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WATER-IN-OIL-IN-WATER MULTIPLE EMULSIONS OF IBUPROFEN FOR PAEDIATRICS USING AFRICAN WALNUT SEED OIL

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ABSTRACT

Many prolonged-release dosage forms have employed multiple emulsions (MEs) systems. Hence, this study formulated water-in-oil-in-water (w/o/w) MEs of ibuprofen using African walnut seed oil (AWSO) for paediatrics use. The MEs were prepared by a two-step emulsification method, using Span 80® and Tween 80® as primary and secondary emulsifiers, respectively. The MEs were evaluated by their physical properties, drug entrapment efficiency, stability and drug release profile. From the study, stable MEs of ibuprofen (100 mg / 5 ml) can be prepared with 25 % w/v Span 80® as the primary emulsifier, and 8, 10 or 12 % w/v Tween 80® as the secondary emulsifier. The optimum ratios of oil to water in the primary emulsion were 1:1 and 3:2, while that of primary emulsion to external aqueous phase were 1:1 and 1:2. The amount of the ibuprofen released from the MEs was ≤ 35.6 % at 5 hours. The study offers ibuprofen emulsions which may require once daily dosing compared to other available paediatric dosage forms of the drug which require three to four times dosing daily. It also provides information on AWSO as a possible drug carrier in the formulation of w/o/w MEs of ibuprofen for paediatrics.

INTRODUCTION

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) used widely in paediatrics for management of pains and fever. It is recommended for use in children 3 months and above weighing 5 kg upwards at a dose of 5 – 10 mg / kg orally every 6 – 8 hours with a maximum daily dose of 30 mg / kg [1]. Commonly available ibuprofen formulations for paediatric use are syrup and suspension (containing 100 mg/ 5 ml dose). Ibuprofen powder is insoluble in water but soluble in organic solvents [2]. This may justify why it is commonly available in capsule, tablet, syrup and suspension dosage forms. It also has a bitter taste which may be unacceptable to paediatrics, hence the need for effective taste masking. Sweeteners, which are used for taste masking, may cause dental caries and diabetes in children. A more effective way of masking the taste of drugs and also reducing the dosing frequency is very much desirable for paediatric drug formulations. A way to achieve this is by multiple emulsion formulations.

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Multiple emulsions (MEs) are dispersed systems in which the dispersed phase contains smaller internal droplets of similar nature with the continuous phase [3]. Among other types of MEs, the water-in-oil-in-water (w/o/w) and oil-in-water-in-oil (o/w/o) types are the commonest. The w/o/w MEs comprise of internal oil globules containing water droplets and are surrounded by an external aqueous phase. The o/w/o MEs, however, comprise of internal water globules containing oil droplets and are surrounded by an external oil phase. MEs' complex structure allows for different modifications that make them suitable for many potential uses than simple emulsions. The most commonly studied MEs are the w/o/w types which are widely used for pharmaceutical purposes [4]. MEs have been employed for controlled and prolonged release of drugs from formulations [5, 6, 7] and as intermediate step in microencapsulation process [8]. Drugs that have unpleasant taste such as bitterness have also been incorporated in the internal phase of a multiple emulsion system to mask the taste [9]. The basic rationale for these applications is that the drug has to transverse interfacial barriers and other phases (than where it was incorporated) before its liberation from the formulation and subsequent bioavailability [10].

The oil employed is one of the major factors that affect ME formulations. Based on ease of emulsification and stability concerns, mineral oils have been more employed than vegetable oils in formulating MEs [11]. However, vegetable oils are more easily biodegradable and have been found to be rich in nutrients which are not available in mineral oils. African walnut seed oil (AWSO), for example, is a rich source of essential dietary fatty acids – oleic, linoleic and alpha-linolenic acids [12]. Dietary minerals such as potassium, magnesium, and calcium have been found in significant quantities in walnut seed [13]. Its polyphenols content is also higher than other common nuts [14].

AWSO is the oil obtained from matured seeds of African walnut, *Plukenetia conophora* Müll.Arg. (formerly called *Tetracarpidium conophorum*) of Euphorbiaceae family. It is native to Nigeria and Cameroon [15]. The high nutritional value and numerous health benefits of walnut plant make it an important research focus. Literature search of the oil as a pharmaceutical excipient revealed that it has not been effectively used in drug formulation. Most of the researches on the plant stopped at its proximate composition analysis and bioactivities. AWSO, being highly nutritive and safe for human consumption, can be employed in formulation of oral w/o/w MEs of water-insoluble drug like ibuprofen. The study therefore aimed to formulate stable prolonged release ibuprofen formulations using AWSO.

MATERIALS & METHODS

Ibuprofen powder (donated by Fidson Healthcare Plc., Sango-Otta, Nigeria); polyoxyethylene sorbitan monooleate (Tween 80®), sorbitan monooleate (Span 80®), methyl-p-amino benzoic acid, propyl-p-amino benzoic acid (BDH Chemical Limited, Poole, England); phosphate buffer solution (pH 7.4); African walnut seed oil and distilled water.

Extraction of AWSO

Matured African walnut seeds purchased at Atakumosa market, Ilesa, Osun State were dried and pulverised with a blender (USHA, India). The seed oil was extracted by soxhlet extraction using n-hexane as the solvent (solvent residue was removed from the oil using a rotary evaporator (Buchi Rotavapor R110, Switzerland) at 50 °C). The physicochemical properties of the oil were determined using the British Pharmacopoeia methods [2].

Formulation of w/o/w MEs of Ibuprofen

MEs of AWSO without the drug (i.e. blank) were prepared with a simple hand mixer (Saisho, Hong Kong) using a two-step emulsification method [7, 16]. Span 80® and Tween 80® were used as the primary and secondary emulsifiers, respectively. The concentrations of the primary and secondary emulsifiers, stirring time, ratios of oil phase to water phase of the primary emulsion and primary emulsion to aqueous phase of the secondary emulsion, were as indicated in Table 1. The primary water-in-oil (w/o) emulsion was prepared by addition of water to the oil containing Span 80® with continuous stirring at 1700 revolution per minute (rpm). This was then added to water containing Tween 80® with continuous stirring at 1400 rpm to form the final MEs.

After 28 days of storage, the blank MEs that did not show oil phase separation were selected for preparing 50 mg/ 5 ml, 100 mg/ 5 ml 150 mg/ 5 ml w/o/w MEs of ibuprofen (Table 2). Becher [17] suggested that oil-soluble and water-soluble materials, respectively, be placed in the oil phase and aqueous phase before adding the phases together. The drug being insoluble in water was incorporated in the oil phase of the primary emulsion prior to addition of the internal aqueous

phase. The primary emulsion was stirred at 1700 rpm for 5 minutes while the secondary emulsion was stirred at 1400 rpm for 30 seconds. Preservatives, 0.1%w/v methyl-p-amino benzoic acid and 0.05%w/v propyl-p-amino benzoic acid, were added to the external aqueous phase and the oil phase, respectively. Preservatives were added to prevent the growth of microorganisms in the w/o/w MEs. Methyl-p-amino- and propyl-p-amino- benzoic acid, which are most commonly used preservatives, were added to the external aqueous phase and the oil phase, respectively.

