



Studies on *In-Vitro* Interaction of Artemether-Lumefantrine with Food Components

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ABSTRACT

This work evaluates *in vitro* interaction of food components with artemether-lumefantrine (AL) in tablet. Pelletized food components (*i.e.*, starch, albumin, sunflower oil and carbonated drink) with AL tablet were observed using Fourier transform infrared (FTIR) spectroscopy while spectra obtained were compared using essential FTIR (eFTIR) software. The influence (*i.e.*, instantaneous change in pH (IPH) and acid buffering capacity (ABC)) of food components on Fasted State Simulated Gastric Fluid (FaSSGF) and Fed State Simulated Intestinal Fluid (FeSSIF) were assessed alongside dissolution kinetics of actives in AL tablets. Artemether and lumefantrine produced identical spectra features with reference infra red (IR) spectra. No change in spectra features of AL on carbohydrate, vitamin A and oil treatment but noted with albumin and the carbonated drinks. IPH values for albumin revealed statistical higher value ($p < 0.05$) than other food components in FaSSGF. ABC for albumin treatment was statistically lower ($p < 0.05$) than for other food components in FaSSGF. Artemether overall release was significantly reduced by starch, lactose and carbonated drink ($p < 0.05$) while that of lumefantrine was significantly increased by sunflower oil ($p < 0.05$). There was interaction between AL and albumin, or carbonated drinks which can be of importance in drug treatment outcome.

Key Words: Artemether-lumefantrine; Food component; *In vitro* interaction; Dissolution kinetics; Instantaneous pH change, Acid buffering capacity

INTRODUCTION

The oral route is the most convenient for drug administration when treating uncomplicated *Plasmodium falciparum* malaria in the tropics. Consequently, a majority of World Health Organization (WHO) recommended drugs are presented as solid formulations for oral administration (Katzung, 2009). Artemether-lumefantrine (AL) is a co-formulated artemisinin combination therapy (ACT) available for paediatric and adult use and widely prescribed across sub-Saharan Africa (Katzung, 2009).

Factor that are critical to the kinetics of dissolution as presented by the Nernst Brunner and Levieh modifications of the Noyes-Whitney model include physiochemical properties of the dissolving compound (*i.e.*, pKa, solubility, crystalline energy, specific surface area) and certain prevailing conditions of the gastrointestinal (GI) tract) (Kostewicz *et*

al, 2002). Physiological parameters that play an important role include pH, surface tension and volume of the GI luminal fluid, solubilization and buffer capacity of substances present in the GI tract (Dressman *et al*, 2001). These parameters change following ingestion of food (Schmidt and Dalhoff, 2002). Amphiphilic bile components (*i.e.*, bile salts and lecithin) may increase in concentration following a meal (Martinez and Amidon, 2002). Biliary secretion has been demonstrated to increase the *in vivo* dissolution rate for some poorly soluble compounds. In order to develop a predictive *in vitro* model to forecast the *in vivo* performance of drugs, the Fasted State Simulated Gastric Fluid (FaSSGF), Fed State Simulated Gastric Fluid (FeSSGF), Fasted State Simulated Intestinal Fluid (FeSSIF) and Fed State Simulated Intestinal Fluid (FeSSIF) were proposed and are now widely employed (Vertzoni *et al*, 2005). FeSSIF-V2 (version 2) was further advanced following its close compositional resemblance to intestinal fluid (Jantratid *et al*, 2008).

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In the present study, the aim was to assess *in vitro* interaction of food components with AL tablet.

MATERIALS AND METHODS

Materials

AL tablet (Coartem®) was purchased in Uyo, Southern Nigeria. The food components were similarly purchased in Lagos, Nigeria. FaSSIF/FeSSIF/FaSSGF and FeSSIF-V2 powder were products of biorelevant.com, UK. Details of the drug and food components are presented in Table 1. Acetonitrile, tetrahydrofuran (THF) and potassium dihydrogen phosphate were of HPLC grade, products of Sigma Aldrich, Germany. All other reagents were of analytical grade.

