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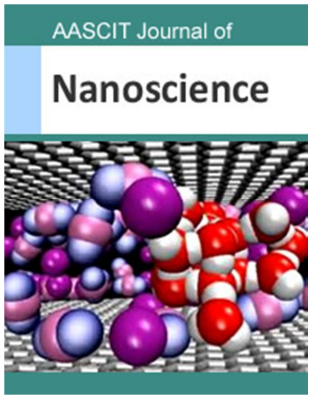

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Formulation and Evaluation of Erythromycin Stearate Soy Lecithin Nanoemulsion

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Citation

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Abstract

The stability and antibacterial activities of erythromycin oil-in-water nanoemulsion in batches F1, F2, F3 (160 mg of erythromycin) and a control having varying amounts of soy oil, soy lecithin and Tween 80 made up to 20 ml with distilled water, homogenising at 7500 rpm for 30 min. They were centrifuged and verified for electrical conductivity, pH, viscosity, droplet size, polydispersity index (PDI), zeta potential, acute toxicity, *in vitro* antibacterial study against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus pneumoniae* together with *in vivo* antibacterial activity in rats besides a commercial erythromycin suspension. The nanoemulsions exhibited no cracking, creaming, phase separation or flocculation. Conductivities and viscosities remained stable through 90 days. The viscosity improved as the oil content increased across the formulations resulting in enlarged particle size and PDI of 237.2 nm and 0.467 for F1, F2 and F3 each had particle sizes of 290.6 nm and PDI of 0.670. The pH was fairly stable between 5 and 6. There was no sign of toxicity or death with the highest dose of the nanoemulsions. There was enhanced antimicrobial activity against *S. aureus* and *S. pneumoniae* similar to those of the commercial erythromycin suspension with the activities of the nanoemulsions higher at 0.14mg/ml compared to 0.78mg/ml of the commercial product. The *in vivo* antibacterial activity of nanoemulsions compared with the commercial product in rats. With the stability of the nanoemulsions up to 90 days, it could be concluded that the erythromycin soy lecithin nanoemulsion had improved solubility, stability and bioavailability.

1. Introduction

The availability of poorly soluble drugs is on the increasing rate of discovery of newer drugs, giving rise to up to 60-70% of drugs that are poorly soluble in the aqueous medium, causing limitations in the conventional formulation methods in providing satisfactory bioavailability and targeted clinical outcomes [1, 2]. Currently, there are advanced delivery technologies, such as lipid-based drug delivery systems (LBDDS) which have become a key technique in the development of new chemical entities. The

LBDDS technologies engage lipid molecules alone or in combination with other biocompatible materials, to present drugs in a more harmonious form to the biological system. It is a commercially proven delivery technology providing enhanced bioavailability for poorly soluble compounds and clinical advantages over conventional formulation approaches [3]. The LBDDS is composed of lipids and surfactants. It may also contain a hydrophilic co-solvent. Many of them are typified as self-emulsifying drug delivery systems (SEDDS) such that they form an emulsion upon gentle agitation in water. Emulsions are considered metastable systems with droplet sizes of 100-1000nm. Other formulations such as self-micro emulsifying drug delivery systems (SMEDDS) form microemulsions that are thermodynamically stable systems. Microemulsions have droplet diameters <100 nm and are visually transparent or translucent [4]. The drug is generally present in the dosage form dissolved in the formulation and should remain solubilized after the dispersion of the dosage form in the GIT. Absorption by the intestinal mucosal cells is enabled by the quick release of drug from the high surface area of the microemulsion droplets.

The LBDDS has some advantages. It has been shown to improve solubility and bioavailability. In addition to these, it offers many other clinical advantages over conventional formulations for many poorly soluble compounds, including lowering of therapeutic dose due to improved drug absorption, etc.

The LBDDS include lipid solutions, emulsions, self-emulsifying systems (SEDDS and SMEDDS), liposomes and solid lipid-based particles. These formulations provide better bioavailability due to improved solubilization of a compound and increased surface area causing emulsification and micro-emulsification of the lipid formulation in the GIT [5].

Factors affecting drug absorption from a lipid-based formulation include particle size, the degree of emulsification, the rate of dispersion and precipitation of drug upon dispersion [6-8]. Lipid-based formulations may include oil solutions, suspensions, emulsions, self-micro and nano emulsifying drug delivery systems [9-11]. Some of the drugs that are successfully marketed as lipid-based formulations include Efavirenz (Sustiva®), Saquinavir (Fortovase ®), Ritonavir (Norvir ®), Clofazimine (Lamprene ®) [5].