Evaluation of the MEs of Ibuprofen

Determination of degree of creaming

A calibrated test-tube was filled with the multiple emulsion up to a labelled mark and the volume ratio of the 'less cloudy' aqueous phase (H_a) to the total volume of the emulsion (H_0) at room temperature was recorded at 24 hours, 7 days, 14 days and 28 days. The percentage creaming (%) was calculated for each formulation as:

$$\text{Percentage creaming} = \frac{H_a}{H_0}(100) \qquad \text{Equation 1}$$

Each result was an average of three determinations.

Droplet size analysis

The droplet sizes of the multiple emulsion droplets were measured with a light microscope to which digital AmScope camera (Omax, China) was attached, 24 hours after preparation. The emulsion was diluted to 1 in 100 using 70% v/v aqueous propylene glycol [18]. A minimum of six fields of view were observed using the camera. The number of multiple emulsion droplets and simple emulsion droplets (those that appeared as o/w type) formed was determined by counting each droplet per category. The fraction of multiple emulsion droplets formed was hence calculated.

Table 1: Blank AWSO multiple emulsions

Code	Primary emulsion			Secondary emulsion		
	Oil : Water (v/v) ratio	Span 80® (%w/v)	Stirring time (min)	(W/O) : Water (v/v) ratio	Tween 80® (%w/v)	Stirring time (min)
A1	1:1	25	10	1:1	12	2
A2	1:1	25	10	3:7	12	2
A3	1:1	25	10	1:2	12	2
A4	3:2	25	10	1:1	12	2
A5	3:2	25	10	3:7	12	2
A6	3:2	25	10	1:2	12	2
B1	1:1	25	10	1:1	10	2
B2	1:1	25	10	3:7	10	2
B3	1:1	25	10	1:2	10	2
B4	3:2	25	10	1:1	10	2
B5	3:2	25	10	3:7	10	2
B6	3:2	25	10	1:2	10	2
C1	1:1	25	10	1:1	8	2
C2	1:1	25	10	3:7	8	2
C3	1:1	25	10	1:2	8	2
C4	3:2	25	10	1:1	8	2
C5	3:2	25	10	3:7	8	2
C6	3:2	25	10	1:2	8	2
D1	1:1	25	10	1:1	6	2
D2	1:1	25	10	3:7	6	2
D3	1:1	25	10	1:2	6	2
D4	3:2	25	10	1:1	6	2

D5	3:2	25	10	3:7	6	2
D6	3:2	25	10	1:2	6	2
E1	1:1	15	10	1:1	10	2
E2	1:1	15	10	3:7	10	2
E3	1:1	15	10	1:2	10	2
E4	3:2	15	10	1:1	10	2
E5	3:2	15	10	3:7	10	2
E6	3:2	15	10	1:2	10	2
F1	1:1	20	10	1:1	10	2
F2	1:1	20	10	3:7	10	2
F3	1:1	20	10	1:2	10	2
F4	3:2	20	10	1:1	10	2
F5	3:2	20	10	3:7	10	2
F6	3:2	20	10	1:2	10	2
G1	1:1	30	10	1:1	10	2
G2	1:1	30	10	3:7	10	2
G3	1:1	30	10	1:2	10	2
G4	3:2	30	10	1:1	10	2
G5	3:2	30	10	3:7	10	2
G6	3:2	30	10	1:2	10	2

Table 2: Composition of multiple emulsion formulations of ibuprofen

Code	Emulsifiers' concentration (% w/v)	Oil : Water (v/v) ratio (primary emulsion)	(W/O): Water (v/v) ratio	Ibuprofen concentration (mg/ 5 ml)
A1a	$S_{25}T_{12}$	1 : 1	1 : 1	50
A1b	$S_{25}T_{12}$	1 : 1	1 : 1	100
A1c	$S_{25}T_{12}$	1 : 1	1 : 1	150
A2a	$S_{25}T_{12}$	1 : 1	3 : 7	50
A2b	$S_{25}T_{12}$	1 : 1	3 : 7	100
A2c	$S_{25}T_{12}$	1 : 1	3 : 7	150
A3a	$S_{25}T_{12}$	1 : 1	1 : 2	50
A3b	$S_{25}T_{12}$	1 : 1	1 : 2	100
A3c	$S_{25}T_{12}$	1 : 1	1 : 2	150
A6a	$S_{25}T_{12}$	3 : 2	1 : 2	50
A6b	$S_{25}T_{12}$	3 : 2	1 : 2	100
A6c	$S_{25}T_{12}$	3 : 2	1 : 2	150
B1a	$S_{25}T_{10}$	1 : 1	1 : 1	50
B1b	$S_{25}T_{10}$	1 : 1	1 : 1	100
B1c	$S_{25}T_{10}$	1 : 1	1 : 1	150
B4a	$S_{25}T_{10}$	3 : 2	1 : 1	50
B4b	$S_{25}T_{10}$	3 : 2	1 : 1	100
B4c	$S_{25}T_{10}$	3 : 2	1 : 1	150
C1a	$S_{25}T_{8}$	1 : 1	1 : 1	50
C1b	$S_{25}T_{8}$	1 : 1	1 : 1	100

C1c	$S_{25}T_8$	1 : 1	1 : 1	150
C4a	$S_{25}T_8$	3 : 2	1 : 1	50
C4b	$S_{25}T_8$	3 : 2	1 : 1	100
C4c	$S_{25}T_8$	3 : 2	1 : 1	150
G1a	$S_{30}T_{10}$	1 : 1	1 : 1	50
G1b	$S_{30}T_{10}$	1 : 1	1 : 1	100
G1c	$S_{30}T_{10}$	1 : 1	1 : 1	150

S and T are Span 80® and Tween 80®, respectively; (W/O) is the fraction of the primary water-in-oil emulsion.

Also, the geometric mean droplet diameter (M) and the geometric standard deviation (δ_g) were calculated using the formulae derived by Smith and Jordan [18] for polydispersed system:

$$\text{Log M} = \frac{\sum_i n_i \log x_i}{\sum_i n_i} \quad \text{Equation 2}$$

$$\text{Log } \delta g = \sqrt{\frac{\sum_i n_i (\log x_i - \log M)^2}{\sum_i n_i}} \quad \text{Equation 3}$$

Where n_i is the number of droplets whose diameter lies in an interval of which the midpoint is x_i.

Stability to centrifugation

This was done by centrifuging 2.5 ml of each freshly prepared formulation in a centrifuge apparatus (Gallenhamp, England) at 4000 rpm for 15 minutes. The formulations in which there was cracking were noted.

Determination of entrapment efficiency (EE)

A 2.5 ml sample of each formulation was centrifuged at 4000 rpm for 15 minutes and allowed to stand overnight. The sample used for the EE determination was prepared by withdrawing 0.5 ml of the centrifuged MEs from the base and diluting with 50 ml of phosphate buffer solution (pH 7.4) before filtering with a filter paper (Whatman® 1,0.22 mm pore size) [7]. A 1 in 10 dilution of the filtrate was done before the absorbance was measured at 222 nm using a UV spectrophotometer (UV-1800, Shimadzu). The entrapment efficiency was calculated using the equation below [4, 19] and each value was an average of three measurements.

$$\% \text{ EE} = \frac{\text{Total drug incorporated –Free Drug}}{\text{Total drug incorporated}} (100) \quad \text{Equation 4}$$

The free drug was the amount of the drug found in the filtrate.

