RESEARCH ARTICLE

Physico-chemical Characterisation of Self-Emulsifying Microemulsion of Antimalarial

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ABSTRACT

Emulsions are employed as drug carriers in pharmaceutical formulations, particularly because they can increase the oral bioavailability of poorly absorbed medications.[1] One of the most often used techniques to improve the oral bioavailability of medications, particularly anti-malarial ones, is the use of lipid-based drug delivery. The current research study focuses on using the lipid-based formulation strategy, i.e., self-emulsifying micro emulsions to formulate such anti-malarial drugs and characterise them to study their stability so that the possibility of bioavailability can be studied more. The current research emphasizes on characterisation techniques like transmission electron microscopy (TEM), turbidity index (%TI) and globule size determination as simpler and industrially implacable techniques to study the stability of the self-emulsifying properties of such formulations. In-order to obtain stable formulations with self-emulsifying behaviour the most important factors like the concentrations of surfactants and co-surfactants were one of the variables which were varied in-order to observe their effect on the overall stability of the microemulsions. This research is a step towards the usage of self-emulsifying microemulsions of anti-malarial drugs, for enhancement of oral bioavailability and encourages other researches to use this formulation strategy to improve bioavailability.

Keywords: Emulsions, Antimalarial, *Plasmodium.*

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INTRODUCTION

Especially for Biopharmaceutical Classification System (BCS) II and IV compounds, self-emulsifying micro emulsions are often used as a verbal medication formulation approach to overcome the oral bioavailability issue. Studying an appropriate drug delivery system that can address the two main causes of poor oral bioavailability—poor aqueous solubility and poor permeability across intestinal lumen, and pre-systemic metabolism becomes crucial because BCS class II and IV molecules struggle with their poor solubilises, and many of them also exhibit low oral bioavailability. The two main causes of the low oral bioavailability are these considerations. The study done by White NJ *et al*.,[1] in their research work mentions the pharmacokinetics of few anti-malarial drugs which face bioavailability issues due to their poor solubility in water and being highly lipophilic also.

Malaria, caused by *Plasmodium* genus, is one of the deadliest diseases in the world. In sub-Saharan Africa, nearly ~77% of these deaths occur in children, under 5 years of age. On average, a child dies from malaria every ~60 seconds, and survivors face the risk of long-term mental and cognitive impairment.[2] Nowadays, antibiotics such as 4-aminoquinoline, arylaminoalcohol, 8-aminoquinoline, artemisinin, antifolates, respiratory chain inhibitors and antibiotics are been consumed by the infected subjects. However, such drugs also face many limitations like : short half-life, poor absorption, and poor oral health, which may render the treatment ineffective.

Despite the availability of these drugs, malaria has not been eliminated, partly due to their limitations. Frequent drugs also exhibit side effects and toxicity which may cause patients to withdraw the therapy, hence lipid-based drug delivery systems using self-emulsifying microemulsions can enhance the therapeutic properties of such drugs and improve their bioavailability by increasing their solubility. Such delivery systems have an extra edge to remain in highly reduce size *in-situ* and get absorbed *via* lymphatic route of transportation.

While such drug delivery systems, i.e., self-emulsifying micro emulsions are having distinct advantages of being highly thermodynamically stable, they on the same hand also have a distinct way of formulating using the most appropriate surfactants and cosurfactants in right proportions. It is highly essential that such dispersions either O/W or W/O type should retain

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Their globule size in-situ and do not coalesce after they are been diluted in the intestinal juices of the GUT.