In the LBDDS methods, one of the most encouraging technologies is the nanoemulsion drug delivery system which is useful to enhance solubility and bioavailability of lipophilic drugs [12]. It comprises fine oil-in-water dispersions, having droplets covering the size range of 100-600 nm. Nanoemulsions, usually spherical, are a group of distinct units used for pharmaceutical biomedical aids and vehicles that have possible usefulness in cosmetics, diagnostics, drug therapies and biotechnologies [13]. The terms sub-micron emulsion (SME) [14], mini-emulsion [15] and ultra-fine emulsion [16] have been used as substitute expression. It is a varied blend of lipid and aqueous phase with its uniformity attained with the use of emulsifying agents. It has been upheld that with the help of nanoemulsion as a delivery system retention time of a drug in the body can be amplified, so low amount of drug is required for the therapeutic action [17]. Presently this dosage form is used for the delivery of various biopharmaceuticals such as vaccines, DNA encoded drugs [18], antibiotics, cosmetics and other topical preparations [19]. It can also be used to deliver drugs through several routes including oral [17], ocular [20] and transdermal [21, 22].

Preparations of the oil-in-water type of nanoemulsion have long been in use [13, 14, 23, 24] but the water-in-oil type of nanoemulsion was recently considered [25, 26]. Both oil-in-water and water-in-oil type of nanoemulsion have many advantages in both the pharmaceutical and cosmetics sciences. The explanations for their many applicability include that nanoemulsion, due to their very small droplet size, does not often exhibit the creaming and sedimentation kind of instability which is very common with the conventional emulsion and even microemulsion. This stability is because of the lesser influence of gravitational force on the nanoemulsion droplets due to its very small droplet size. This also inhibits the coalescence of droplets. In the process of coalescence, droplets come together and form a large droplet with increased size that results in the breakdown of the emulsion. But the small droplet size of nanoemulsion avert the coalescence among them and prevent the distortion and then surface fluctuation. More advantages include the fact that dispersibility of nanoemulsion is very high as compared to the microemulsion [27-34]. The setback with the nanoemulsions includes the high cost of production since the type of surfactants and co-surfactants needed to produce them is difficult to procure and also complex equipment is needed to reduce them to the required droplet size [35].

The main purpose of producing the nanoemulsion is to attain a droplet size range of 100-600 nm and offer the stability state it requires. Several processes are applied to achieve these and comprise sonication and a high pressure-homogenizer [36-39]. Being thermodynamically unstable, the features of nanoemulsions will depend on the production technique. The following parameters should be embraced to analyse any product of nanoemulsion at the time of manufacture [40] and consist of phase behaviour study, particle size analysis, surface charge measurement, transmission electron microscopy (TEM), drug content and viscosity. The unpredictability of the nanoemulsions is due to some issues that include creaming, flocculation, coalescence and Oswald ripening [41-43]. Among them, the Oswald ripening is the key mechanism of nanoemulsion instability because the other impediments are abated by the small size of nanoemulsion and use of a non-ionic type of surfactant. Creaming of nanoemulsion is hindered by the rapid diffusion rate of smaller droplets. Van der Waals forces are liable for the attraction of droplets and lead to the flocculation of the emulsion. But in the case of nanoemulsion non-ionic surfactant, it does not create any kind of attractive force,

hence no flocculation occurs. The droplet size of nanoemulsion also averts the flocculation because these small droplets show high curvature and Laplace pressure opposes the deformation of large droplets [44]. Coalescence of droplets of nanoemulsion can be stopped by a thick multilamellar surfactant film adsorbed over the interface of droplets [45]. Erythromycin is sparingly soluble in water due to its lipophilic nature. Under the Biopharmaceutics Classification System (BCS) guidance, it has been classified as a class II drug, having low solubility with high permeability. It is also simply destabilised by acids and hence, is very unstable in the gastric environment. These contribute to its poor absorption and eventual low bioavailability. Erythromycin is administrated at 500 mg 6 hourly to offer its antibacterial action. Patient's compliance with this dosage regimen is difficult, leading to drug misuse. Therefore, there is a need to formulate erythromycin into a better dosage form that could be administrable at a lower dosage with longer dosage intervals.

This research work is therefore aimed at formulating erythromycin oil in water nanoemulsion system and to evaluate its stability and antibacterial activity.

2. Materials and Methods

2.1. Materials

The following materials were used as procured and included: erythromycin stearate (Leisha Pharm Solutions, India), soy lecithin, soya beans oil (Sunola Foods Ltd), tween 80 (William& Sons, England), isopropyl alcohol, Mueller-Hinton Agar MHA (Oxoid, Difco USA), Blood Agar (BA, Titan Biotech)

2.2. Methods

Prior to the formulations, solubility profile and the calibration of erythromycin was determined by dissolving a 1000 mg quantity of erythromycin stearate in 10 ml of methanol. A 1 ml volume of the solution was made up to 10 ml in 9 ml of water until very dilute concentrations was obtained with the least concentration of 0.001mg/ml.

The absorbance was recorded for each of the concentrations using water as blank in a UV – spectrophotometer (C109, Brazil) at 280nm. A graph of absorbance against concentration was plotted and K was calculated.