Accelerated stability study

The effect of storage temperature on physical properties and stability of the most stable 100 mg/ 5 ml ibuprofen w/o/w MEs was assessed. Duplicate samples of each selected formulation were dispensed into graduated test-tubes and each was stored at 10°C and 40°C for 4 weeks. At different time intervals, the formulations were monitored for degree of creaming, changes in viscosity; pH; colour and droplet size. Entrapment efficiency of each formulation after the 4 weeks storage period was also determined.

Viscosity measurement of the MEs

It had been established that the viscosity of a non-Newtonian system such as emulsion can only be determined by rotational viscometers at different shear rates [20]. Thus, NDJ-5S digital rotary viscometer (China) operating at different speeds – 6 rpm, 12 rpm, 30 rpm and 60 rpm was used. The rotor of the viscometer (inserted in the formulation at a fixed position) was subjected to a torque moment (rotation about a specific point) proportional to the viscosity of the formulation. The torque moment was measured by the sensors and processed into the viscosity (mPa.s) as displayed on the screen. At 28 ± 1 °C, each measurement at the varying speeds (increasing order) was done at 20 minutes interval. After allowing the sample to rest for 5 minutes, the viscosity was again determined at the different speeds in decreasing order also at 20 minutes interval.

In vitro drug release study

The drug release from the MEs was done in vitro by dialysis method using Spectra/ Por® membrane tubing (Fisher Scientific, UK) [21, 22]. Veego (India) digital dissolution test apparatus was employed. The dissolution test conditions were as specified in Table 3. Samples withdrawn from the dissolution medium were balanced by replacement with an equal volume of buffer solution maintained at equal temperature. The drug content in each withdrawn sample was determined spectrophotometrically at 222 nm. The percentage of ibuprofen released from the MEs at pre-determined times was calculated and each value was an average of three determinations.

Drug release kinetics

The dissolution data obtained from the in vitro drug release study were computed using Microsoft Excel spreadsheet and DDSolver software [23, 24]. Zero-order, first-order, Higuchi, Higuchi with lag time, and Korsmeyer-Peppas release kinetics models were utilised for the release kinetics of ibuprofen from the w/o/w emulsions and are expressed mathematically as follows:

a. Zero-order kinetic model [25, 26]

$$Q_t = Q_0 + k_o t \quad \text{Equation 5}$$

Q_t is the amount of drug released in time, t; Q_0 is the initial amount of drug; and k_o is the zero-order release constant.

Table 3: Dissolution test conditions

Parameter	Material/test condition
Dissolution medium	Phosphate buffer solution (pH 7.4)
Dissolution medium volume	700 ml
Temperature	37.5 ^{0}C
Method	Basket
Speed	50 rpm
Volume withdrawn	5 ml
Sampling times (min)	15, 30, 60, 90, 120, 180, 240 and 300

b. First-order kinetic model [27, 28]

$$\ln Q_t = \ln Q_0 - k_1 t \quad \text{Equation 6}$$

Q_t is the amount of drug released in time, t; Q_0 is the initial amount of drug; and k_1 is the first-order release constant. The drug release is proportional to the remaining amount of drug in the formulation [27].

c. Higuchi model [27, 29]

$$Q_t = k_H \sqrt{t} \quad \text{Equation 7}$$

Q_t is the amount of drug released in time, t; and k_H is the Higuchi release constant.

d. Higuchi model with lag time [27]

$$Q_t = k_H \sqrt{(t - T_{lag})} \quad \text{Equation 8}$$

Q_t is the amount of drug released in time, t; T_{lag} is the lag time; and k_H is the Higuchi release constant.

e. Korsmeyer-Peppas model [28, 30]

$$\frac{M_t}{M_\infty} = k_{KP} t^n \quad \text{Equation 9}$$

$\frac{M_t}{M_\infty}$ is the drug fraction released in time, t; k_{KP} is the Korsmeyer-Peppas release constant; and *n* is the drug release exponent.

The Adjusted Coefficient of Determination ($R^2_{adjusted}$), Model Selection Criterion (MSC) and Akaike Information Criterion (AIC), which are goodness of fit parameters, were used to select the best drug release model from the MEs [23]. The release model with the highest $R^2_{adjusted}$ (≥ 0.99), MSC (≥ 3.00) values and lowest AIC value was chosen as the best-fit model [28]. The goodness of fit of the models for each formulation was also validated using the DDSolver software to predict the percentage of ibuprofen released at 120 min.

Statistical analysis

The release parameters obtained were subjected to single factor Analysis of Variance (ANOVA) at 0.05 alpha value with $p < 0.05$ considered statistically significant.

RESULTS AND DISCUSSION

Physicochemical properties of AWSO used for the study

The physicochemical properties of the AWSO were as presented in Table 4. The golden yellow colour of the AWSO is similar to what was obtained in literature [31]. It did not have optical activity, indicating the absence of chiral compounds in the oil. The weight per millilitre (0.8668 ± 0.0043 g/ml) and relative density (0.8640 ± 0.0056) of the oil indicate that it is less dense than water. This is of importance in the creaming behaviour of an emulsion prepared with the oil. An o/w emulsion will show upward creaming if the density of the oil is less than that of water and vice-versa [32]. The oil had good saponification value which falls within the range given in literature (189 – 198 mg KOH/g) [33]. The acid value obtained for the oil was 75.954 ± 0.001 mg KOH/g, indicating susceptibility of the oil to oxidation being a fixed oil. The oil should be kept in air-tight coloured glass containers below 30 °C. Also, the ester value obtained for the oil used in this study showed that it is suitable for oral preparations. Nkafamiya et al. [34] reported that oils having higher ester value are more suitable for consumption.

Water-in-oil-in-water MEs of ibuprofen using AWSO

The study attempted to formulate stable MEs of ibuprofen with AWSO. The stability of the MEs was evaluated by centrifugation, creaming percentage, entrapment efficiency, microscopic droplet size analysis, and storage at different temperatures. All the formulations had entrapment efficiencies (EE) greater than 60% and the EE increased with increase in drug concentration (Table 5). During preparation of the MEs,

the drug was found to be partially soluble in AWSO. A possible strong bond between the oil and the drug could be responsible for the observed drug entrapment efficiencies of the MEs. However, the formulated MEs, except A1a, A1b, B1a, B4a, B4b, B4c, G1a, G1b and G1c, cracked after being subjected to centrifugation at 4000 rpm for 15 minutes. Centrifugation has been used for determining 'good' and 'bad' emulsions in a series of trial formulations by inducing creaming, coalescence and/or oil phase separation.

Table 4: Physicochemical properties of AWSO

Physicochemical Properties	Value
Colour	Golden yellow
Relative density at 27 ± 1°C	0.8640 ± 0.0056
Weight per millilitre at 27±1.5 °C	0.8668 ± 0.0043 g/ml
Optical rotation at 27 ± 1.5 °C	Nil
Saponification value	193.7 ± 5.0 mg KOH/g (189–198 mg KOH/g)
Acid value	75.954 ± 0.001 mg KOH/g
Ester value	117.76 ± 5.012 mg KOH/g
Kinematic viscosity at 28 ± 1 °C	0.00289 ± 0.00002 mm^2s^{-1}
pH at 27± 1.5 °C	6.12

Values in parenthesis are the literature values

'Bad' emulsions can be described as those that readily show oil phase separation or cracking when subjected to centrifugation while 'good' emulsions do not show oil phase separation or cracking. The application of centrifugal force in relation to creaming of an emulsion is based on Stokes' equation [35]. Centrifugation exaggerates the value of 'g' in Stokes' equation, which makes the relationship between the behaviour of an emulsion under centrifugation compared against normal storage conditions to be unpredictable. Based on this, the MEs that cracked may not necessarily be considered unstable.