Methods

Preparation of standard solution of internal standard, artemether and lumefantrine

Ten milligram of halofantrine was transferred into a 10 mL volumetric flask containing 6.0 mL of methanol to dissolve. The powder was dissolved and thereafter made up to mark to produce stock concentration of 1000 µg/mL. Reference standard powder of artemether 20 mg and lumefantrine 120 mg were weighed accurately into different flasks. Artemether and lumefantrine powder were dissolved to produce stock solutions of 2 mg/mL and 12 mg/mL, respectively.

Preparation of mixed standard solutions of AL and (IS)

Equal volumes (2 mL) of artemether and lumefantrine stock solutions were dispensed into 5 mL sample bottles to produce 1 and 6 mg/mL of the standards, respectively. Serial dilutions of the mixed standard and reference standard solutions were made to obtain graded concentrations of AL ranging from 0.01/10 to 2/10 mg/mL. Mixed standard solutions were prepared using acetonitrile and THF (50:50, %) as diluents. The resulting solutions were spiked with IS solution to give 5 mg/mL.

Preparation of hydrochloric acid/sodium chloride solution pH 1.6

A weight of 1.0 g of sodium chloride was dissolved in 0.450 L of distilled water. The pH was adjusted to 1.6 with HCl. Either HCl or NaOH were spiked as applicable to give the exact pH 1.6, thereafter made up to 0.5 L with distilled water.

Preparation of sodium hydroxide/glacial acetic acid/sodium chloride-Buffer solution pH 5

A weight of 2.020, 4.325, and 5.937 g of sodium hydroxide pellet, glacial acetic acid and sodium chloride, respectively, were dissolved in 0.450 L of distilled water. The pH was adjusted with either of 1N HCl or NaOH solution.

Preparation of FaSSGF, FeSSIF and FeSSIF-V2

A weight of 1.120 g of FaSSIF/FeSSIF powder was dissolved in 0.25 L of acid buffer (pH 1.6). This was stirred until the powder was completely dissolved. The solution was made to 0.5 L mark. A weight of 5.60 g of FaSSIF/FeSSIF/FaSSGF powder was dissolved in 0.25 L of buffer (pH 5.0). The solutions were made up to volume with the buffer and allowed to stand for 2 h before use. FeSSIF-V2 was prepared by dissolving 5.0 g of FeSSIF-V2 powder in 5.0 L of distilled water.

Spectroscopic determination

Infra red (IR) studies were performed using Fourier transform infra-red (FTIR) spectrophotometer, FTIR-84005 (Schimadzu, Japan). One milligram of AL powder and 200 mg of dried KBr powder were mixed and compressed into a tablet shaped disk and scanned at a speed of 2 mm/s over a wavenumber region from 4000 to 500 cm⁻¹. Essential FTIR (eFTIR) software was employed for spectral analysis.

Dissolution kinetics of AL in the presence of FeSSIF-V2

A total weight of 2 g of each solid food components or 1.7 mL of sunflower oil or 35 cL of carbonated drinks was dispersed in 500 mL FeSSIF-V2 in a flask. One tablet of the AL product was subsequently placed in the dissolution apparatus; USP type 2. The dissolution profile was determined by sampling 5 mL of the medium at 0, 5, 15, 30, 45, 60 and 90 min. Samples were analyzed using HPLC system.

Statistical analysis

The data obtained were analyzed using Minitab for Windows statistical package (Minitab, Inc., USA). *Post hoc* comparison was performed with Hsu's test.

RESULT

Albumin had a pH change of 1.51 units in FaSSGF (Table 2). Similarly, it increased the pH in FaSSGF from 1.62 to 3.13 units and revealed an increase of 0.17 units in FeSSIF. Albumin also demonstrated significantly lower ($p < 0.05$) ABC compared to the carbohydrates in FaSSGF (Table 2). The ABC of Coca-Cola® was significantly higher ($p < 0.05$) than Fanta®. Carbonated drinks treatment presented the highest ABC. The extent of artemether release in the presence of fructose was statistically higher than for starch ($p < 0.05$). Albumin and fructose treatment had significantly higher total artemether release adjudging by the area under the curve (AUC) values (Table 4), ($p < 0.05$).