The solubility profile of erythromycin in the various admixtures of soya oil and lecithin was determined. This was carried out by preparing a 10 ml volume of the soya oil-lecithin mix with different ratios of: 10:1, 9:1, 8:2, 7:3, 6:4 and 5:5. A 10ml of the oleaginous admixture in each case was placed in a beaker and 1g of erythromycin was added to it and was mixed using a magnetic stirrer. The mixture was kept overnight at room temperature. The samples were centrifuged at 3000 rpm for 30 min using the 800-B electronic centrifuge. The supernatant was pipetted and the absorbance of each supernatant was read at λ_{max} of 280 nm after calibrating with the oil mix of the same ratio. The concentration of erythromycin dissolved in each mix was calculated using beers calibration curve formula:

$$Absorbance = k \times concentration\ (mg/ml).$$

2.2.1. Formulation of Nanoemulsion

The different batches of nanoemulsion was prepared using the formula

Formulation 1 (F1)	
Erythromycin stearate	160.00mg
Soya bean oil	2.00ml
Soy lecithin	2.00ml
Tween 80	2.00ml
Freshly distilled water to	20.00ml

Formulation 2 (F2)	
Erythromycin stearate	160.00mg
Soya bean oil	2.50ml
Soy lecithin	2.50ml
Tween 80	2.00ml
Freshly distilled water to	20.00ml

Formulation 3 (F3)	
Erythromycin stearate	160.00mg
Soya bean oil	3.50ml
Soy lecithin	3.50ml
Tween 80	2.00ml
Freshly distilled water to	20.00ml

For each of the formulation, the soya bean oil and the soy lecithin were blended. Erythromycin stearate (160.00 mg) was solubilised in the mix. The Tween 80 was added and the water phase was also added and homogenised at 7500 rpm for 30 min to obtain small nanosized droplets using a homogenizer (T 25 Digital Ultra-Turrax®-Ika, Germany).

The following tests were conducted on the respective batches of nanoemulsions formulations:

2.2.2. Centrifugation Test

Each batch was centrifuged at 1000, 2000 and 3000 rpm for 10 min to determine whether it will show signs of creaming or phase separation. The nanoemulsions were observed visually for change in appearance.

2.2.3. Electrical Conductivity

The electrical conductivity of the nanoemulsions were measured using a portable conductometer at room temperature. The readings were taken every 24 hr. for 30 days and later on days 60 and 90.

2.2.4. pH Measurement

The pH was measured using a digital pH meter at room temperature. Daily readings were taken for 30 days and later at day 60 and 90.

2.2.5. Viscosity Measurement

The viscosity was measured using a U-tube viscometer at room temperature. The readings were taken every 24hrs for 30 days and then on days 60 and 90.

2.2.6. Determination of Droplet Size, Polydispersity Index and Zeta Potential

Droplet size, polydispersity index and zeta potential was determined by photon correlation spectroscopy which analyses the fluctuations in light scattering due to a Brownian motion of particles using a Zetasizer (ZEN1600 Nano series, Malvern Instruments Ltd, UK). For each determination, the formulation was diluted at the ratio of 1:200 using deionized water inside the cuvette and was carried out in triplicate. A micro filter of pore size 0.22 µm was used to filter the deionized water. The refractive index of the oil and water were set at 1.46 and 1.33, respectively and was carried out thrice. These were repeated on days 0, 30 and 90 during storage on shelf conditions at 25°C.

2.2.7. Determination of Antimicrobial Activity (Agar-Well Diffusion Assay)

Antibacterial activity of the formulations was evaluated by the cup plate agar diffusion method as applied by Aida et al [46]. Human pathogenic bacteria Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi and Streptococcus pneumoniae were used.

The bacterial cultures were adjusted to 0.5 McFarland turbidity standard and 0.1mL of each of the test organisms was seeded onto sterile Mueller-Hinton Agar (MHA) (Oxoid, Difco, USA) plates, with the exception of S. pneumoniae that was seeded onto Blood Agar (BA)(Titan Biotech) plates (diameter: 90mm). Six wells (6 mm in diameter) were made and labelled A1-A6 on each of the MHA and BA plates.

Aliquots of 100 µl of the formulation dilutions, at different concentrations, were applied in each of the wells in the culture plates previously seeded with the test organism. The cultures were incubated at 37 ^{o}C for 18-24 hrs. Antibacterial activity was determined by measuring the zone of inhibition (ZOI) around each well (excluding the diameter of the well). For each concentration, three replicate trials were conducted against the test organism.

2.2.8. Acute Toxicity Test

The acute toxicity study was performed using the Lorke's method [47] as described by Chinedu *et al* [48]. Nine white male albino mice were used. They were divided into three animals per group. The three groups were administered with 10, 100 and 1000 ug/kg body weight respectively.

The animals were observed for change in behaviour and mortality for 24 hrs.

2.2.9. Determination of *in Vivo* Antibacterial Activity

Adult male Wistar rats weighing 150-200 g were obtained from the Department of Veterinary Medicine, University of Nigeria Nsukka, Nigeria were housed and fed and observed for 48 hrs.

The animals were assembled into 5 groups (A-E) comprising 5 animals per group. Group A was treated with F1, group B (treated with F2), group C (treated with F3), group D (treated with commercial erythromycin suspension) and group E (treated with water).