The MEs exhibited upward creaming which is characteristic of a typical o/w simple emulsion [32]. The oil, being less dense than water (Table 4), might have contributed to this behaviour. Increasing the ratio of primary emulsion in the final multiple emulsion decreased the creaming percentage of the formulations on storage (A1 < A3 <A2). The creaming percentage also decreased with increase in the drug concentration in the MEs. The viscosity of the final MEs which depends on the ratio of the primary emulsion to the external aqueous phase will increase when this ratio is higher. Higher drug concentration would also increase the viscosity of the primary emulsion and ultimately that of the final MEs. Based on Stokes' law, creaming will be expected to be less at increased viscosity [36]. And it is possible that the drug has a synergistic effect on the emulsifiers' ability to decrease the interfacial tension between the internal aqueous phase and the oil phase of the w/o/w emulsion. Reduction in interfacial tension has been reported to increase stability of emulsion systems generally [37].

The internal and external droplet diameters of the MEs increased on storage for 28 days at room temperature (Table 6). This may be due to coalescence which is the thinning and disruption of the liquid film between the MEs' droplets resulting to the merging of two or more droplets into larger ones [38]. A higher oil fraction (from 0.5 to 0.6); increase in the emulsifiers' concentrations and amount of ibuprofen resulted in MEs with larger droplets. The decrease in the fraction of multiple emulsion droplets with increase in the concentration of primary emulsifier from 25 to 30 % w/v may be attributed to the ability of 'excess' lipophilic emulsifier to increase swelling capacity of the oil phase and favour formation of simple o/w emulsions [39]. Two or more swollen oil phases in a globule may merge, thus increasing the internal droplet diameter, or rupture, thus releasing the internal aqueous phase to the external aqueous phase and slip back to simple o/w system with smaller droplets.

Accelerated stability study

There was continuous decrease in both the internal and external droplet diameters of most of the formulations at 10 °C and 40 °C compared with those stored at room temperature (Table 7 cf. Table 6). This may be due to leakage of the internal aqueous phase to the external aqueous phase [40] causing shrinkage of the emulsion droplets. Omotosho [41] reported that an expected increase in droplet size of MEs by coalescence might be counteracted by a decrease in size due to leakage of internal aqueous phase (in the oil) to the external aqueous phase. Colour change and oil phase separation took place only in the formulations stored at 40 °C. The change in colour, which appeared on the 14th day, may be due to oil phase separation which is encouraged by elevated temperatures [7].

The percentage entrapment efficiency of the MEs changed only slightly after storage at extreme temperatures for 28 days (Figure 1). This can be indicative of the ability of AWSO based w/o/w emulsions to entrap the drug without leaking it to the external aqueous phase, even at adverse temperatures. Since the

drug was incorporated in the oil phase and not the internal aqueous phase, leakage of the internal to the external aqueous phase will not likely have significant effect on the entrapment efficiency. The viscosity of the formulations also increased at these temperatures. This may be due to reduction in the internal and external droplet diameters and it has been reported that smaller droplet size distribution offers more resistance to flow due to an increase in total interfacial area [42].

Table 5: Stability to centrifugation, creaming, and entrapment efficiency of ibuprofen MEs

Code	Centrifugation	Percentage creaming (%)				Entrapment Efficiency (%)
		24 hours	7 days	14 days	28 days	
A1a	-	0	0	0	0	67.6 ± 0.0
A1b	-	0	0	0	0	76.8 ± 0.3
A1c	+	0	0	0	0	82.6 ± 0.0
A2a	+	89.2 ± 1.2		Cracked		83.7 ± 0.0
A2b	+	65.0 ± 2.4	79.2 ± 1.2	80.8 ± 1.1	81.6 ± 0.0	87.7 ± 0.0
A2c	+	16.7 ± 0.0	24.2 ± 1.2	24.2 ± 1.2	24.2 ± 1.2	91.4 ± 0.0
A3a	+	88.3 ± 0.0	91.7 ± 0.0	91.7 ± 0.0	91.7 ± 0.0	83.2 ± 0.2
A3b	+	7.5 ± 1.1	7.5 ± 1.1	7.5 ± 1.1	8.3 ± 0.0	86.2 ± 0.1
A3c	+	6.7 ± 2.3	6.7 ± 2.3	7.5 ± 1.1	7.5 ± 1.1	89.9 ± 0.0
A6a	+	45.0 ± 2.4	45.0 ± 2.4	45.0 ± 2.4	45.0 ± 2.4	80.0 ± 0.1
A6b	+	8.3 ± 0.0	9.2 ± 1.2	10.0 ± 2.4	10.9 ± 1.2	89.0 ± 0.0
A6c	+	0	0	0	0	89.6 ± 0.0
B1a	-	0	0	0	0	84.3 ± 0.1
B1b	+	0	0	0	0	89.7 ± 0.0
B1c	+	0	0	0	0	87.1 ± 0.0
B4a	+	0	0	0	0	77.1 ± 0.0
B4b	-	0	0	0	0	90.5 ± 0.0
B4c	-	0	0	0	0	96.1 ± 0.0
C1a	+	0	0	0	0	76.9 ± 0.0
C1b	+	0	0	0	0	86.9 ± 0.0
C1c	+	0	0	0	0	92.6 ± 0.1
C4a	+	0	0	0	0	77.2 ± 0.1
C4b	+	0	0	0	0	84.7 ± 0.0
C4c	+	69.2 ± 1.2		Cracked		93.5 ± 0.1
G1a	-	0	0	0	0	86.1 ± 0.0
G1b	-	0	0	0	0	91.7 ± 0.1
G1c	-	0	0	0	0	93.7 ± 0.0

+ = cracking present; - = cracking absent

In vitro release of ibuprofen from the w/o/w MEs

The in vitro release of ibuprofen from the w/o/w MEs was characterised by a slow release with only A6b and A1b releasing 35.6 ± 0.3 % and 34.9 ± 0.6 % of the drug respectively at 5 hours. This release pattern may be attributed to a slow release of the hydrophobic drug from the oil phase through the aqueous phase and diffusion through the emulsifiers' interfacial film [9, 22]. This can be used to prolong release of active ingredients from the dosage form [7]. It has been reported that the middle oil membrane strength in addition to the partition and diffusion coefficient of the drug are important factors that affect drug release from MEs [10, 19]. However, increasing the concentration of the secondary emulsifier increased the amount of the drug released from the formulation as can be seen in formulations A1b, B1b and C1b (Tables 2 and 8). Similar result was also observed by Tirnaksiz and Kalsin [40]. This shows that the concentration of the hydrophilic secondary emulsifier is critical in drug release from w/o/w MEs.