Dissolution efficiency (DE) values of 1.06 and 0.96, respectively for albumin and fructose treatments gave indication of satisfactory artemether release. Lumefantrine dissolution throughout the 90 min run time was less than 20% for the

food components except sunflower oil with a value of 59.7% (Figures 1b and 2b).

In AL spectra presented (Figure 3A), there were broad peaks for artemether aliphatic O-H stretching vibration and aliphatic C-H bending vibrations. Treatment with albumin (Figure 4B) and carbonated drinks (Figure 5B) showed deletion of the O-H bond vibration. Carbonated drinks further caused a deletion of the C-H, C-O and C-O-O bond vibrations. There were prominent C-H bending and O-H stretching vibrations for artemether in the AL spectra (Figure 4A) as well as the spectra for the food components treatments (Figure 3A-E and 4C), except for albumin (Figure 4B). The endoperoxide bond stretching was also deleted by carbonated drink treatment. The band due to C=O for carbonated drink treatment had wavenumber shifted downwards beyond 7 cm^{-1} .

DISCUSSION

There are several reports on the impact of food and beverages on the luminal content physicochemical characteristics (Vertzoni *et al*, 2005; Kong and Singh, 2010). Foods with high protein content and high viscosity have been reported to cause higher gastric acid secretion (Marciani *et al*, 2001). This study assesses the instantaneous pH change and buffering capacity of food component and carbonated drinks in simulated gastric and intestinal fluids on the *in vitro* course of artemether and lumefantrine release from AL tablet.

The pH of the simulated media was altered instantaneously by food components to varying extent. Albumin showing a pH change of 1.51 units in FaSSGF is expected to present a significant change in the ratio of ionized to unionized drugs in solution. A measurable effect on the release profile of the components of AL tablets being studied is therefore expected. Carbohydrate and sunflower oil components did not reveal any significant change in pH of the two media studied.

Some studies have also reported that the pH of proximal jejunum increased to 6.5 after the administration of dried milk, vegetable oil and dextrose to healthy volunteers (Dressman *et al*, 1990). The buffering capacity of the different food components and drugs evaluates the influence of these agents on postprandial hydrochloric acid secretion in the stomach following the ingestion of drug doses concomitantly with food (Parapini *et al*, 2008). In this study, albumin increased the pH in FaSSGF sufficiently to affect the release profile of actives. Similarly, albumin had a pH increase of 0.17 units in FeSSIF. The effect of temporal changes in GI tract pH profile and the physicochemical basis of altered pH effect on drug absorption have been reported (Charman *et al*, 1997). The findings of this study are in agreement with those of Charman and co-workers with respect to the manifestations of the physicochemical properties of the studied drugs (*i.e.*, food

component in FeSSIF-V2) which may affect their bioavailability. Treatment with sunflower oil increased the *in vitro* artemether and lumefantrine release when compared with the other food components (Figures 1 and 2). This establishes the fact that lumefantrine possess some intrinsic aqueous solubility problems (Premji *et al*, 2008).

FTIR spectra of AL in this study (Figure 3) revealed frequencies that were consistent with the vibrational data reported by earlier studies of artemisinin and artesunate (Lawal *et al*, 2012). This study revealed that FTIR spectrum of AL conformed to the eFTIR analysis of the important bonds/functional group bands (Socrates, 1994; Sohrabi *et al*, 2005). The positions of these peaks in the food component treatment formed the basis for the assessment of possible food-drug interaction. AL treatment with albumin (Figure 4B), and carbonated drinks (Figure 5B) showed deletion of the O-H bond vibration. This is suggestive of molecular interaction in the albumin treatment. Deletion of certain characteristic bond vibrations of a molecule due to the presence of additives are marks of molecular interaction (Koutsoupakis *et al*, 2002). Spectra band due to C=O for carbonated drink treatment had wavenumber shifted downwards beyond 7 cm^{-1} , which is strong indication of molecular interaction.

