Determination of Equilibrium Solubility of Anti-Malarial Drug(S) in Different Physiological Media

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Abstract

Malaria has been a highly fatal disease for many decades, especially in emerging countries. When we look back and see the drugs that have already been on the market to kill parasites, there are not many new drugs that have been recently introduced by the various pharmaceutical companies. The drugs that are on the market have already been around for quite some time and have their own pros and cons. Most of these drugs have been consistently exhibiting a common problem of poor oral bioavailability, which is moreover dependent on their poor aqueous solubility and/or first-pass effect. Hence, this research work was focused on the assessment of equilibrium solubilities of various anti-malarial drugs (already on the market), especially those drugs that bear a wide range of C log P values. These anti-malarial drugs sourced from different manufacturers (i.e., artemisinin, amodiaquine, halofantrine, and chloroquine) are highly lipophilic in nature and have the least affinity for polar solvents like water, because of which they are least soluble in aqueous media and solvents. The absorption of drugs through the oral route is due to their limited aqueous solubility, which, in the absorption process, becomes a rate-limiting step. The study evaluates the equilibrium solubilities of 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 study was conducted as per the shake flask method at 37 °C, and the results concluded that the drug(s) equilibrium solubility was observed to be relatively low in purified water when compared to other physiological pH media. The drug(s) exhibited the highest equilibrium solubility in the simulated GIT dissolution medium. The study indicated that such anti-malarial drugs, which bear higher C Log P values, also tend to solubilize or exhibit higher solubility in the presence of gastric, intestinal, and lipoidal media because the drug undergoes solubilization under the available surfactants present in the medium, e.g., sodium taurocholate and/or likewise. All model drugs exhibited a similar trend and solubilization effect, which indicated the potential of lipid-based drug delivery systems as one of the most preferred delivery systems.

Keywords: Low aqueous solubility, Equilibrium solubility, Physiological pH solubility profile, Biorelevant media, Lipoidal media, Antimalarial drugs.

1. Introduction

Malaria, caused by Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax, and/or Plasmodium knowlesi species, is a deadly disease that commonly affects humans in many countries in tropical and subtropical regions worldwide. Malaria continues to be the leading cause of morbidity and mortality worldwide. In fact, malaria has caused between 21 and 260 million cases and nearly 400,000 deaths per year over the past decade.

In 2020, about 94% of malaria cases were recorded in sub-Saharan Africa. Nigeria (about 27%), the Republic of the Congo (about 12%), Uganda (about 5%), Mozambique (about 4%), and Nigeria (about 3%) are responsible for approximately 51% of all malaria cases worldwide. Pregnant women and children under 5 years of age in these regions are responsible for approximately 85% of deaths[1].

In total, around more than 100 Plasmodium species exist, but among those, only five have been known to exhibit human infections, viz., P. vivax, P. ovale, P. knowlesi, and P. malariae, including the falciparum species. These infections demonstrate a vivid and dedicated drug response, cellular morphology, immune response, and pattern of their relapses, but they can be very well prevented and/or treated using fixed-dose combination therapies of artemisinin and other drugs [2, 3, 4]

Factors contributing to this disadvantage include poor quality of antimalarial drugs, particularly lack of bioavailability (poor water solubility, permeability, and/or un-stability towards GUT enzymes), and serious side effects that render patients ineffective. To solve all these problems, nanotechnology-based drug delivery systems have emerged as important therapeutic tools in malaria control. In fact, the benefits of nanotechnology as a drug delivery system include increasing efficacy, reducing drug toxicity, improving patient compliance, and overcoming the development of drug resistance. In addition, nano-drug delivery systems can provide cell adhesion sources and materials to bind certain ligands on their surfaces, leading to poor transport and/or selective drug delivery at target or other sites.

Although malaria is a curable disease that has not been completely eradicated because the parasites are becoming resistant to the different current artemisinin-based combination therapy drugs, these anti-malarial drugs, being highly oil-soluble in nature (indicative of their higher C log P values), exhibit limited oral absorption because solubility is their rate-limiting step in the absorption process. However, this poor absorption or low oral bioavailability can be attributed to their poor aqueous solubility too; hence, studying the equilibrium solubility of such drugs becomes highly important in order to draw meaningful conclusions and decide new lipid-based formulation strategies that can induce higher oral bioavailability if aqueous solubility is increased in simulated gastric, intestinal, and lipoidal media.