Table 6: Geometric droplet size and ratio of multiple droplets to simple droplets of multiple emulsion formulations of ibuprofen

Code	Geometric mean droplet diameter, M (μm)				Fraction of multiple droplets formed	
	24 hours		28 days			
	Internal droplet	External droplet	Internal droplet	External droplet	24 hours	28 days
A1a	6.36 ± 2.13	40.16 ± 1.98	6.93 ± 2.92	90.99 ± 1.57	0.03	9.36
A1b	6.51 ± 1.88	10.12 ± 1.81	7.24 ± 3.13	20.65 ± 3.10	0.71	1.67
A1c	9.59 ± 3.56	30.23 ± 1.86	10.00 ± 3.55	64.12 ± 1.86	0.38	1.39
A2a	37.15 ± 6.62	137.09 ± 1.09	Cracked		0.04	Cracked
A2b	2.92 ± 1.42	14.29 ± 2.28	4.34 ± 2.31	109.40 ± 1.56	0.44	11.30
A2c	No visible multiple droplets		7.73 ± 2.46	38.46 ± 1.52	0.00	19.69
A3a	2.57 ± 1.00	34.91 ± 1.22	5.13 ± 2.32	81.10 ± 1.41	0.02	10.67
A3b	5.55 ± 2.54	17.66 ± 2.52	6.23 ± 2.55	57.15 ± 1.65	0.16	15.60
A3c	3.52 ± 1.65	44.77 ± 1.51	6.22 ± 2.43	98.63 ± 1.67	0.04	0.51
A6a	3.50 ± 1.72	31.12 ± 1.32	9.38 ± 3.34	141.25 ± 1.10	0.07	Ruptured
A6b	8.75 ± 2.38	23.88 ± 1.57	5.68 ± 3.13	45.50 ± 2.21	1.23	Ruptured
A6c	12.47 ± 2.66	42.17 ± 1.26	19.63 ± 2.94	89.74 ± 1.97	5.17	Ruptured
B1a	4.47 ± 1.74	23.17 ± 1.77	18.79 ± 2.29	97.72 ± 1.61	0.11	Ruptured
B1b	2.57 ± 1.00	40.17 ± 1.49	8.84 ± 3.51	56.49 ± 1.83	0.17	0.84
B1c	3.72 ± 1.68	60.26 ± 1.69	17.14 ± 3.27	87.10 ± 1.29	0.04	10.38
B4a	2.57 ± 1.00	77.09 ± 1.53	11.30 ± 4.89	120.23 ± 1.33	0.01	12.00
B4b	No visible multiple droplets		7.11 ± 3.55	84.33 ± 1.70	0.00	4.55
B4c	2.57 ± 1.00	54.20 ± 1.71	14.89 ± 3.06	61.66 ± 2.38	0.00	2.08
C1a	3.20 ± 1.58	26.30 ± 2.33	4.35 ± 1.79	47.21 ± 1.79	0.08	0.52
C1b	3.28 ± 1.73	23.93 ± 2.26	5.82 ± 1.82	48.75 ± 2.26	0.06	0.40
C1c	3.10 ± 1.58	40.46 ± 2.04	3.52 ± 1.65	58.48 ± 1.49	0.03	0.48
C4a	3.01 ± 1.49	29.85 ± 2.52	3.74 ± 1.76	33.88 ± 2.47	0.15	0.26
C4b	4.31 ± 1.73	41.30 ± 2.21	4.44 ± 1.84	41.98 ± 1.92	0.14	2.50
C4c	3.45 ± 1.69	39.36 ± 1.50	Cracked	0.20	Cracked	
G1a	No visible multiple droplets	2.57 ± 1.00	50.70 ± 1.53	0.00	0.02	
G1b	2.57 ± 1.00	40.27 ± 1.76	3.55 ± 1.91	51.64 ± 1.34	0.06	0.02
G1c	2.72 ± 1.27	13.37 ± 2.21	2.77 ± 1.32	37.50 ± 1.97	0.03	0.06

It had been reported that the hydrophilic secondary emulsifier in higher concentrations can exceed its critical micelle concentration, form micelle that may in turn solubilize the lipophilic primary emulsifier and move it into the continuous aqueous phase. This will likely result in a decrease in the concentration of the primary emulsifier and subsequently lead to rupture of the oil layer and loss of the internal contents to the external environment [43]. Increasing the oil ratio in the primary emulsion also increased the percentage of ibuprofen released from the w/o/w emulsions. It was explained earlier that increase in oil ratio in the primary emulsion increased droplet diameters.

The increase in percentage release can thus be due to thinning and possible rupturing of the interfacial film around the globules with increase in droplet diameter. Formulation A6b, for example, had ruptured ME droplets when observed microscopically after 28 days (Table 6). This must be the reason it had the highest cumulative drug release.

Table 7: Effects of storage temperature on geometric droplet diameter of ibuprofen w/o/w emulsions

Code	M (µm) 10 °C				M (µm) 40 °C			
	4 days		28 days		4 days		28 days	
	Int.	Ext.	Int.	Ext.	Int.	Ext.	Int.	Ext.
A1b	2.64 ±1.18	14.00 ± 1.66	2.64± 1.18	7.93 ± 2.34	2.65 ± 1.20	17.22 ± 1.67	2.61± 1.15	4.94 ± 2.42
A3b	2.57 ±1.00	9.55 ± 1.43	2.57± 1.00	2.84 ± 1.48	2.77 ± 1.32	19.59 ± 1.95	2.57± 1.00	9.38 ± 1.45
A6b	2.57 ±1.00	11.0 ± 1.54	2.57± 1.00	3.71 ± 2.00	2.61 ± 1.13	11.38± 2.31	2.57± 1.00	4.50 ± 2.83
B1b	2.72 ± 1.27	19.14 ± 1.49	2.60± 1.12	9.04 ± 2.66	2.57 ± 1.00	19.54 ± 1.45	2.57± 1.00	5.45 ± 2.99
C1b	2.57 ± 1.00	10.35 ± 1.39	2.61± 1.13	5.81 ± 2.68	2.62 ± 1.16	8.41 ± 1.98	2.57± 1.00	8.15 ± 2.70

M: geometric droplet diameter (µm); Int.: internal droplet; Ext.: external droplet

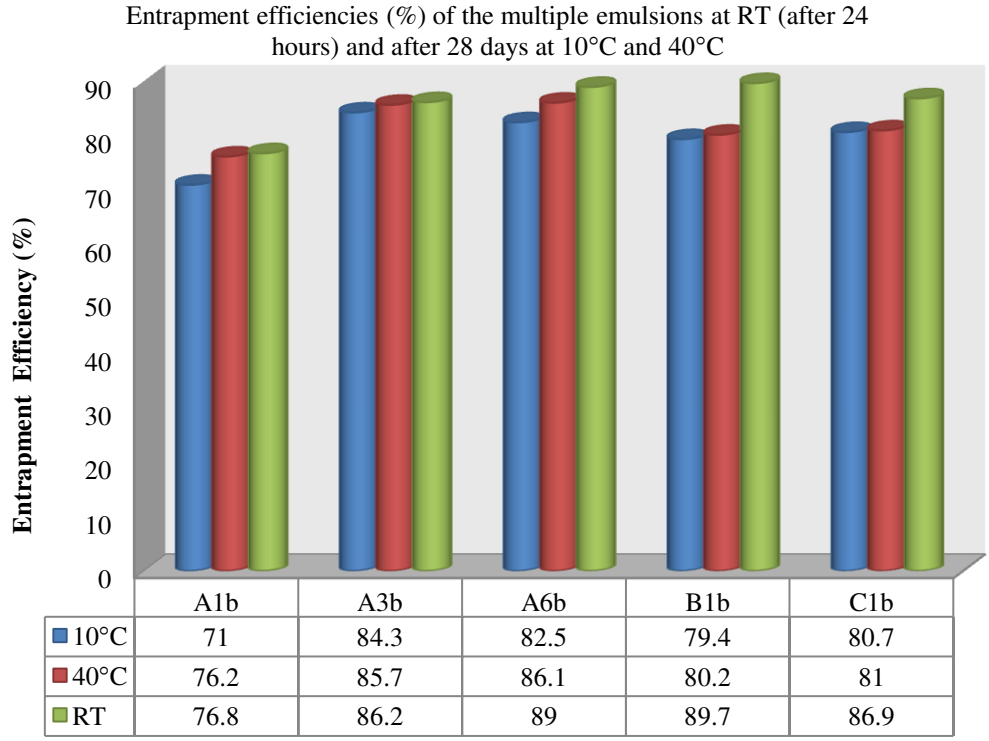

Figure 1: Comparison of the entrapment efficiencies of MEs A1b, A3b, A6b, B1b, and C1b at room temperature (RT) after 24 hours and after 28 days storage at 10°C and 40°C

