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REVIEW ARTICLE

GENERATION, CHARACTERIZATION AND PHARMACOKINETIC STUDY OF OFLOXACIN-LOADED CASTOR OIL BASED NANOEMULSION

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ABSTRACT

The challenges associated with effective drug delivery necessitates the development and adoption of modern approach. This research aims to improve the bio-potency of ofloxacin (OF) against drug resistant strains of microorganism using nanoemulsion. The emulsification was achieved by mechano-chemical process. The morphological characterization of emulsion was carried out using Cryogenic-TEM (Cryo-TEM). The electro-kinetic properties and emulsion size was done using Zetasizer and Photon Correlation Photo-spectrometer (PCS). Drug-emulsion interaction study using FTIR. The antimicrobial studies of Ofloxacin (OF), non-drug loaded emulsion (COAB), drug-loaded emulsion (COAB+OF) was carried out on *P. aeruginosa* and pharmacokinetics were established using HPLC. Cryo-TEM micrograph showed spherical morphology with mean particle size of 166.8 and 177.5 nm for COAB and COAB+OF respectively. While PCS and Zetasizer recorded a mean value of 0.156 and -26.1 mV for COAB and 0.341, and -2.72 mV for COAB+OF respectively. This shows that the colloids are nano-sized, charged and metastable. FTIR results shows O-H absorption at 3570 – 3200 cm^{-1}, likewise CH_3 and CH_2 at 3050-2895 cm^{-1} for OF, COAB and COAB+OF respectively. This suggests encapsulation and wholeness with no drug-excipient interactions. The antimicrobial study shows COAB+OF to me more bio-potent that OF with zone of inhibition value of 12.8±0.3 mm compared to 8.0±0.2 mm in OF at 62.50 mg/ml. HPLC showed a maximum OF concentration (C_{max}) of 2.27 µg/mL at 150 min which is the drug release maxima. This study showed that encapsulating ofloxacin in castor oil based nanoemulsion system improves its bio-potency.

KEYWORDS

Emulsification, Nanoemulsion, Morphology, Encapsulation, Pharmacokinetics

1. INTRODUCTION

Nanoemulsion are thermodynamically bi-phasic liquid system which is a dispersion of liquid in liquid; oil-in water (O/W) or water-in–oil (W/O), with a dispersed phase in nanometer range (Bolla et al., 2020; Bamisaye et al., 2018; Tamilvanan and Benita, 2004). The different liquid phases are combined in a definite ratio to form a homogenous (in nature) liquid with the help of an emulsifying agent. They form spontaneously and present no discernible change in size distribution, charge, shape and spatial arrangement of its dispersed phase (Pandey et al., 2020; Jaiswal et al., 2015; Chime et al., 2014). The above mentioned features as well as the metastability, ease of preparation (spontaneous emulsification), high solubilization rate of drug molecules and excellent surface characteristics (size, charge and shape) of emulsion promotes the solubility, encapsulation, effective drug targeting and release, with an improved bioavailability of drugs and bio-active agent trapped in the organic phase of the emulsion system (Lee and McClements, 2010). This accorded it a wide application in the field of medicine and pharmacy as potential vehicle for delivery of biomolecules. Nanoemulsion has shown to be one of the frequently preferred types of ophthalmic, oral and transdermal drug delivery system as a result of its excellent characteristic which includes advanced rate of bioavailability and improved shelf life with high solubility and permeability across the reticuloendothelial cell barriers (Choradiya and Patil, 2021; Halnor et al., 2018). Many poor soluble or hydrophobic drugs are now soluble when transformed into nanoemulsion (Sonneville-Aubrun, 2004, Inayat and Mallikarjuna, 2012).