The aim of the current research was to review the comparative examination of equilibrium solubilities of Artemisinin, Amodiaquine, Halofantrine, and Chloroquine across a wider physiological pH range, ranging from the acidic side, like pH ~1.0, pH ~2.0, and pH ~4.5, to the neutral side, pH ~6.8, and the alkaline side, pH ~7.4, including the simulated gastric, intestinal, lipolytic digestive medium, and purified water, and also introduce different formulation strategies with the objective of increasing oral bioavailability if aqueous solubility is increased.

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 said research purpose.

Determination of equilibrium solubility of anti-malarial drugs:

Sample preparation (for each drug): The test drug suspensions (i.e., for each drug) were prepared by adding excess solids to the medium, which is in a stoppered 250-ml conical flask containing 50 ml of medium.

The sample preparation was carried out in triplicate to provide at least three solubility results for each test solution. In order to facilitate the dissolution of the solids, the suspension was actively mixed under continuous stirring by placing the conical flask under a mechanical stirrer set at 1000 rpm. Initially, stirring was continued until 24 hours. The temperature of the suspension was well controlled during this dissolution phase (37℃ ± 0.5°). The maintenance of temperature throughout the experimentation was achieved by placing the conical flask over a highly precise thermostat-controlled hot water bath.

After 24 hours, the drug suspension was filtered using a suitable sintered flask assembly, and the filtrate was transferred into a glass test tube labeled accordingly. The transfer pipet was also pretreated with a sample solution before use.

End point determination for saturation (equilibrium):

Samples were tested using a suitable analytical method, and the aliquots were tested until the last two samples showed a change of less than 5% over 24 hours, or less than 0.2%/h. The above procedure was followed for each of the antimalarial drug(s) and for each of the mediums enlisted below.

i. On the acidic pH side: pH ~1.0, ~2.0 prepared by hydrochloric acid, of $1/10^{th}$ and $1/100^{th}$
ii. Sodium acetate buffer solution of pH ~4.5 and potassium phosphate buffer solution of pH ~6.8 and pH ~7.4, as per USNF Chapter <22-26>
iii. Fasted simulated gastric fluid media as per USNF Chapters 22–26.
iv. Fed simulated gastric fluid media as per USNF Chapters 22–26.
v. Fasted simulated intestinal fluid media as per USNF Chapters 22–26
vi. Fed simulated intestinal fluid media as per USNF Chapters 22–26.
vii. Simulated intragastric lipolytic digestive or lipoidal media
viii. Purified water.

3. Preparation of different physiological buffers and biorelevant mediums for solubility assessment.

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 added approximately 800 ml of deionized or purified water. 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 added 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 labeled 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 were measured using a precision weighing balance, and the salt(s) were added 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 labeled 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 slat; 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[5]

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.

4. Results and Discussions

The measured equilibrium solubility of artemisinin, amodiaquine, halofantrine, and chloroquine in different physiological media is mentioned in the table below. 1, 2, 3, and 4, respectively. Refer to figure no(s). 1, 2, 3, and 4 also.

Table No. 1: Equilibrium solubility of Artemisinin in different media

S. No.	Different Physiological buffer / solution	Solubility (mg/ml)
1	Hydrochloric acid solution, of $1/10^{th}$ normality	0.002
2	Hydrochloric acid solution, of $1/100^{th}$ normality	0.003
3	Buffer solution of sodium acetate, adjusted to pH ~4.5	0.002
4	Buffer solution of pot. phosphate, adjusted to pH ~6.8	0.002
5	Buffer solution of pot. phosphate, adjusted to pH ~7.4	0.003
6	Simulated fasted-state gastric fluid medium	0.12
7	Simulated fed-state gastric fluid medium	0.13
8	Simulated fasted-state intestinal fluid medium	0.15
9	Simulated fed-state intestinal fluid medium	0.15

S. No.	Different Physiological buffer / solution	Solubility (mg/ml)
10	Simulated digestive intra-gastric lipolytic media	0.14
11	Purified water	0.002