Spectra due to starch treatment were similar to those of other food components (except albumin) with no significant differences in the wavenumber when compared with the reference.

CONCLUSION

Carbonated drinks *have* significantly reduced the dissolution profile of artemether and lumefantrine from tablet. Starch resulted in good drug release for artemether but poor lumefantrine release profile.

AUTHOR'S STATEMENT

The authors have no conflict of interest to disclose.

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Table 1: Details of the food components used in the study

Name of food component	Manufacturing Date	Expiry Date	Batch number	Origin
Albumin	01-2014	12-2016	Alpha Chemika	India
Glucose	05-2013	12-2016	Quigyuang Ltd	China
Lactose	03-2014	02-2016	Triveni Chemicals	China
Fructose	08-2013	07-2017	Park Services	Ukraine
Starch	05-2013	05-2017	Park Services	Ukraine
Sunflower oil	01 2012	01-2017	JiannHairu Co.	China
Carbonated drink	05-2014	11-2015	Coca-cola Co.	Nigeria

Table 2: Instantaneous pH change and buffering capacity due to the presence of food components

Food component	pH in medium		pH change in medium		Volume of hydrochloric acid (µL)	
	FaSSGF	FeSSIF	FaSSGF	FeSSIF	FaSSGF	FeSSIF
Starch	1.60	5.12	0	+0.12	1.6	5.5
Fructose	1.61	5.12	-0.01	+0.12	2.0	8.2
Lactose	1.61	5.09	-0.01	+0.9	1.8	10.8
Albumin	3.13	5.17	+1.51	+0.17	0.3	6.4
Sunflower oil	1.57	5.16	-0.04	+0.16	2.0	11.4
Carbonate drinks						
(Coke)	1.36	4.83	+0.26	-0.17	8.0	11.6
(Fanta)	1.90	4.49	+0.28	-0.51	2.8	14.8

NB: pH of FaSSGF =1.62 and FeSSIF= 5.12 (+ and/or - signify an increase and decrease in pH, respectively, due to introduced substances).

Table 3: Drug release profile of AL in the different media conditions

Food component /drugs	Drug release profile							
	Artemether			Lumefantrine				
	C ₄₀ (%)	T ₇₀ (min)	AUC (%.min/mL)	DE	C ₄₀ (%)	T _∞ (min)	AUC (%.min/mL)	DE
AL alone	40	65	4547.34	1.0	14	90	2260.99	1.0
Starch	78	28	938.02	0.21	9	90	955.28	0.42
Fructose	75	15	4380.93	0.96	11	90	1057.69	0.47
Lactose	58	47	941.90	0.21	9	90	1042.43	0.46
Albumin	53	55	4821.54	1.06	8	90	662.13	0.29
Sunflower oil	63	42	2261.92	0.50	14	90	3483.40	1.54
Carbonated drink	24	-	1763.215	0.39	10	90	1127.315	0.50

NB: C₄₀, T₇₀, T_∞, AUC and DE are concentration of drug dissolved at 40 min, time to achieve 70% dissolution, time to highest dissolution, total drug dissolved and the dissolution efficiency, respectively

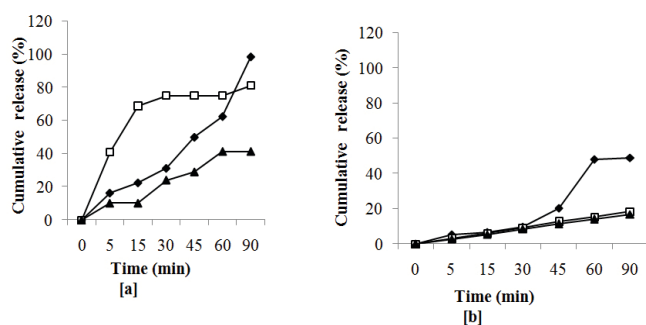


Figure 1: Dissolution profile for AL tablet. ♦ = fasted, □ = fructose and ▲ = carbonated drink; (a) artemether and (b) lumefantrine.