Furthermore, some strains of disease causing microorganism have shown to have developed resistance to certain antibiotics. *Pseudomonas aeruginosa* is not exonerated because it is considered as one of the major causes of certain infections in the urinary tracts, burns, and wounds as well as hospital acquired pneumonia. This is due to its immunity to a vast array of antibiotics (Stryjewski and Sexton, 2003; Bryan et al., 2016). It is one of the major pathogens that contributes to high rate of morbidity and mortality in immunocompromised individuals (Goosens, 2003). More so, ofloxacin, a synthetic drug belongs to the second generation fluoroquinolones. It possesses a broad spectrum of activities against both

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gram positive and gram negative bacteria (Hooper, 2002). However, regular usage of these antibiotics to fight disease causing microorganisms encourages the metamorphoric process of the noxious pathogen strains to a drug-resistant species thereby necessitating the increase in the dosage of the antibiotic (drug) to fight the disease-causing pathogen. This is a significant concern. However, to effectively alleviate this microbial load, nanotechnology has now been of assistance in the field of science by developing hybrid-antibiotics; drug (antibiotic)-loaded nanoemulsion system. This increases drug therapeutic property by improving bioavailability as well as its potency against disease causing microorganisms (Yan et al., 2018; Yamanaka et al., 2005; Mudshinghe et al., 2011; Kim and Berg, 2000). It is however pertinent to carry out the pharmacokinetic study of the emulsion to ascertain it's encapsulating and drug delivery potential. This study is therefore aimed at investigating the comparative antimicrobial potential of COAB, COAB+OF, and OF on *P. aeruginosa* and *P. aeruginosa* (ATCC 15442) as well as its absorption and release in the plasma of adult Wistar rats.

2. Materials and Methods

2.1 Materials

Polyethylene glycol (PEG 400) and Polyethylene (20) sorbitanmonooleate (Tween 80), Polyethylene (20) sorbitanmonolaurate (Tween 20) were purchased from Evergreen Chemical industry, Idumota Lagos, Nigeria. Castor oil was purchased at Precious pharmacy, Lagos, Nigeria. Freeze-dried *Pseudomonas aeruginosa* (ATCC15442) was provided from the Department of Microbiology, Federal University of Agriculture, Abeokuta. The clinical isolates of *Pseudomonas aeruginosa* were collected from Federal Medical Centre (FMC), Idi Aba, Abeokuta.

2.2 Emulsification

The emulsification was carried out by weighing 0.75 g (3 % w/w) castor oil into a beaker, and then, 1.00 g (4 % w/w) of T20, 4.00 g (16% w/w) of T80, and 1.00 g (4% w/w) of PEG 400 and 18.25 g (73 % w/w) of distilled-deionized water were gently added. The mixture was homogenized using a magnetic stirrer (Faithful Huanghua SH-4C) at 800 rpm for 60 min (Tri et al., 2014). The followed reaction scheme is shown in Figure 1.