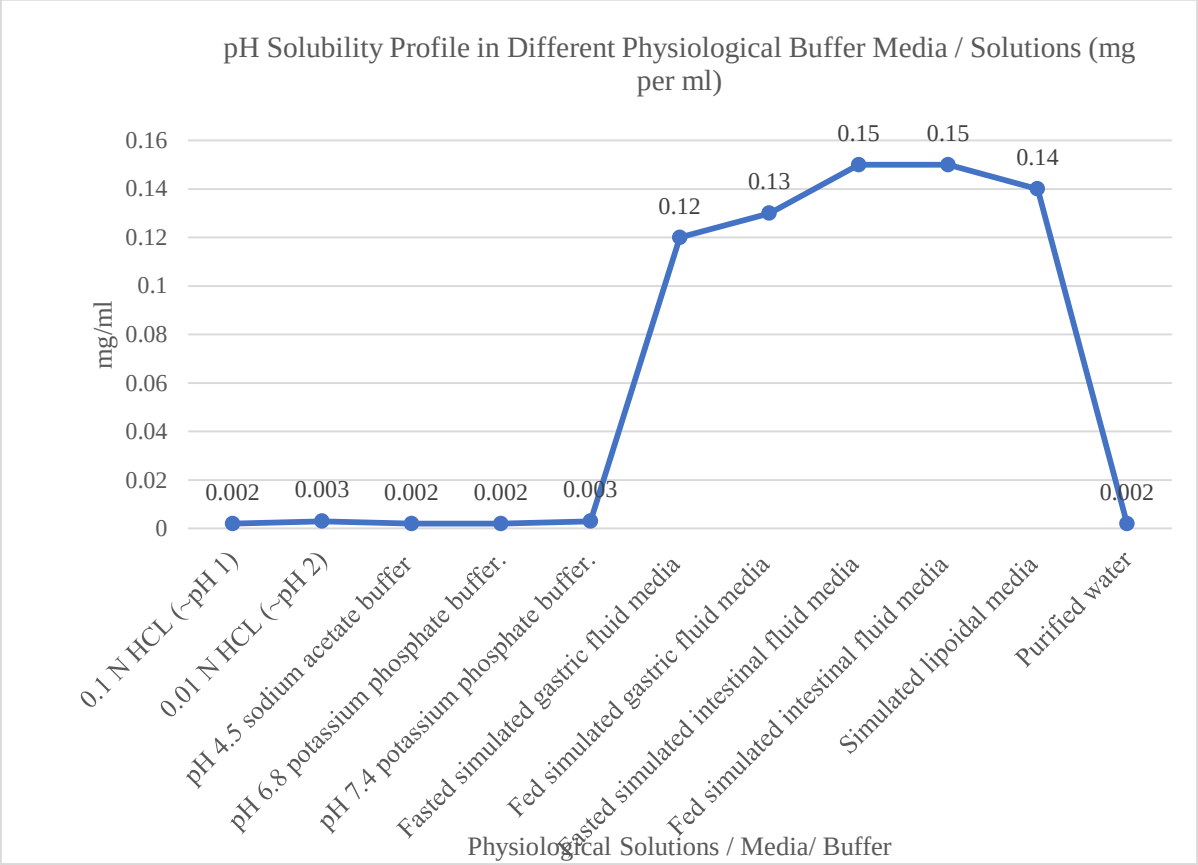

Fig. No. 1: Graphical representation of equilibrium solubility of Artemisinin in different media

Table No. 2: Equilibrium solubility of Amodiaquine in different media

S. No.	Different Physiological buffer / solution	Solubility (mg/ml)
1	Hydrochloric acid solution, of 1/10th normality	0.020
2	Hydrochloric acid solution, of 1/100th normality	0.017
3	Buffer solution of sodium acetate, adjusted to pH ~4.5	0.024
4	Buffer solution of pot. phosphate, adjusted to pH ~6.8	0.020
5	Buffer solution of pot. phosphate, adjusted to pH ~7.4	0.023
6	Simulated fasted-state gastric fluid medium	0.125
7	Simulated fed-state gastric fluid medium	0.138
8	Simulated fasted-state intestinal fluid medium	0.156
9	Simulated fed-state intestinal fluid medium	0.176
10	Simulated digestive intra-gastric lipolytic media	0.171