Table 8: The release parameters of formulated ibuprofen w/o/w emulsions

Formulation code	$T_{15\%}$ (min)	% drug released in 120 min	% drug released in 300 min
A1b	5.1 ± 0.5	15.0 ± 0.3	34.9 ± 0.6
A3b	2.3 ± 0.1	15.2 ± 0.2	28.8 ± 0.2
A6b	5.6 ± 0.1	21.3 ± 0.5	35.5 ± 0.3
B1b	2.1 ± 0.2	12.8 ± 0.0	29.3 ± 0.3
C1b	1.8 ± 0.3	10.4 ± 0.4	23.9 ± 0.2

Drug release kinetics of formulated ibuprofen MEs

The release mechanism was best described by Korsmeyer-Peppas model (Table 9). The release exponent, *n,* values obtained for the formulations ranged from 0.582 to 0.918 indicating non-Fickian diffusion release mechanism [27] and this has been associated with more than one type of release process [44]. For example, formulation C1b fitted into Korsmeyer-Peppas, zero order and first order models while

formulation A6b fitted into Korsmeyer-Peppas and Higuchi with lag-time models. This can be attributed to the many factors influencing drug release from multiple emulsion systems [9]. The values of *n* decreased with increasing the concentration of the secondary emulsifier; increasing the oil ratio in the primary w/o emulsion; and decreasing the primary emulsion ratio in the final multiple emulsions. This could be due to effect of these factors on the miscibility of the multiple emulsion system with the dissolution medium [28].

Table 9: Derived values of release kinetics model fitting parameters from release data of ibuprofen from w/o/w emulsions

Model	Parameter	Formulation codes				
		A1b	A3b	A6b	B1b	C1b
Zero-order	K_0 (min^{-1})	0.122	0.104	0.136	0.098	0.084
	$R^2_{adjusted}$	0.965	0.877	0.784	0.980	0.994
	AIC	29.1	35.7	42.9	22.0	11.1
	MSC	3.1	1.8	1.3	3.7	4.8
	$D_{120\ min\text{-}pre}$ (%)	14.70 (0.30)	12.54 (2.66)	16.37 (4.93)	11.75 (1.05)	10.03 (0.37)
First-order	K_1 (mg/min)	0.001	0.001	0.002	0.001	0.001
	$R^2_{adjusted}$	0.980	0.918	0.877	0.987	**0.998**
	AIC	24.9	32.5	38.4	18.7	**2.4**
	MSC	3.6	2.2	1.8	4.1	**5.9**
	$D_{120\ min\text{-}pre}$ (%)	15.96 (0.96)	13.54 (1.66)	18.26 (3.04)	12.53 (0.27)	10.58 (0.18)
Higuchi	K_H ($mg/min^{1/2}$)	1.715	1.489	1.969	1.363	1.155
	$R^2_{adjusted}$	0.888	0.938	0.985	0.870	0.853
	AIC	38.5	30.2	21.6	36.9	36.0
	MSC	1.9	2.5	3.9	1.8	1.7
	$D_{120\ min\text{-}pre}$ (%)	18.79 (3.79)	16.32 (1.12)	21.57 (0.27)	14.93 (2.13)	12.66 (2.26)
Higuchi with lag time	K_H ($mg/min^{1/2}$)	2.081	1.581	2.041	1.661	1.453
	$R^2_{adjusted}$	0.876	0.974	0.997	0.913	0.958
	AIC	40.0	24.1	8.4	34.4	26.8
	MSC	1.7	3.3	5.6	2.1	2.8
	$D_{120\ min\text{-}pre}$ (%)	17.70 (2.70)	16.32 (1.12)	21.54 (0.24)	14.20 (1.40)	12.00 (1.60)
	T_{lag} (min)	47.6	13.4	8.7	46.9	51.7
Korsmeyer-Peppas	K_{KP} (mg/min^n)	0.333	0.632	1.292	0.202	0.130
	n	0.815	0.666	0.582	0.866	0.918
	$R^2_{adjusted}$	**0.985**	**0.976**	**0.998**	**0.989**	0.997
	AIC	**23.0**	**23.5**	**7.0**	**18.3**	6.6
	MSC	**3.9**	**3.4**	**5.8**	**4.1**	5.3
	$D_{120\ min\text{-}pre}$ (%)	16.45 (1.45)	15.29 (0.09)	20.92 (0.38)	12.76 (0.02)	10.55 (0.15)

$R^2_{adjusted}$: adjusted coefficient of determination; AIC: Akaike Information Criterion; MSC; Model Selection Criterion;$D_{120\ min\text{-}pre}$ (%): predicted percentage drug release in 120 min; Values in bold case represent best goodness of fit for each formulation; Values in parentheses are absolute deviation (AD) from experimental values.

Decrease in the concentration of Tween 80® changed the release mechanism from Korsmeyer-Peppas to first order model (A1b cf. B1b cf. C1b). This implies that the concentration of the hydrophilic secondary emulsifier is critical in the drug release properties (especially amount release and release pattern) of w/o/w MEs. There was no significance difference ($p > 0.05$) between the predicted percentage of ibuprofen released in 120 min ($D_{120\ min\text{-}pre}$ (%)) and the

experimental values, thus indicating the suitability of the models in describing the release mechanism of ibuprofen from the w/o/w MEs. Also, the dissolution test duration may not be necessary to be more than 5 hours as the percentage release can be predicted using suitable models.