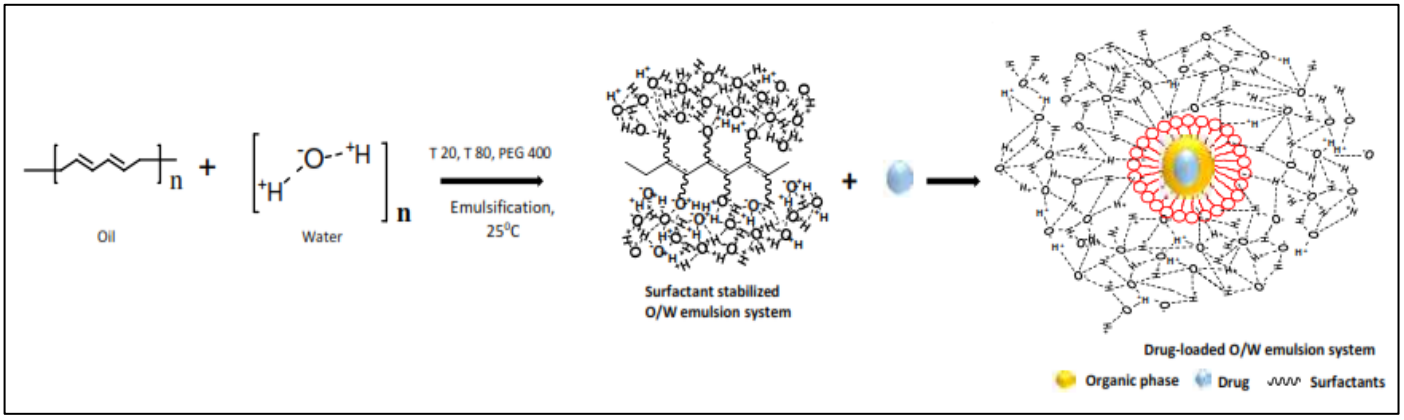


Figure 1: The reaction scheme and the components of nanoemulsion system

2.3 Droplet size and Polydispersity Index (PDI) measurement

The droplet size and PDI of O/W nanoemulsions were measured at 25 °C by photon correlation spectroscopy (PCS) using a using zetasizer (Malvern Instruments, UK). Each 1 mL of the formulations; both the drug loaded and non-drug loaded emulsions were diluted with 25 mL distilled water in a volumetric flask. The mixture was gently mixed. Triplicate measurements were performed using He–Ne laser at a wavelength of 633 nm and at a scattering angle of 173°.

2.4 Zeta potential measurement

The zeta potential was measured by electrophoretic mobility using (Malvern Instruments, UK). All analyses were done in triplicate and each 1 mL sample was diluted in 25 mL of NaCl (1 mM) ultra-filtered (0.22 m).

2.5 Drug-excipient interaction

The degree of crosslinking of the drug-excipient interaction was carried out using FTIR (Schimadzu, IR 8400S, Europe) on Cp, SOAB and SOAB+Cp by mixing the samples with KBr (Potassium Bromide, Spectroscopy grade). The mixture was pelletized using hydraulic-press prior subjecting it to scanning.

2.6 Morphological characterization

The morphology of the emulsions was determined at the Aaron-Klug Institute of Microscopy, using Cryofixation-Transmission Electron Microscope (Cryo-TEM). Cryo-grids were prepared using a Vitrobot (Thermo Fisher (FEI) Eindhoven, Netherlands). 3 µL of sample was applied to a glow-discharged R2/2 copper Quantifoil grid (SPI, USA). The vitrified samples were stored in liquid nitrogen. While the cryo-samples were viewed using a FEI Tecnai F20 transmission electron microscope (Thermo Fisher (FEI), Eindhoven, Netherlands) with a field emission gun operating at 200 kV. Microscopy was done and images were recorded with a Gatan camera using the Digital Micrograph software suite (Gatan, UK).

2.7 Inoculums preparation

Each strain of bacteria was prepared overnight at 37 ^{0}C. The presence of turbidity in broth culture was adjusted equivalent to 0.5 McFarland standards to obtain standard suspension by adding sterile normal saline in Mueller-Hilton agar slants. The McFarland 0.5 standard provides turbidity comparable to bacterial suspension containing 1.5 x 10^8 CFU/mL and used at different dilutions in the proposed evaluation tests.

2.8 Antimicrobial Study

2.8.1 Inoculums preparation

Each strain of bacteria was prepared overnight at 37 ^{0}C. The presence of turbidity in broth culture was adjusted equivalent to 0.5 McFarland standards to obtain standard suspension by adding sterile normal saline in Mueller-Hilton agar slants. The McFarland 0.5 standard provides turbidity comparable to bacterial suspension containing 1.5 x 10^8 CFU/mL and used at different dilutions in the proposed evaluation tests.

