.0
	Final HLB Value	**10.0**	100.0

Preparation of Lipoidal Formulation of Chloroquine:

Procedure: Based on the above composition, Cremophor RH 40 was heated up to 35 °C until it melted, and added to it were MCT oil, liquid paraffin, and cetyl alcohol. When the mixture was translucent and warm, drugs were added to it and mixed vigorously in a mortar and pestle for approximately 5 minutes until an emulsion was formed. The HLB value was

calculated theoretically for the lipoidal formulation, which was found to be 10. This lipoidal formulation was nomenclated as batch no. A-4 (HLB~10). Refer to table no. 4.

Table No. 4: Pharmaceutical Composition of HLB system of Chloroquine (A-4)

S. No.	Lipids Excipients / Drug	Individual HLB value	% w/w
1.	Chloroquine	-	6.0
2	Cremophor RH 40	9	24.0
3	MCT oil	12	20.0
4	Liq. Paraffin	10	40.0
5	Cetyl Alcohol	15	10.0
	Final HLB Value	**10.0**	100.0

Determination of the percent drug release of different lipoidal formulations at different physiological pHs:

The model drug was weighed and filled manually in an empty hard gelatin capsule and inserted into an USP-approved basket.

The dissolution apparatus was set at the following parameters:

RPM: 100 rpm

Apparatus type: basket

Volume: 900 ml

Media: Dissolution studies were run using wider physiological buffer and biorelevant media, i.e., in the acidic region pH ~1, ~2, and ~4.5, in the neutral range pH~6.8, and in the alkaline range pH ~7.4, including simulated fasted-state gastric fluid, simulated fasted-state intestinal, simulated fed-state gastric fluid, simulated fed-state intestinal fluid, simulated lipolytic-digestive fluids/media, and purified water.

Total drug release time: 60 min.

Temperature: 37 °C.

As a control, the same drugs were also filed in empty hard gelatine capsules, and the percent drug release was also studied for comparison purposes, as depicted in table no. 5.

Table No. 5: Results of percent drug release of lipoidal formulations of Artemisinin, Halofantrine, Amodiaquine and Chloroquine.

S. No.	Formulations of Artemisinin	Hydrochloric acid (1/10th normality)	Hydrochloric acid (1/100th normality)	Buffer solution of pH ~4.5	Buffer solution of pH ~6.8	Buffer solution of pH ~7.4	Fa-SSGF	Fa-SSIF	Fe-SSGF	Fe-SSIF	Lipoidal	Purified Water
1	Artemisinin API 200mg in Hard Gelatin capsule.	2.7	2.6	2.5	2.6	2.5	11	14	22	27	27	ND
2	Amodiaquine API (100 mg) in Hard Gelatin capsule	1.1	2.1	2	1.9	2.1	9	11	20	21	21	ND
3	Halofantrine API (500 mg) in Hard gelatin capsule	0.2	0.5	0.5	0.8	0.8	0.8	0.8	0.8	0.9	2.2	ND

S. No.	Formulations of Artemisinin	Hydrochloric acid ($1/10^{th}$ normality)	Hydrochloric acid ($1/100^{th}$ normality)	Buffer solution of pH ~4.5	Buffer solution of pH ~6.8	Buffer solution of pH ~7.4	Fa-SSGF	Fa-SSIF	Fe-SSGF	Fe-SSIF	Lipoidal	Purified Water
4	Chloroquine API (500 mg) in Hard Gelatin capsule	0.5	0.7	0.5	1.1	1.1	1.1	1.1	1.1	1.1	2.2	ND
5	Lipid Formulation -A1	1.1	1.9	2.2	2.0	2.0	5.0	8.0	10	18	93	ND
6	Lipid Formulation -A2	1.1	1.9	2.2	2.0	2.0	5.0	6.0	5.0	11	90	ND
7	Lipid Formulation -A3	13	15	10	11	10	81	86	88	97	98	ND
8	Lipid Formulation -A4	21	19	10	11	10	85	88	89	97	98	ND

ND: not detected.

4. Results and Discussions

The above tabulated data depicts that the percent drug release of lipoidal formulation(s) depicts more drug release than control samples (which are non-lipoidal formulations). The drug release is profoundly seen more in biorelevant media, especially those that contain sodium taurocholate, which acts as a surfactant by solubilizing the drug in vitro and hence may be responsible for enhanced drug dissolution when compared to formulations (corresponding control samples) where there are no lipids. The lipids in the formulations are basically amphiphilic in nature, wherein their lipophilic moieties interact with the lipoidal moiety of the drug molecule, and the hydrophilic portions of the lipids interact with the hydrophilic portions of the drug, on the well-known principle of "*like dissolves like."*

The measured percent drug release of the lipoidal formulation of the antimalarial drugs indicates that such anti-malarial drugs, when administered orally, have a higher chance of getting absorbed more than conventional dosage formulations (i.e., non-lipoidal) because the lipids and drug in situ interact with each other to probably form a micelle wherein the drug is entrapped within the vicinity and surrounded by lipid polymeric chains. These micelles have a better probability of passing through the intestinal cells into the lymphatic system and circulatory systems owing to their bypass of first-pass metabolism (if any). However, the current research study only focuses on drawing meaningful strategies and conclusions; there has been no in-vivo study conducted to validate this hypothesis.

5. Conclusion

Based on the results obtained, it is evident that anti-malarial drugs like Artemisinin, Amodiaquine, Halofantrine, and Chloroquine exhibit higher drug release in vitro, especially in simulated fasted-state gastric fluids, simulated fasted-state intestinal fluids, simulated fed-state gastric fluids, simulated fed-state intestinal fluids, and simulated lipoidal-digestive fluids/media, when compared to non-lipoidal formulations of the same. This hints at the fact that such highly lipophilic drugs, when formulated in lipid-based delivery systems, have an opportunity to exhibit better absorption through the oral route when compared to conventional drug delivery dosages like tablets. However, more research, especially in vivo studies, is yet to be carried out in this direction.

References

[1] WHO`s World Malaria report. World Health Organization. 2021 Dec p. 322.
[2] Klayman DL. Qinghaosu (artemisinin): an antimalarial drug from China. Science. 1985 May 31;228(4703):1049-55.
[3] White NJ, Hien TT, Nosten FH. A Brief History of Qinghaosu. Trends Parasitol. 2015 Dec;31(12):607-610.
[4] Li J, Zhou B. Biological actions of artemisinin: insights from medicinal chemistry studies. Molecules. 2010 Mar 8;15(3):1378-97
[5] Winstanley P, Edwards G, Orme M, Breckenridge A. The disposition of amodiaquine in man after oral administration. Br J Clin Pharmacol. 1987 Jan;23(1):1-7.
[6] Karbwang J, Na Bangchang K. Clinical pharmacokinetics of halofantrine. Clin Pharmacokinet. 1994 Aug;27(2):104-19.
[7] Tulpule, A., Krishnaswamy, K. Effect of food on bioavailability of chloroquine. Eur J Clin Pharmacol. 1982 ;23 : 271–273.
[8] Rezhhdo et al. Journal of Controlled Release. 2016 ; 28. 544–560.
[9] Diakidou, A et. al. Simulation of gastric lipolysis and prediction of felodipine release from a matrix tablet in the fed stomach. Eu. J. Pharm. Sci. 2009 ; 37. 133-140.