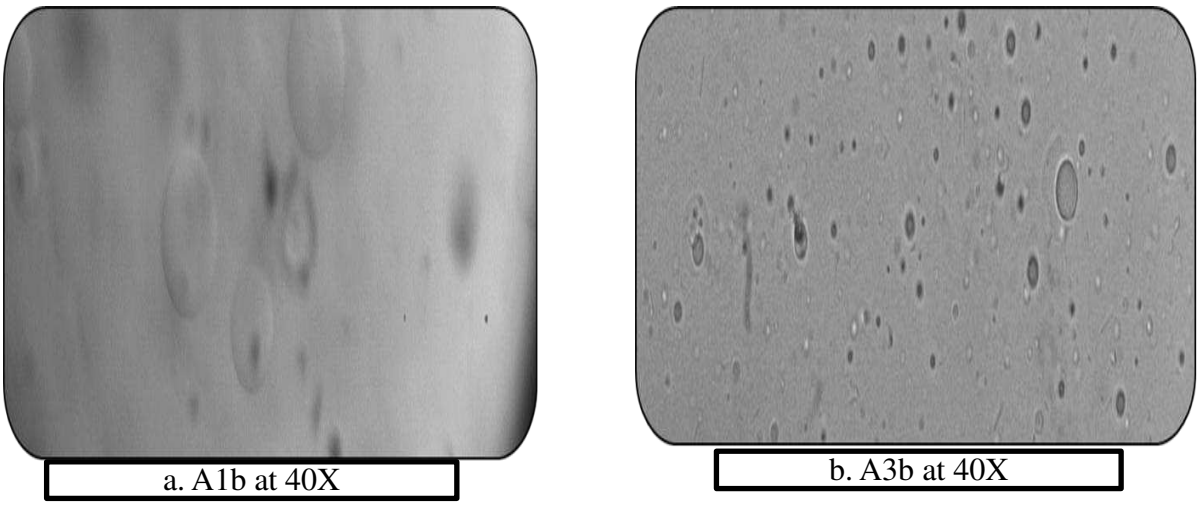

Plate 1 (a and b): Photomicrographs of A1b and A3b, respectively, after 24 hours at room temperature

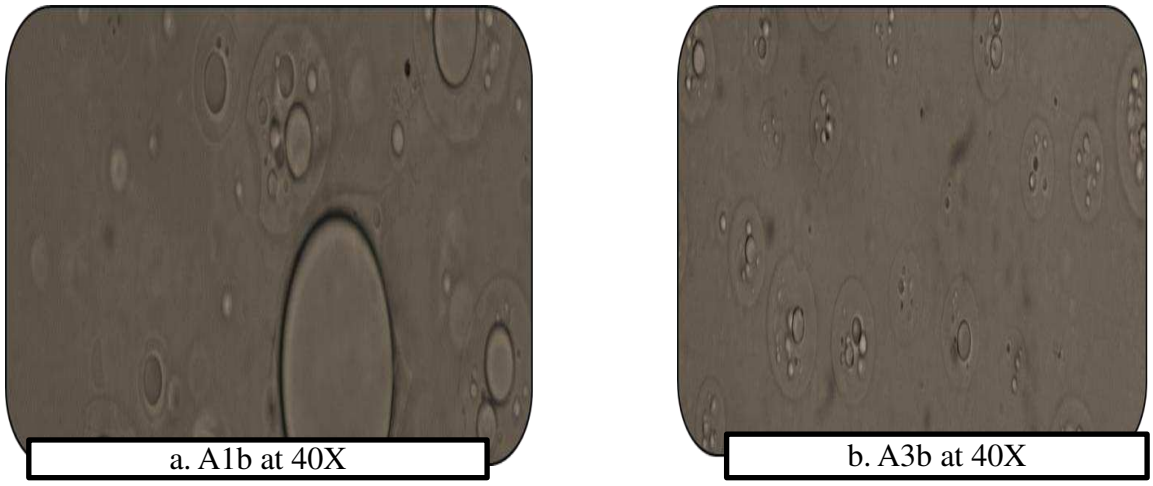

Plate 2 (a and b): Photomicrographs of A1b and A3b, respectively, after 28 days at room temperature

CONCLUSION

AWSO is highly nutritive and readily available, and thus can be used in place of mineral oils in oral liquid preparations. Literature revealed that it has not been effectively used for pharmaceutical purposes. This study employed AWSO as the drug carrier in formulation of oral w/o/w multiple emulsions of ibuprofen. Stable w/o/w MEs of ibuprofen at 100 mg/ 5 ml with AWSO, based on this study, can be prepared with 25 % w/v Span 80®, and 8, 10 or 12 % w/v Tween 80® as the primary and secondary emulsifiers, respectively. The optimum ratios of oil to water and primary emulsion to external aqueous phase were found to be 1:1 and 3:2, and 1:1 and 1:2, respectively. Based on the yield of multiple emulsion droplets, 100 mg/ 5 ml w/o/w MEs of ibuprofen (A1b and A3b) prepared with 25 % w/v Span 80® and 12 % w/v Tween 80® as the primary and secondary emulsifiers (Plates 1 and 2), respectively are suggested for further development as prolonged release paediatric ibuprofen formulation.

Stability of the w/o/w MEs was found to be affected by many factors including the ratio of the oil to water in the primary emulsion, fraction of the o/w primary emulsion in the final formulation, concentrations of the lipophilic and hydrophilic emulsifiers used in the primary and secondary emulsions, respectively, and storage temperature. All the formulations had entrapment efficiencies above 60 % with increase in ibuprofen concentration leading to increase in entrapment efficiency. Storage of the multiple emulsions at extreme temperature conditions (10 °C and 40 °C) caused instability in the

formulations. The optimum storage temperature for the formulated ibuprofen w/o/w MEs was room temperature.

The concentration ratio of the primary and secondary emulsifiers is critical in drug release properties of w/o/w multiple emulsion systems. The release of ibuprofen from the w/o/w MEs was characterised by a slow release with only ≤ 35.6 % drug release after 5 hours from the formulations. The release mechanism of the drug from the formulations was best described by Korsmeyer-Peppas release model and the release mechanism was found to be non-Fickian diffusion which is usually associated with more than one type of release process.

Based on the slow release pattern of this system, it can be used to formulate prolonged release ibuprofen emulsion which may require once daily dosing compared to other available paediatric dosage forms of the drug which require three to four times dosing daily. The inherent instability of the ibuprofen MEs is a challenge for further study. The study, however, has been able to provide information on AWSO as a possible drug carrier in the formulation of w/o/w MEs of ibuprofen for paediatrics.

FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest

REFERENCES

[1] BNFC, British National Formulary for Children "Ibuprofen.": https://bnfc.nice.org.uk>drug>ibuprofen, cited 2 October, 2018.

[2] BP, British Pharmacopoeia Volumes I, II and V. Pharmaceutical Press, London, pp. 802, appendices V (A-H), X (B-G) (2013)

[3] Madaan V, Chanana A, Kataria MK, Bilandi A. Emulsion Technology and Recent Trends in Emulsion Applications. *International Research Journal of Pharmacy*, **5**, 7 (2014)

[4] Nimberkar TP, Wanjari BE, Sanghi DK, Gaikwad NJ. Formulation and evaluation of sustained release multiple emulsion of hydroxprogesterone. *Int J Pharm Pharm Sci*, **4**(1), 76-80 (2012)

[5] Omotosho JA, Florence AT, Whateley TL. Absorption and lymphatic uptake of 5-fluorouracil in the rat following oral administration of w/o/w multiple emulsions. *Int. J. Pharm.*, **61**, 51–56 (1990)

[6] Asuman B, Ongun MS. Delivery systems for antigens. Multiple emulsions: Technology and applications (Abraham A ed.) John Wiley and Sons, Inc., USA, pp. 293–306 (2008)

[7] Bhowmick M, Sonakpuriya P, Pandey G, Joshi A., Dubey B. Formulation and evaluation of multiple emulsion of valsartan. *International Journal of PharmTech Research*, **5**(1), 132-146 (2013)

[8] Neeraj B, Siddharth P, Shikha A, Dishant G. A Review on Multiple Emulsions. *International Journal of Pharmaceutical Erudition*, **3**(2), 22-30 (2013)