Additionally, studies have shown that self-emulsifying drug delivery systems, or SEDDSs, can successfully enhance drug delivery *via* a range of routes, such as the vaginal, rectal, ophthalmic, nasal, and topical/transdermal routes of administration.[3] The following advantageous methods of lipophilic compounds absorption take place once a molecule is solubilized: increased intracellular drug concentration and residence time by surfactants, which results from the inhibition of P-glycoprotein and/or cytochrome (CYP) P 450 enzymes; lipid stimulation of lipoprotein/chylomicron production for lymphatic uptake; improved membrane fluidity, which facilitates drug absorption transcellularly; opening tight junctions, which facilitates drug transport paracellularly.[4]

Zhu *et al.* microemulsion based formulations are excellent carriers of drug substances and being the most ideal candidates for increase in oral bioavailability.[5] Apart of this advantage these formulations are also advantageous inview to impart stability of oral formulations.

According to the recent year studies conducted by Ujhelyi *et al.* in their work described that the globule size (which is a dispersed phase of O/W emulsions) is primary the main criteria to nomenclate such pharmaceutical emulsions like if the globule size is in sub-micron range, they are called as "self-emulsifying micro-emulsions" and if the size of the globule are in nanometre range then they are termed as "self-emulsifying nano-emulsions".[6] In one more study, conducted by Tran and Park *et al.* in 2021, it was studied that microemulsions when agitated at very low stirring and diluted with large volumes of water the system remains as transparent micro emulsions indicative of stable system.[7] Page & Szepes *et al.* in their research study discussed that there are various limitations in GUT like the acidic pH of stomach and the peristaltic movements which play vital role in providing the necessary energy to the micro emulsion for being in self-emulsifying state and thermodynamically stable.[8] Another research done by Mahmood and Bernkop *et al.* in the year 2019, explained that among micro and nano-emulsions, it was more convenient to industrially scale-up micro emulsions when compared to nano-emulsions, liposomes, carbon-nanotubes and micellar-dispersion systems because preparing microemulsions are just like making simple solutions from industrial scalability point of view.[9] In a study by De Oliveira MC *et al.* described that macro molecules like peptidyl drugs, proteins and oligosaccharide-based medicines have also been researched to dissolve in appropriate dispersed phase of emulsions and formulate them finally into micro and/or nano emulsions for target drug delivery via lymphatic route.[10] In one of research conducted by Lyons *et al.* who prepared O/W and W/O emulsion of a GMDP peptidyl macromolecule when they administered in the duodenum exhibited tremendous increase in the oral bioavailability.[11] Another study carried out by Fan Y *et al.* described in their research work that W/O microemulsions of calcitonin had the unique characteristics of withstanding the vigorous acidic conditions of the GIT and prevented the degradation and finally leading to increase in bioavailability.[12] These different studies and research indicated that micro-emulsions have the unique characteristics of protecting the encapsulated drug in the globules from different oxidative and pH dependant further degradation in-situ as mentioned by Sarciaux J *et al.* [13]

Therefore, microemulsions have been well known in the literature for having a distinct advantage of protective property and increase in absorption through lymphatic circulatory system as mentioned by Talegaonkar S *et al.*[14]

In another very recent findings, conducted by Holm *et al* concluded that the well-known drug Halofantrine when formulated into emulsions using long chain triglycerides exhibited higher bioavailability in dogs.[15] Rao *et al.* investigated and elucidated in their paper the administration of lipophilic and hydrophilic drugs as drug carriers due to their enhanced bioavailability, extended shelf life, ease of preparation, and better drug solubilization capability.[16] Artemisinin an-another natural anti-malarial chemical, used as a chemotherapeutic in medicine, which is known for its poor solubility, stability, and bioavailability. To solve these problems and increase the drug's effectiveness, nanotechnology was used. Asgharkhani E *et al.* study aimed, to ascertain the effects of several non-ionic surfactants on the physicochemical properties of artemisinin niosomes.[17]

Anti-malarial drug(s) are ideal candidates to be chosen for enhancement of bioavailability because they have been well known from decades and hence twelve formulations were prepared inview to characterise them for their self-emulsifying properties. Below (Table 1) is the list of 12 formulations of anti-malarial drugs.