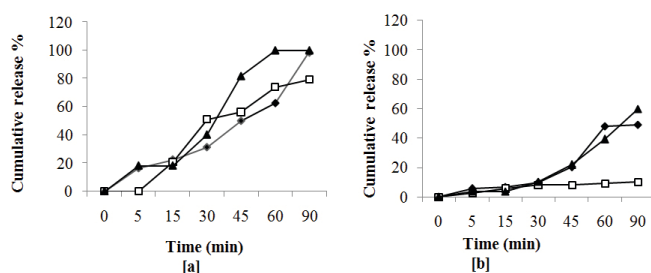


Figure 2: Dissolution profile for AL tablet; ♦ = fasted, □ = albumin and ▲ = sunflower oil (a) artemether and (b) lumefantrine.

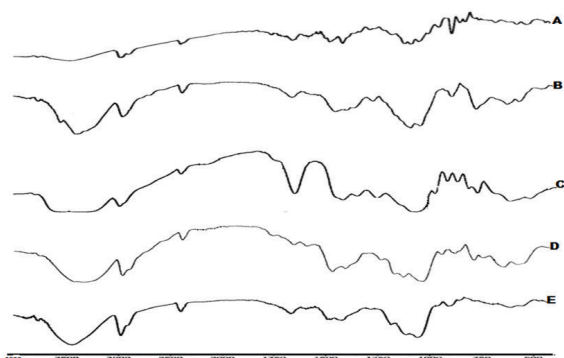


Figure 3: FTIR spectra for; (A) AL alone, (B) lactose with AL, (C) fructose with AL, (D) glucose with AL and (E) starch with AL.

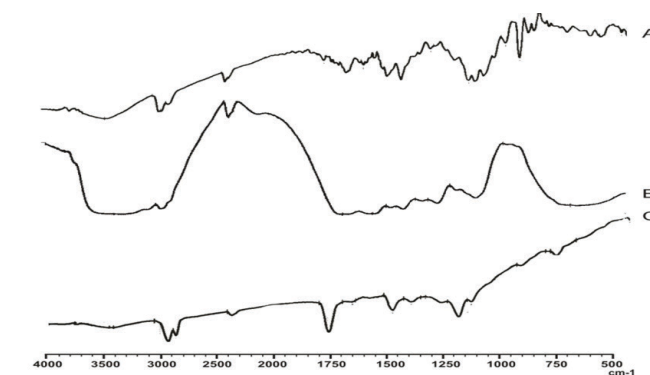


Figure 4: FTIR spectra of (A) AL alone, (B) albumin with AL and (C) sunflower with AL.

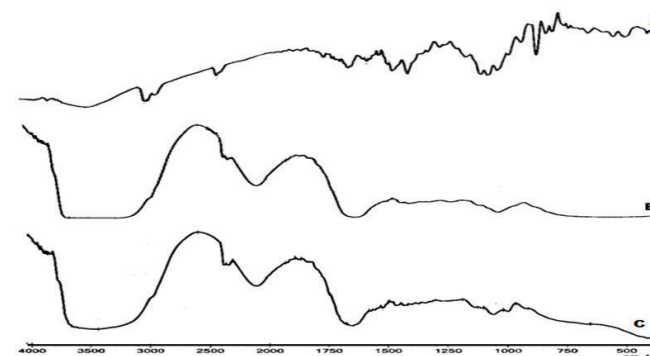


Figure 5: FTIR spectra of (A) AL alone, (B) CocaCola® with AL and (C) Fanta® with AL.