Blood was withdrawn from the animals, pre-infection, and viable bacterial cell count was determined by agar dilution method with Muller-Hinton agar (Oxoid, Difco, USA). The animals were infected by injecting them with 0.5ml of bacterial suspension intraperitoneally. The bacterial suspension was prepared from an overnight cultures of typed *S. aureus* obtained from the Department of Pharmaceutical Microbiology, Nnamdi Azikiwe University Awka, Nigeria on Muller-Hinton agar.

The animals were allowed, monitored and fed for 72 hrs after which blood was aseptically withdrawn and the viable bacilli in the serum was counted on Muller-Hinton agar plates to determine the establishment of infection.

In the animals with established active infection, treatment was initiated. The drugs were administered orally at a dose of 25 mg/kg twice daily and blood was withdrawn and cultured daily and viable bacteria cell counted on Muller-Hinton agar plates until treatment was established.

3. Results and Discussions

The solubility of erythromycin in different proportions of the oil mix was carried out to determine the solubility of the drug in the various ratios of oil combinations. According to Audumbar D. M *et al* [49], the most important criteria for screening of excipients is the solubility of the poorly soluble drug in oil and surfactants. As shown in Figure 1, the solubility of erythromycin stearate was found to be highest in 5 parts of oil and 5 parts of lecithin which is 10 mg/ml. Erythromycin has poor solubility in water, about 2 mg/ml at room temperature [50].

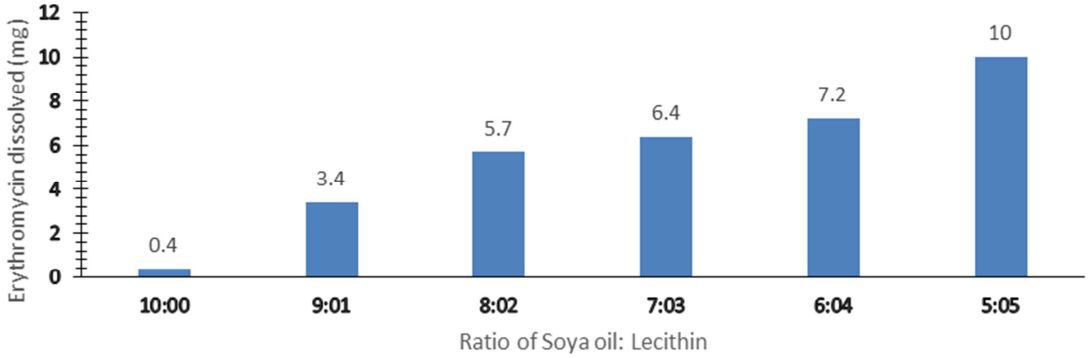

Figure 1. *Solubility of erythromycin in different proportions of Lecithin and Soya oil.*

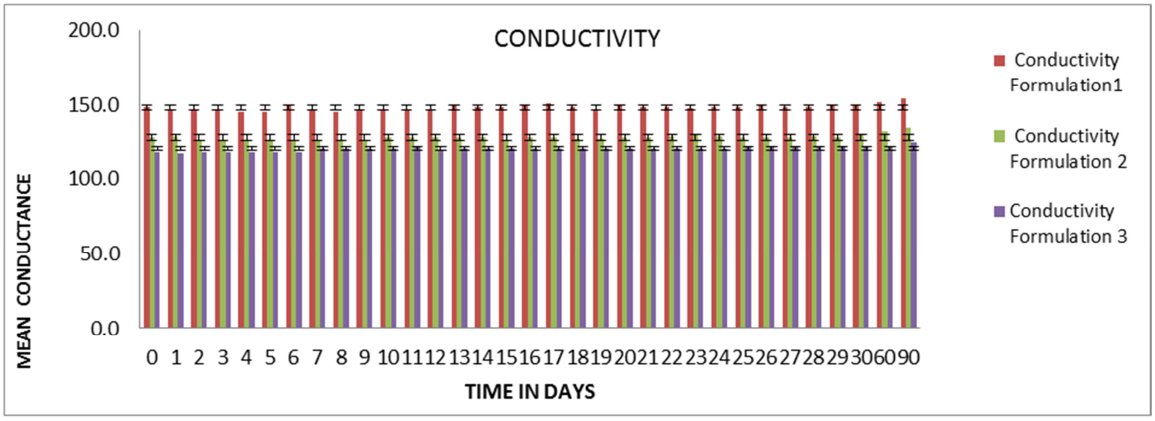

Figure 2. *Graph showing conductivity (µS/cm) readings of the various the formulations (F1, F2, F3) against time (days).*