MATERIAL AND METHODS

Artemisinin was purchased from M/s Rhyme Organics, Hyderabad. Amodiaquine was purchased from M/s. Merix Labs, Hyderabad and Halofantrine was procured by Sigma Aldrich, Hyderabad. Chloroquine was supplied by M/s. Dev-Life Corporations, Mumbai, Maharashtra.

Characterisation of self-emulsifying emulsions

Turbidity test

Turbidity is a measurable tool, that can be used to estimate the extent and duration of self-emulsification. Turbidity was measured using a turbidity meter after adding 250 to 5 mL of each emulsion as mentioned in Table 1 at ambient temperature with continuous stirring at 50 rpm on a magnetic stirrer. 450 nm was the wavelength at which the turbidity absorbance reading was noted as mentioned in Table 2. Each emulsion after dilution was subjected to ambient conditions for 7 and 14 days.

Particle size determination using TEM

'One of the biggest problems, in the study of microemulsions is the determination of the spatial structure of micelles, and information on the subject is currently very scarce. In this research work, various preparations for transmission electron microscopy (TEM) analysis of microemulsions of antimalarial were examined.

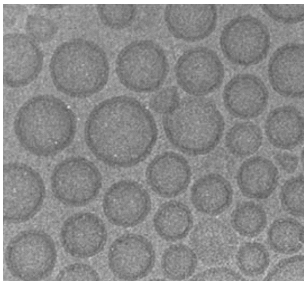
Table 1: Anti-malarial formulations (emulsions)

S. No.	*Drug in emulsions*	*Type of emulsion*	*Surfactant and cosurfactant ratio in each emulsion*	*Labeled*
1	Artemisinin	O/W	3:1	A3
2		O/W	2:1	A2
3		O/W	1:1	A1
4	Halofantrine	O/W	3:1	H3
5		O/W	2:1	H2
6		O/W	1:1	H1
7	Amodiaquine	O/W	3:1	M3
8		O/W	2:1	M2
9		O/W	1:1	M1
10	Chloroquine	O/W	3:1	C3
11		O/W	2:1	C2
12		O/W	1:1	C1

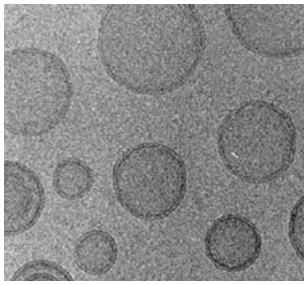
Table 2: Turbidity index and physical appearance of emulsions

S. No.	*Emulsion*	*At initial*		*At 7 days ambient temperature*		*At 14 days ambient temp*	
		Turbidity index%	*Appearance*	*Turbidity index%*	*Appearance*	*Turbidity index%*	*Appearance*
1	A3	-0.21	Clear	-0.20	Clear	-0.19	Clear
2	A2	-0.27	Clear	-0.25	Clear	-0.21	Cloudiness
3	A1	-0.26	Clear	-0.21	Cloudiness	-0.21	Cloudiness
4	H3	-0.21	Clear	-0.20	Clear	-0.19	Clear
5	H2	-0.27	Clear	-0.25	Clear	-0.21	Cloudiness
6	H1	-0.26	Clear	-0.21	Cloudiness	-0.21	Cloudiness
7	M3	0.09	Clear	0.11	Clear	0.10	Clear
8	M2	0.08	Clear	0.11	Clear	0.45	Cloudiness
9	M1	0.11	Clear	0.33	Cloudiness	0.41	Cloudiness
10	C3	-0.08	Clear	-0.09	Clear	-0.09	Clear
11	C2	0.10	Clear	0.11	Clear	0.32	Cloudiness
12	C1	0.09	Clear	0.10	Cloudiness	0.31	Cloudiness

Note: The negative value above is indicative of lower or very slight turbidity, when compared to distilled water.