[9] Vaziri A, Warburton B. Slow release of chloroquine phosphate from taste masked w/o/w multiple emulsion. *Journal of Microencapsulation*, **11**(6), 641 (1994)

[10] Laugel C, Baillet A, Piemi MPY, Marty JP, Ferrier D. Oil-in-water-in-oil multiple emulsions for prolonged delivery of hydrocortisone after topical application: comparison with simple emulsions. *Int. J. Pharm.*, **160**, 109-117 (1998)

[11] Florence AT, Whitehill D. The formulation and stability of multiple emulsion. *Int. J. Pharm.*, **11**, 277 (1982)

[12] Aryapak S, Ziarati P. Nutritive value of Persian walnut (*Juglans regia* L.) Orchards. *American-Eurasian Journal of Agricultural and Environmental Science*, **14**(11), 1228-1235 (2014)

[13] Ayoola PB, Onawumi OOE, Faboya OOP. Chemical evaluation and nutritive values of *Tetracarpidium conophorum* (Nigerian walnut) seeds. *Journal of Pharmaceutical and Biomedical Sciences*, **11**(15), 1-5 (2011)

[14] Grace MH, Warlick CW, Neff SA, Lila MA. Efficient preparative isolation and identification of walnut bioactive components using high-speed counter-current chromatography and LC-ESI-ITTOF-MS. Plants for Human Health Institute, Food Bioprocessing and Nutritional Sciences, **158**, 229-238 (2014)

[15] Onawumi OOE, Faboya OOP, Ayoola PB. Chemical evaluation and nutritive values of African walnut leaf (*Plukenetia conophora* Müll.Arg.). *International Journal of Herbal Medicine*, **1**(3), 122-126 (2013)

[16] Neeraja P, Amaleshwari M, Ravali G. Formulation and evaluation of nifedipine multiple emulsions. *International Journal of Pharmaceutical, Chemical and Biological Sciences*, **4**(3), 673–680 (2014)

[17] Becher P. Emulsions: Theory and Practice. American Chemical Society Monograph, No.135. Rheinhold Publishing Corporation, New York (1957)

[18] Oladimeji FA. Formulation studies on essential oil of *Lippia multiflora* Moldenke for management of scabies. Ph.D thesis, Obafemi Awolowo University, Ile-Ife, Nigeria (2003)

[19] Sinha VR, Kumar A. Multiple Emulsions: An overview of formulation, characterization, stability and applications. *Int. J. Pharm.*, **64**(3), 191–199 (2002)

[20] Sherman P. Rheology of emulsion. Emulsion science. (Sherman P ed.) Academic Press, London, pp. 217-351 (1968)

[21] Omotosho JA, Law TK, Whateley TL, Florence AT. Methotrexate transport from the internal phase of multiple w/o/w emulsions. *Journal of Microencapsulation*, **6**(2), 183-192 (1989)

[22] Nakhare S, Vyas SP. Prolonged release of rifampicin from internal phase multiple w/o/w emulsion systems. *Indian Journal of Pharmaceutical Sciences*, **57**(2), 71-77 (1995)

[23] Zhang Y, Huo M, Zhou J, Zou A, Li W, Yao C. DDSolver: An add-in program for modeling and comparison of drug dissolution profiles. *Association of American Pharmaceutical Scientists Journal*, **12**(3), 263-271 (2010)

[24] Zuo J, Gao Y, Bou-chacra N, Löbenberg R. Evaluation of DDSolver software applications. *Biomedical Research International*, http://dx.doi.org/10.1155/2014/204925, cited 21 December, 2015

[25] Gurny R, Doelker E, Peppas NA. Modelling of sustained release of water-soluble drugs from porous, hydrophobic polymers. *Biomaterials*, **3**, 27–32 (1982)

[26] Brazel CS,Peppas NA. Modeling of drug release from swellable polymers. *Eur. J. Pharm. Biopharm*, **49**, 47–58 (2000)

[27] Costa P, Lobo JMS. Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Biopharm*, **13**, 123-133 (2001)

[28] Bankole VO, Oladimeji FA. Evaluation of the kinetics and mechanism of piroxicam release from lipophilic and hydrophilic suppository bases. *International Journal of Chem Tech Research*, **10**(1), 189-198 (2017)

[29] Higuchi T. Rate of release of medicaments from ointment bases containing drugs in suspension. *J Pharm. Sci*., **50**, 874 -875 (1961)

[30] 30. Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas, NA. Mechanisms of solute release from porous hydrophilic polymers. *Int. J. Pharm*., **15**(1), 25–35 (1983)

[31] Isong N, Alozie YE, Ekwere Y. Physicochemical properties of African walnut (*Tetracarpidium conophorum*) oil and its suitability for domestic and industrial uses. *Nigerian Journal of Agriculture, Food and Environment*, **9**(3), 12–15 (2013)

[32] Agarwal SP, Rajesh K. Basics of pharmaceutical emulsions: A review. Physical Pharmacy. CBS Publisher, Delhi, India, pp. 177-186 (2007)

[33] Karleskind A. Manuel des corps gras. Association francaise pour l'etude des corps gras, Paris. **2**, 787–792 (1992)

[34] Nkafamiya, MHM, Osemeahon, SA, Modibbo. Percentage oil yield and physicochemical properties of different groundnut (*Arachis hypogea*) species. *African Journal of Food Science*, **4**(7), 418-421 (2010)

[35] Martin A, Swarbick J, Cammarata A. Physical Pharmacy, 3rd edition, Lea and Febiger, Philadelphia, USA, pp. 75-87; 445-68; 492-573 (1983)

[36] Patrick JS. Martin's Physical Pharmacy and Pharmaceutical Sciences, 5th edition. Lippincott Williams and Wilkins, Philadelphia, USA, pp. 509-516; 561–576 (2006)

[37] Paul B. The Science and Practice of Pharmacy, Remington. Lippincott Williams and Wilkins, Philadelphia, USA, pp. 325-335, 759-760 (2005)

[38] McClements DJ. Food Emulsions: Principles, Practice and Techniques. CRC Press, New York, pp. 53-339 (1999)

[39] Vasiljevic D, Parojcic J, Primorac M, Vuleta G. An investigation into the characteristics and drug release properties of multiple w/o/w emulsion systems containing low concentration of lipophilic polymeric emulsifier. *Int. J. Pharm*., **309**, 171–177 (2006)

[40] Tirnaksiz F, Kalsin O. A topical w/o/w multiple emulsions prepared with Tetronic 908 as a hydrophilic surfactant: Formulation, characterization and release study. *Int J Pharm Pharm Sci*, **8**, 299–315 (2005)

[41] Omotosho JA. The effect of acacia, gelatin and polyvinylpyrrolidone on chloroquine transport from multiple w/o/w emulsions. *Int. J. Pharm.*, **62**, 81-84 (1990)

[42] Pal R. Shear viscosity behaviour of emulsions of two immiscible liquids. *Journal of Colloid and Interface Science*, **225**(2), 359-366 (2000)

[43] Hou W, Papadopoulos KD. W/O/W and O/W/O globules stabilized with Span 80 and Tween 80. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **125**, 181-187 (1987)

[44] Okubanjo OO, Odeku OA. Effect of interacting variables on the mechanical and release properties of chloroquine phosphate suppositories, *ActaPharma. Sci.*, **51**, 281-288 (2009)