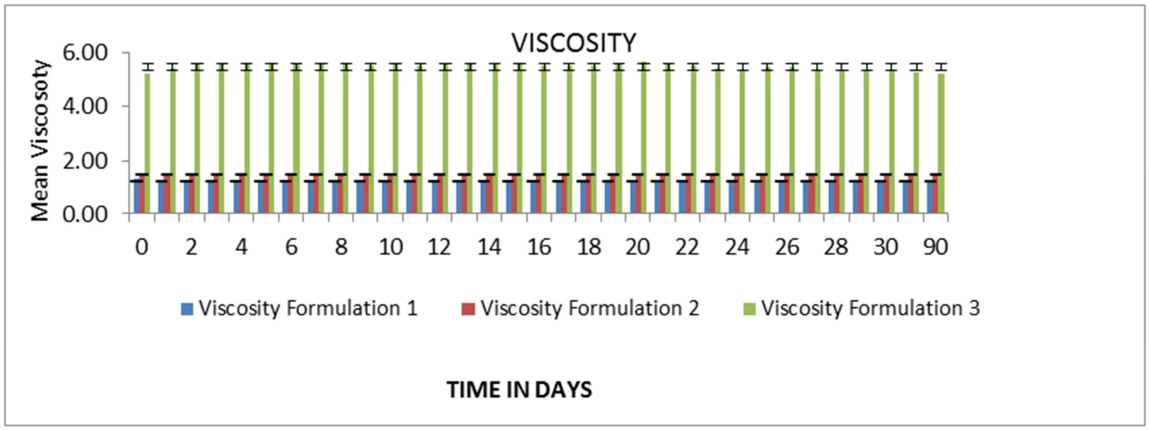

Figure 3. *Graph of viscosity (cP) of the various formulations (F1, F2, F3) against time (days).*

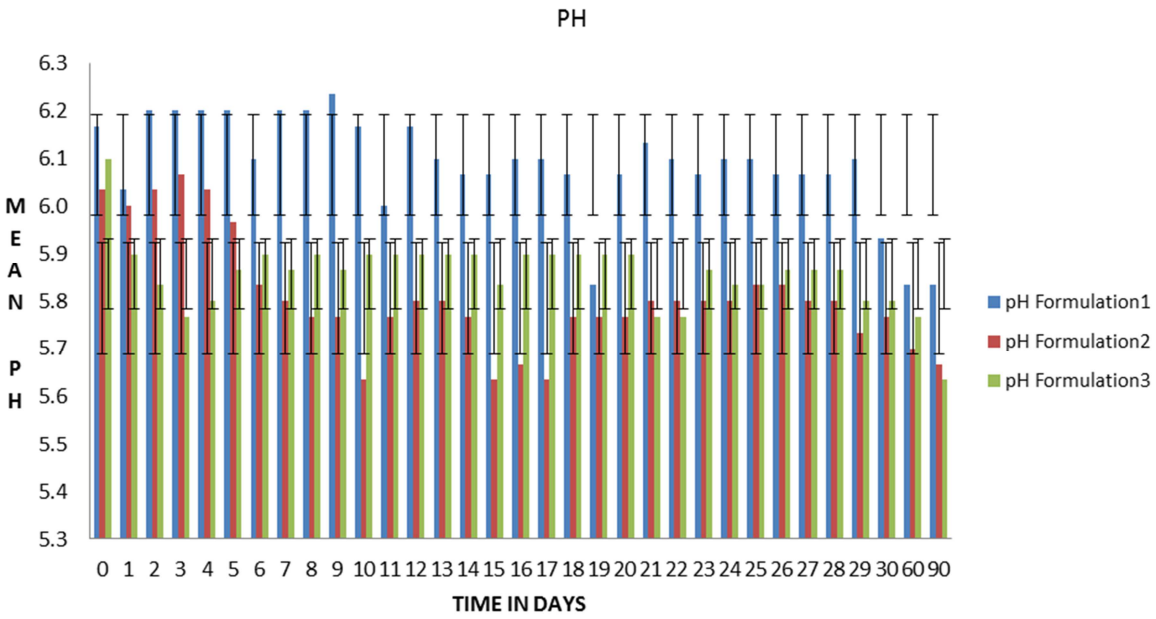

Figure 4. *Graph of pH of the various formulations (F1, F2, F3) against time (days).*

The conductivity of the nanoemulsions was measured to determine the phase system (o/w or w/o) of the nanoemulsion [51]. Oil-in-water nanoemulsions are highly conducting because water is in the external phase. On the other hand, water-in-oil nanoemulsion, where water is in the internal phase is less conducting [52, 53]. From the conductivity results in Figure 2, the nanoemulsion formulations were detected as oil-in-water nanoemulsions. The conductivities remained fairly constant over 90 days period of the study showing the absence of phase inversion. It has been noted

that the absence of phase inversion in nanoemulsions over a period of time is an indicator of the good stability of the nanoemulsion system [54]. It was also observed that increase in the oil component of the nanoemulsion resulted to a reduction in the conductivity of the nanoemulsion.

Viscosity is another good measure of the stability of emulsions as a change in viscosity over a period of time would indicate a breakdown of the nanoemulsion system. The viscosity of all the formulations (F1, F2 & F3) was determined Figure 3. It was observed that viscosity increased as the oil content increases across the formulations, F1 being significantly lowest compared to those of F2. F3 had the highest viscosity. Though low viscosity was observed for all the formulations, however, this coincides with one of the characteristics of nanoemulsion which is low viscosity [55]. The viscosity of the respective formulations remained fairly constant over the 90 days period of study.