S. No.	Different Physiological buffer / solution	Solubility (mg/ml)
11	Purified water	0.004

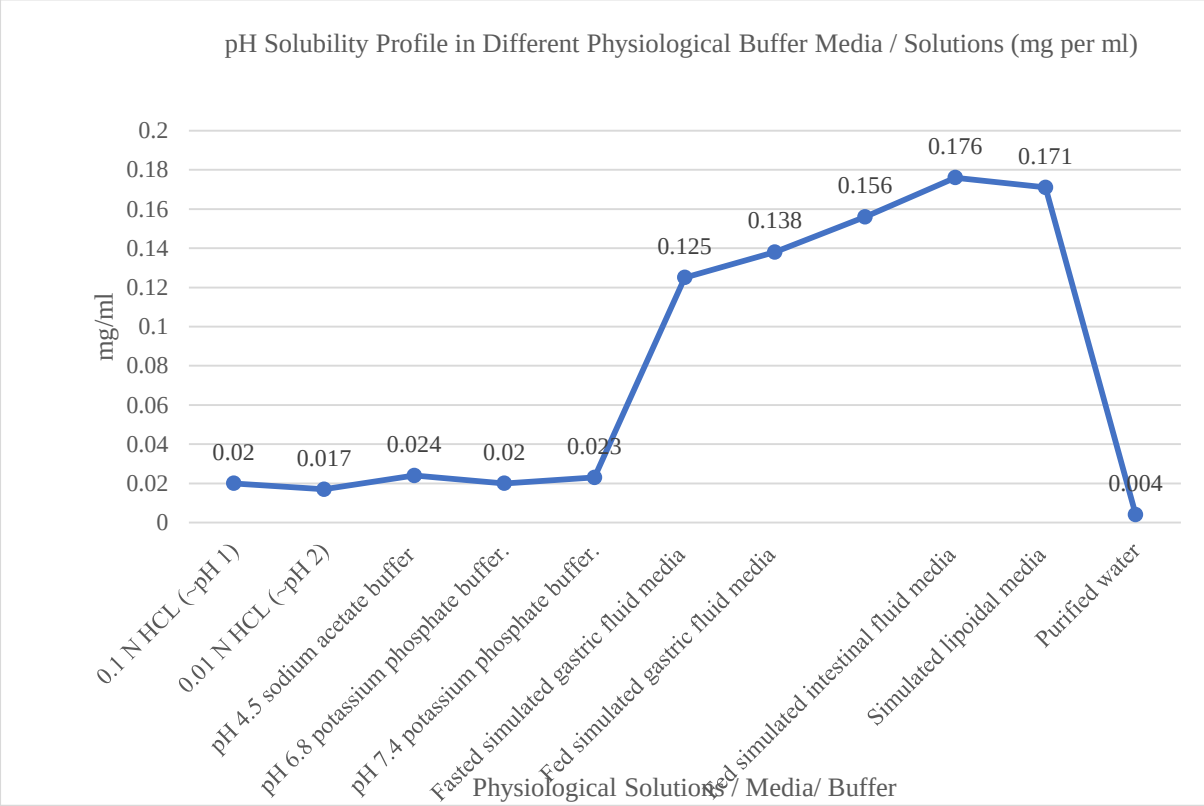

Fig. No. 2: Graphical representation of equilibrium solubility of Amodiaquine in different media

Table No. 3: Equilibrium solubility of Halofantrine in different media

S. No.	Different Physiological buffer / solution	Solubility (mg/ml)
1	Hydrochloric acid solution, of 1/10th normality	0.004
2	Hydrochloric acid solution, of 1/100th normality	0.004
3	Buffer solution of sodium acetate, adjusted to pH ~4.5	0.006
4	Buffer solution of pot. phosphate, adjusted to pH ~6.8	0.005
5	Buffer solution of pot. phosphate, adjusted to pH ~7.4	0.005
6	Simulated fasted-state gastric fluid medium	0.007
7	Simulated fed-state gastric fluid medium	0.008
8	Simulated fasted-state intestinal fluid medium	0.005
9	Simulated fed-state intestinal fluid medium	0.007
10	Simulated digestive intra-gastric lipolytic media	0.006
11	Purified water	0.006