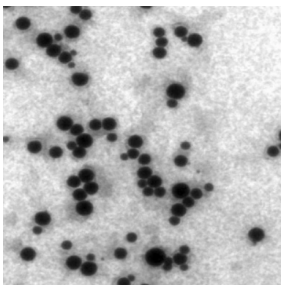


At initial at ambient RT In TEM scale bar 40 nm

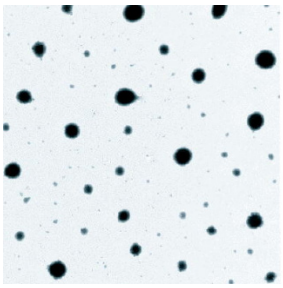


At 14 days at ambient RT In TEM scale bar 20 nm

Figure 1: TEM images of clear formulations of A3 (Artemisinin Surfactant to Co-surfactant ratio of 3:1)



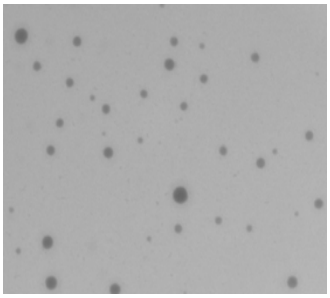
At Initial at ambient RT In TEM scale bar 160 nm



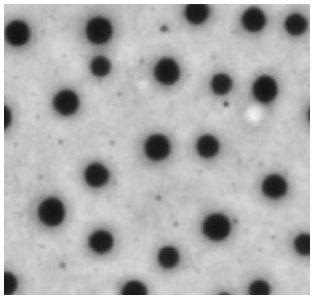
At 14 days at ambient RT In TEM scale bar 210 nm

Figure 2: TEM images of clear formulations of H3 (Halofantrine Surfactant to Co-surfactant ratio of 3:1)

Note: The scales were adjusted with an objective that the oil globules are seen visibly and remain distinct and untouched with adjacent globules so that the inference can be drawn with respect to whether there is any coalescence been triggered at 14 days or not. Instruments make: TEM ; FEI Tecnai G2 S-Twin, 200kV; Gatan Imaging Filter (GIF) Quantum SE 963 fitted with a 2k x 2k CCD camera. The images are modulated with respect to brightness and contrast so that they remain visible and clear after publication prints.

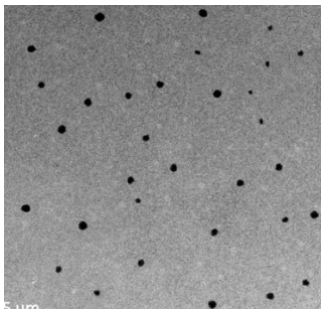


At Initial at ambient RT In TEM scale bar 135 nm

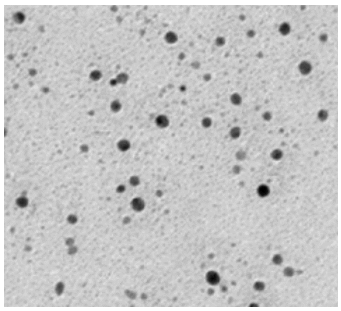


At 14 days at ambient RT In TEM scale bar 80 nm

Figure 3 : TEM images of clear formulations of M3 (Amodiaquine Surfactant to Co-surfactant ratio of 3:1)



At Initial at ambient RT In TEM scale bar 155 nm



At 14 days at ambient RT In TEM scale bar 90 nm

Figure 4: TEM images of clear formulations of C3 (Chloroquine Surfactant to Co-surfactant ratio of 3:1)

Note: The scales were adjusted with an objective that the oil globules are seen visibly and remain distinct and untouched with adjacent globules so that the inference can be drawn with respect to whether there is any coalescence been triggered at 14 days or not. Instruments make: TEM ; FEI Tecnai G2 S-Twin, 200kV; Gatan Imaging Filter (GIF) Quantum SE 963 fitted with a 2k x 2k camera of CCD