Figure 4 is a graphical representation of apparent pH recorded for all the formulations within the period of the study. Monitoring the pH value is important for determining the emulsions' stability because pH changes indicate the occurrence of chemical reactions that can compromise the quality of the final product. The pH of the nanoemulsions was fairly stable between the pH of 5-6 which is an acceptable value and is not irritating to the skin. Though some slight changes in pH were observed on some days, this could be due to hydrolysis of the vegetable oil used in the formulations as vegetable oils usually undergo hydrolysis giving a drop in pH due to the hydrolysis of fatty acid esters into free fatty acid degradation products [54, 56]. However, all the formulations maintained a fairly constant pH over 90 days and this could be an indicator of the good stability of the nanoemulsions, especially in the absence of a preservative as is the case in this formulations.

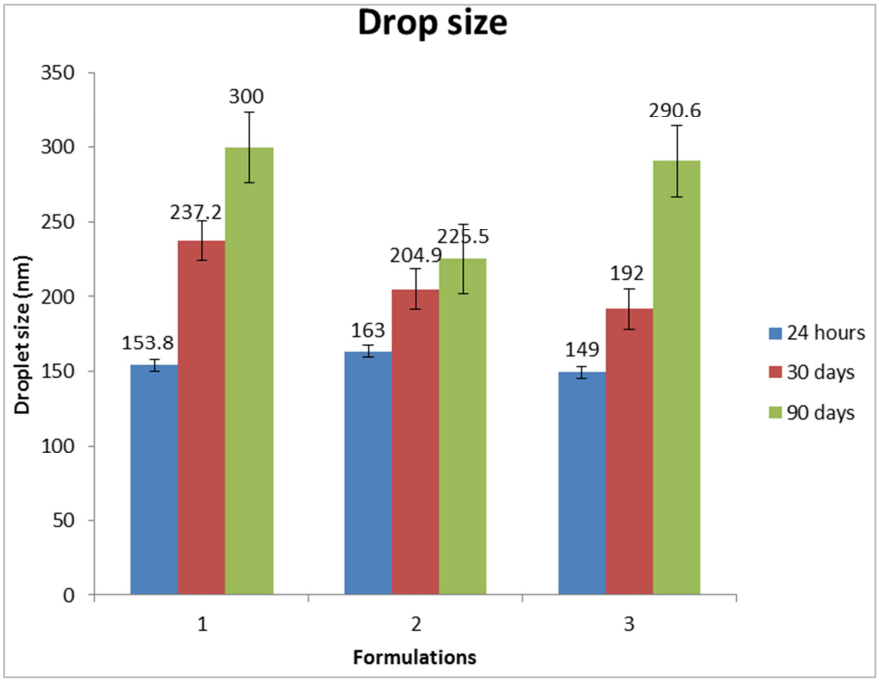

Figure 5. *Showing the drop size of the different formulations (F1, F2 & F3) on different days.*

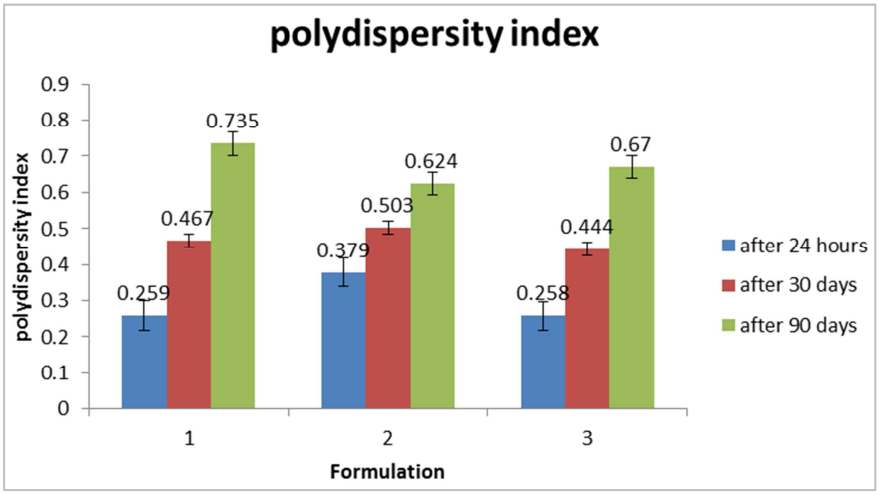

Figure 6. *Showing the Polydispersity index (PDI) after several days.*

Figures 5 and 6 show the droplet size distribution and polydispersity index (PDI) for the respective formulations (F1, F2 & F3) after 24 hrs, 30 and 90 days respectively.

Photon correlation spectroscopy which evaluates the fluctuations in light scattering due to a Brownian motion of particles using a zeta sizer was used to measure droplet size of formulations (F1, F2 & F3) (Figure 5). Nanoemulsions have been defined as oil-in-water (o/w) emulsions with average droplet size between 100 and 500 nm [60]. Other authors stated that formulations with emulsion size 200 nm and below are considered nano-size and above 200 nm is micron-size [61]. The particle sizes were notably below 200nm size for all the formulations in the first day after their preparation. Between days 30 -90, the average droplet size were generally found to be above 200 nm. Though F2 and F3 had more oil carrier in composition than F1, it was expected that this was supposed to lead to an increase in the viscosity of the disperse phase and consequently increase in flow resistance and restricted droplet break-up rate which could be the likely reason for droplet size increase after storage.