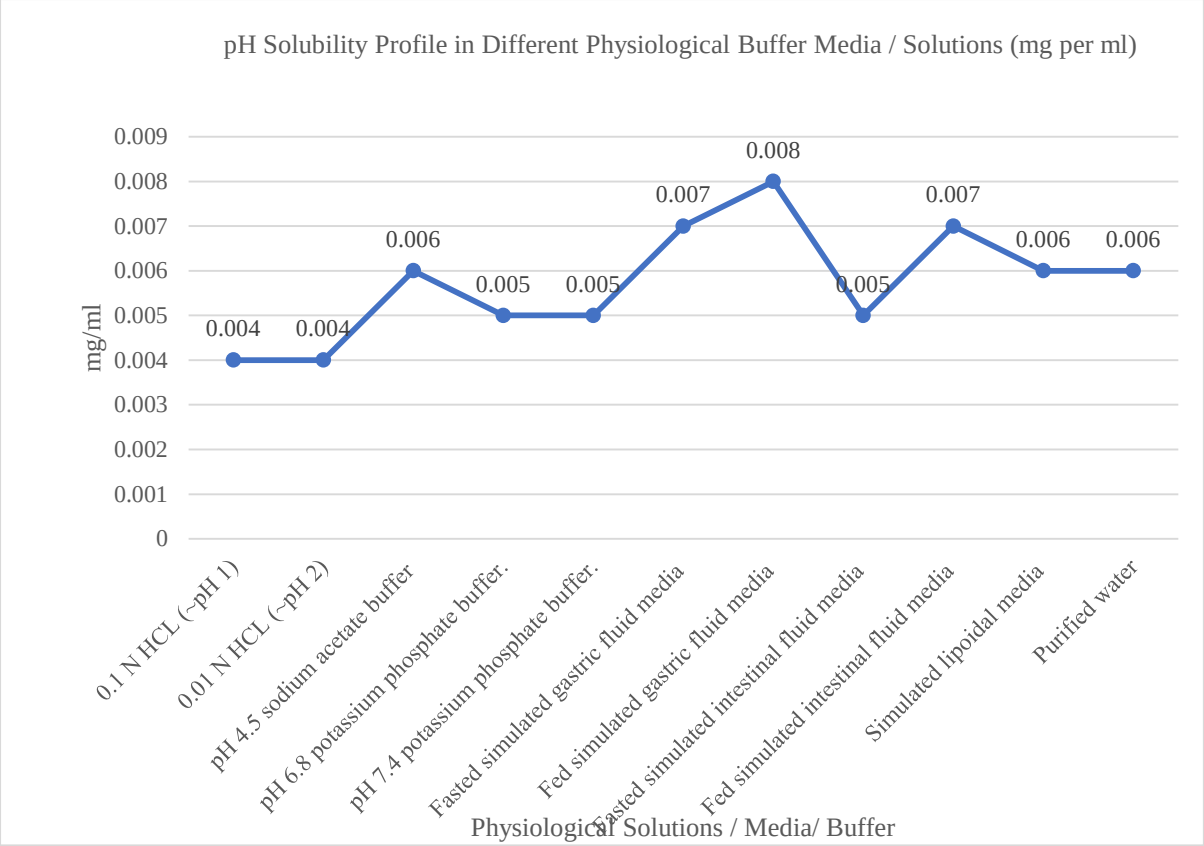

Fig. No. 3: Graphical representation of equilibrium solubility of Halofantrine in different media

Table No. 4: Equilibrium solubility of Chloroquine in different media

S. No.	Different Physiological buffer / solution	Solubility (mg/ml)
1	Hydrochloric acid solution, of 1/10th normality	0.006
2	Hydrochloric acid solution, of 1/100th normality	0.005
3	Buffer solution of sodium acetate, adjusted to pH ~4.5	0.007
4	Buffer solution of pot. phosphate, adjusted to pH ~6.8	0.006
5	Buffer solution of pot. phosphate, adjusted to pH ~7.4	0.007
6	Simulated fasted-state gastric fluid medium	0.123
7	Simulated fed-state gastric fluid medium	0.221
8	Simulated fasted-state intestinal fluid medium	0.201
9	Simulated fed-state intestinal fluid medium	0.208
10	Simulated digestive intra-gastric lipolytic media	0.254
11	Purified water	0.006