Table 3: Determination of oil globule(s) size of emulsions (O/W type) using TEM

	Emulsion	*Average Globule Size (nm) ± %RSD*	
		At initial	*At 14 days*
1	A3	187 nm ± 2.1	176 nm ± 1.1
2	H3	123 nm ± 0.9	119 nm ± 0.3
3	M3	196 nm ± 1.7	210 nm ± 1.2
4	C3	143 nm ± 1.2	188 nm ± 0.6

The formulations prepared and mentioned in Table 1, were subjected to TEM analysis, observations captured in Figures 1-4, were subject to globule size determination via Transmission electron microscopic photograph of the selected microemulsions formed which were found to be visibly clear at 14 days storage time point. The overall idea of selecting the 14 days' time point was to shortlist and evaluate only those formulations which are intended to be self-emulsifying and stable at longer stress conditions. Table 3 captures TEM images of only clear formulations

Table 4: Detection of coalescence of oil globule(s) in TEM photographs

	Emulsion	*Detection of coalescence of globules under TEM*	
		At initial	*At 14 days*
1	A3	All globules were distinctly separated and no coalescence phenomenon seen	All globules were distinctly separated and no coalescence phenomenon seen
2	H3	All globules were distinctly separated and no coalescence phenomenon seen	All globules were distinctly separated and no coalescence phenomenon seen
3	M3	All globules were distinctly separated and no coalescence phenomenon seen	All globules were distinctly separated and no coalescence phenomenon seen
4	C3	All globules were distinctly separated and no coalescence phenomenon seen	All globules were distinctly separated and no coalescence phenomenon seen

The globule size determined for lead emulsions, are captured herewith in Table 3.

The observation for determination of any coalescence of oil globule(s) under TEM photographs are captured in Table 4.

RESULTS AND DISCUSSIONS

The emulsions with higher concentration ratio of surfactants and co-surfactants were the most ideal self-emulsifying microemulsions demonstrated less turbidity and were observed to be extremely clear even after 14 days of storage at ambient conditions. However, emulsions wherein the surfactant and cosurfactants were in ratio of 2:1 and 1:1 were even-though very clear but displayed slight turbidity and struggled to remain clear, after 14 days of storage. This phenomenon was observed because of the action of combinations of surfactants and co-surfactants at the interface of oil globules being in dispersed phase and water being in dispersion phase. Concentrations of 3:1 were found to be proven more stable when diluted by addition of 250 mL of water into 5 mL of each self-emulsifying microemulsions of the four antimalarial drugs. The TEM photographs of the diluted microemulsions displayed distinct oil globules which were well separated with each other and there were no signs of coalescence even after 14 days of storage formulation samples especially for all four formulations wherein surfactant to cosurfactant ratio were 3:1. Hence, basis the TEM photographs, and globule size of all four formulations, it was evident that the micro-emulsions were of sub-micron size in nature and found to be stable.

Aarti M *et al*. researched on a lipid decreasing drug, i.e., finofibrate and prepared different liquid and solid self-emulsifying formulations and tested the globule size through means of scanning electron microscopy (SEM) and studied the particle surface for its sphericity and/rod like structure and surface smoothness. In her work she had explained the versatility and utility of the techniques which can also be used for studying globule size.[18]

Similarly, Chatap V *et al*. also extracted a unique gum from the endosperm of Sesbania grandiflora and utilised the same to prepare and/or formulate different dosages to characterise the same. They also observed significant outcomings after running the SEM technique, wherein they specifically studied the slippery textured granules.[19]

CONCLUSION

Basis the above results obtained, it was clear that the four antimalarial drugs, i.e., artemisinin, halofantrine, amodiaquine and chloroquine which basically are well known in prior art for their limited oral bioavailability if formulated into self-emulsifying micro emulsions (with appropriate concentrations of surfactant to co-surfactant) have high probability to absorb through lymphatic circulation and in-turn exhibit higher bioavailability too.

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