Droplet size is a good indicator of the formulation stability. A fast droplet size increase indicates low stablilty (Ali *et al*, 2012). From the results obtained, all the formulations had droplet sizes in the nano range and PDI of less than 1 after 90 days. This could be an indicator of good stability and uniformity of droplet size within each formulation. It was however observed that increase in the oil component of the formulations resulted in a decrease in particle size and polydispersibility index (PDI) with F1 having a particle size of 153.8nm and PDI of 0.259nm while F3 had particle size 149 nm and PDI of 0.258 having the highest oil concentration.

The PDI, which is a dimensionless measure of the broadness of the size distribution derived from the cumulative analysis of dynamic light scattering ranging from 0-1. The PDI indicates the quality or homogeneity of the dispersion [62]. Precisely, PDI value close to zero denotes the mono-dispersion system and value close to 1.0 suggests that the emulsion has a very broad size distribution. Therefore, PDI values lower than 0.2 indicate homogenous populations, while a 0.3 value represents heterogeneity. The desirable characteristics of the formulation are the high stability of two immiscible components to stay mixed as one phase emulsion. The acceptable PDI value should be less than 0.7 depending on the sample type [57-59]. All the formulations studied presented PDI lower than 0.7 except at 90 days when F1 and F3 displayed PDI values slightly above 0.7. However, increase in particle size and PDI could also be attributed to the method of preparation of the nanoemulsions.

It has been observed that high energy emulsification method yields nanoemulsion with larger particle size and PDI, hence lower stability in comparison to the lower energy emulsification method [63]. Increase in particle size over the 90 days could also result from the lack of preservative used in the preparation as the emulsion could easily be attacked by microbes.

From all the formulations made, F2 showed better particle size with narrow size distribution.

3.1. Centrifugation Test

After centrifuging, no physical instability like cracking, creaming, phase separation or flocculation was observed. This could be as a result of the good homogeneity of the overall system.

3.2. Acute Toxicity Test

The various dilutions were administered to the animals according to their body weights and no deaths occurred even with the administration of the highest dose. This shows that the nanoemulsion is safe at all concentrations (Table 1).

Table 1. *Showing acute toxicity test results.*

Number	1	2	3	4	5	6	7	8	9
Dose (mg/kg)	10.0	10.0	10.0	100.0	100.0	100.0	1000.0	1000.0	1000.0
Remarks	ND	ND	ND	ND	ND	ND	ND	ND	ND

Key: ND = No death

3.3. *In Vitro* Antibacterial Activity

Table 2. *Minium inhibitory concentration (MIC) (mg/ml) of the anti bacterials against different test organisms.*

Formulations	Minimum inhibitory concentration (mg/ml)		
	S.aureus	*S.pneumoniae*	*P.aeruginosa*
F1	0.2239	0.2813	-
F2	0.1816	0.2383	0.2591
F3	0.1764	0.1639	0.1672
Commerical suspension	0.9809	0.8266	-
Plain nanoemulsion without drug	-	-	-

Table 2 shows that erythromycin nanoemulsion had low MICs against the organisms. The formulations recorded lower MICs with an increase in the oil-lecithin mix, with F3 having the lowest MIC against *S.aureus, S.pneumoniae* at 0.1764 mg/ml, 0.1639 mg/ml, respectively when compared to F2 that had 0.1816 mg/ml, 0.2383 mg/ml, F1 with 0.2239 mg/ml, 0.2813 mg/ml, respectively against *S.aureus, S.pneumoniae*. It was observed that plain nanoemulsion lacking the antibiotic (erythromycin) had no activity against the organisms showing that the activity of the erythromycin loaded nanoemulsion is not just due to the excipients. The commercial suspension also showed good activity against *S.aureus, S.pneumoniae* with the MICs of 0.9809 mg/ml and 0.8266 mg/ml respectively for both organisms. It was observed that the commercial suspension and F1 had no activity against *P. aeruginosa,* while F2 and F3 had activity

against the organism as shown by the MICs of 0.2591mg/ml and 0.1672 mg/ml respectively. The enhanced activity demonstrated when compared to the commercial suspension could be attributed to the reduced particle size, higher solubility and bioavailability of erythromycin in the nanoemulsion formulation matrix. It was also observed that F2 and F3 had lower MICs than F1. Therefore it could be deduced that an increase in the oil- lecithin component of the nanoemulsion led to a higher solubility of the drug in the matrix, thus achieving improved penetration into the nutrient agar.

3.4. *In Vivo* Antibacterial Activity

In vivo antibacterial activity of erythromycin nanoemulsion compared with commercial erythromycin suspension in rats.