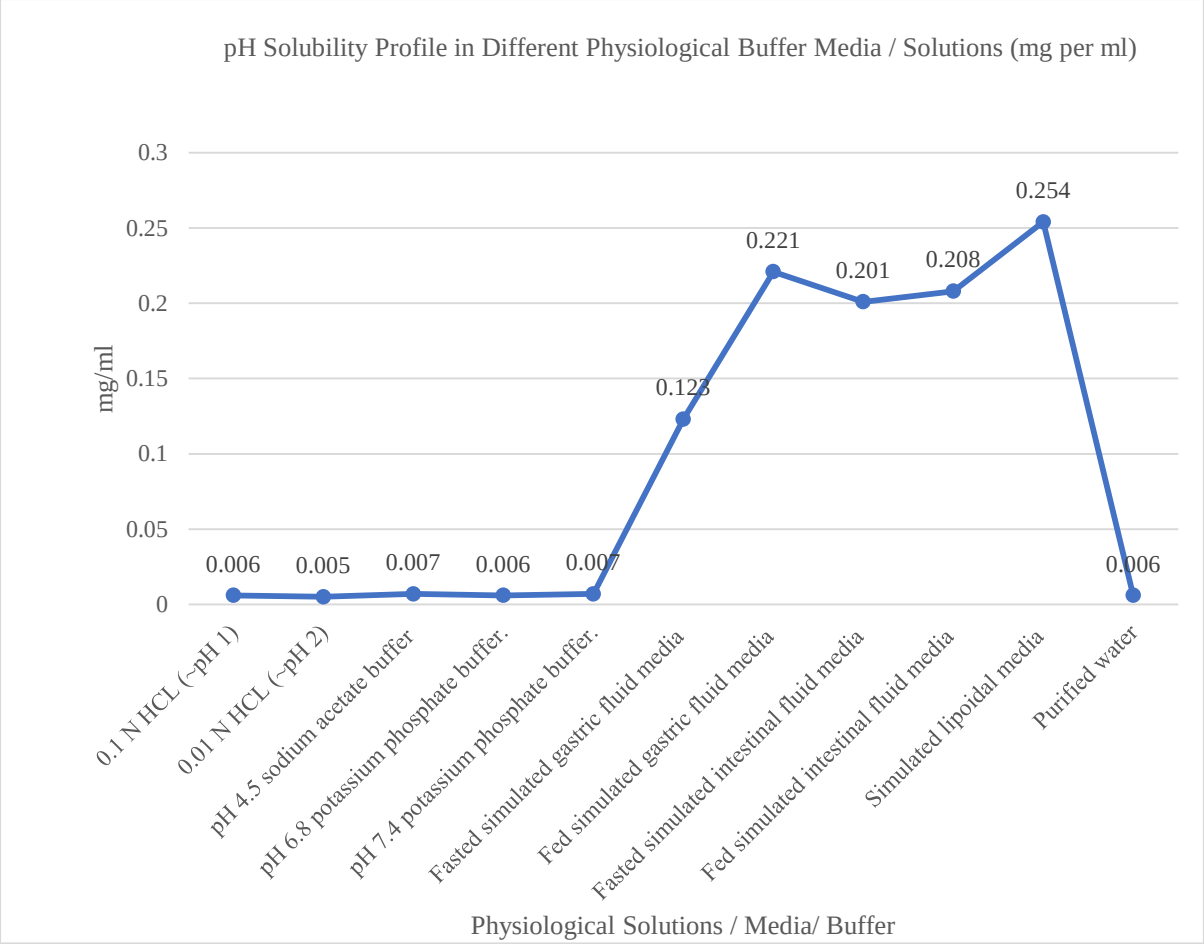

Fig. No. 4: Graphical representation of equilibrium solubility of Chloroquine in different media

The measured equilibrium solubility of the antimalarial drugs exhibited limited solubility in most of the different physiological media, which is indicated by their low solubility values. The solubility was found to be increased, especially in simulated media like fasted and fed gastric and intestinal media, including lipolytic media. The reason attributable to this enhanced solubility was the presence of ingredients like sodium taurocholate and/or lecithin, which act as surface active agents and aid in solubilizing the drugs, which are lipophilic in nature. These lipophilic anti-malarial drugs have the tendency to solubilize in simulated media in the presence of sodium taurocholate and lecithin in order to demonstrate enhanced solubility when compared to purified water.

Ingredients like pepsin and lipase, as part of a few simulated mediums, also aided in the solubilization effect of such lipophilic molecules.

5. Conclusions

Based on the results obtained, it is evident that anti-malarial drugs like Artemisinin, Amodiaquine, Halofantrine, and Chloroquine exhibited higher solubilities, especially in simulated fed-state, fasted-state biorelevant, and lipoidal digestive media, when compared to purified water. This also 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 is yet to be carried out in this direction.

References

[1] Gelband H, Bogoch II, Rodriguez PS, Ngai M, Peer N, Watson LK, et al. Is Malaria an Important Cause of Death among Adults? The American Journal of Tropical Medicine and Hygiene. 2020 Apr 20 ;103(1):41–7.
[2] World malaria report. World Health Organization; 2021 Dec p. 40.
[3] Tuteja R. Malaria- an overview. FEBS Journal. 2007 Aug 30;274(18):4670–9.
[4] Singh B, Daneshvar C. Human Infections and Detection of Plasmodium knowlesi. Clinical Microbiology Reviews. 2013 Apr 1;26(2):165–84.
[5] Diakidou A, Vertzoni M, Bertil Abrahamsson, Dressman JB. Simulation of gastric lipolysis and prediction of felodipine release from a matrix tablet in the fed stomach. European Journal of Pharmaceutical Sciences. 2009 May 1;37(2):133–40.