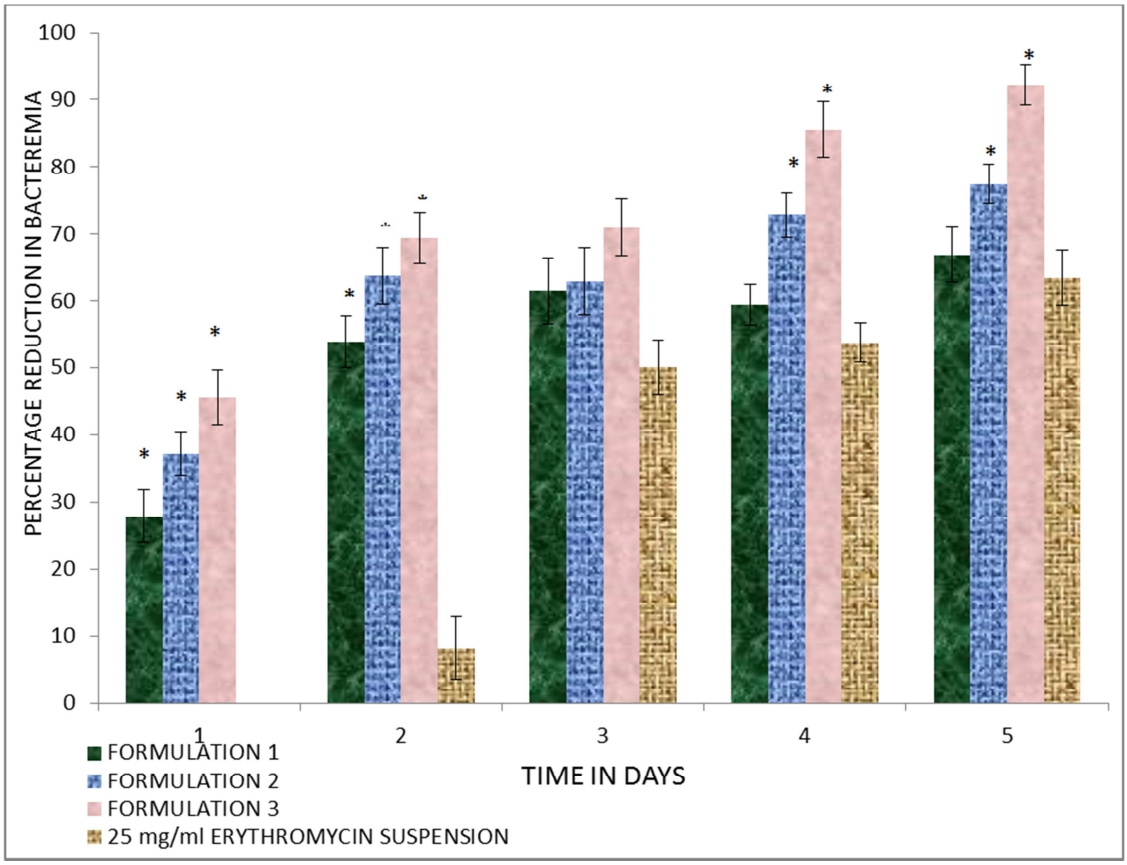

Figure 7. *In vivo antibacterial activity of erythromycin nanoemulsion and commercial erythromycin suspension against S. aureus. (*significantly different at p<0.05 from group treated with commercial suspension).*

Group A= treated with F1; Group B = treated with F2; Group C = treated with F3; Group D = treated with commercial erythromycin suspension
Group E = treated wth water

Figure 7 above shows the total viable count of bacteria in the blood of the animals before and after infection and during treatment using the erythromycin nanoemulsion and commercial erythromycin suspension.

In pre-infection, all animals in the group had no significant (countable) bacterial load in their blood but post infection showed uncountable bacterial load in almost all animals.

On day 1 of the treatment, the animals administered with the nanoemulsions showed a significant decrease in bacterial load when compared with the ones treated with the commercial erythromycin product. On day 2, there was a further decrease in bacteraemia especially for the animals in group C administered with F3. This is in line with the *in vitro* antibacterial activity which showed that F3 have lower MICs. This could be due to an enhanced solubility of the drug in the increased oil-lecithin mix of the formulation hence improved bioavailability.

On day 5 of treatment very high significant decrease in parasitemia was observed both n the nanoemulsion and commercial suspension but the nanoemulsion had lower bacteria count than that of the commercial erythromycin suspension.

On the whole, the nanoemulsion exhibited a better activity when compared to the commercial preparation, this could be due to the reduced particle size in the nano range which could easily penetrate the gastrointestinal walls by overcoming the first pass effects. It could also be due to the improved solubility of the erythromycin in the nanoemulsion system allowing for better solubility in the gastrointestinal tract hence improved absorption and then bioavailability.

It could also be due to the ability of the nanoemulsion to mask the drug, protecting it from the acidic gastric fluids improving its stability in the stomach which is not obtainable in the commercial suspension.

4. Conclusion

Erythromycin soy lecithin nanoemulsion was formulated using a high-speed homogenizer. The particle size of the formulation was within the nano size range and demonstrated much better antibacterial activity *in vitro* and *in vivo* when compared to the commercial erythromycin suspension. The nanoemulsion system was stable for the 3 months period of study. Hence, it could be concluded that the erythromycin soy lecithin nanoemulsion had improved solubility, stability and bioavailability.

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