2.8.2 Determination of Antibacterial activities

Antibacterial activities of the drug samples were evaluated by the well plate agar diffusion method using the earlier method with modifications (Aida et al., 2001). The bacterial cultures were adjusted equal to 0.5 McFarland turbidity standards and inoculated on a nutrient agar plate (diameter of 9 cm) by flooding the plate with 1 mL of each of the standard test organism, swirled and excess inoculum was carefully decanted. A sterile cork borer was used to make wells (6 mm in diameter) on other agar plates. The different concentrations of 500, 250, 125, 62.50, and 31.25 mg/mL of the drug samples were prepared by the dilution method, and the antibacterial activity was determined by measuring the zone of inhibition around each well (NCCLS, 1990).

2.8.3 Determination of Minimum Inhibitory Concentration (MIC)

The MIC of active drug samples was determined by the tube dilution technique, using a modified method (Junaid et al., 2006). The smallest concentration that inhibits the growth was taken as the MIC (Geo et al., 2001).

2.8.4 Determination of Minimum Bactericidal concentration (MBC)

The MBC is the lowest concentration of antibiotic agent that kills at least 99.9% of the organism. This was carried out using the Doughari method (Doughari et al., 2007).

2.8.5 Pharmacokinetics study

The animals used were humanely handled and kept in plastic suspended cages in a well ventilated and clean rat house under suitable conditions of temperature and humidity. They were provided with rat pellets (Arojo Feeds), water *ad libitum* and subjected to natural photoperiod of 12 h light and 12 h dark cycle. All procedures for the maintenance and sacrifice (care and use) of animals were carried out according to the criteria outlined by the National Academy of Science published by the National Institute of Health (National Institutes of Health, 2004). Ethical approval for this study was obtained from Lead City University Ethical Review Board (LCU/ERB/AN0017). Twenty-one adult Wister rats were divided into six (7) groups in which each group is made up of three (3) rats. The animals were acclimatized for two weeks before the commencement of the study. The rats were weighed and marked before oral administration. 30 mL kg per body weight of COAB+OF was orally administered to each rat using cannula. The rats were anesthetized and the blood sample was collected via cardiac puncture at intervals of 30, 60, 90, 120, 150, and 180 min) into lithium heparin bottles and kept temporarily in an ice pack.

Extraction: The refrigerated samples were centrifuged at 300 rpm for 15 min and plasma was pipetted into 2.5 ml Eppendorf tube. 1:1 mixture comprising 500 µL of plasma and 500 µL acetonitrile was homogenized and later centrifuged at 300 rpm for 15 min and the supernatant was collected and stored for further analysis.

2.8.6 Preparation of working standard

0.5 mg of both drugs in 10.0 mL volumetric flask with 0.1M HCl as stock solution were used to produce a stock solution of 500 ppm. Serial dilutions were made from the stock solution to prepare the working standard and the linearity of the method was ascertained. 0.01M potassium dihydrogen phosphate was used for the mobile phase while phosphate buffer and acetonitrile in a ratio of 80:20 (v/v) was used as a stationary phase. 20 µL of each working standard was injected into the liquid chromatographic systems using the mobile phase prepared at a flow rate of 0.7 mL/min at absorbance between 190 – 400 nm from which the maximum wavelength of absorption (Λ_{max} = 280 nm) was obtained (Agilent Technologies, Santa Clara, USA).

2.8.7 Drug concentration in the plasma

The fortification was carried out on the samples in which a known concentration from the stock solution was added to the sample to be analyzed at a ratio of 1:1. 20 µL. The spiked sample mixture was injected and the concentration was determined by HPLC.

2.9 Statistical analysis

The statistically significant difference between groups was analyzed using One-way ANOVA followed by independent-sample test. The level of significance was set at $P < 0.05$. The results are presented as mean ± SD.

3. RESULT

Both charge and droplet size are essential parameters that determine the stability of an emulsion. This is due to their roles in steric repulsion and metastability by reducing the surface tension, and the mean value of these parameters are shown in Figures 3a and b respectively. While the TEM micrograph in Figure 2 shows the morphology of both COAB and COAB+OF.

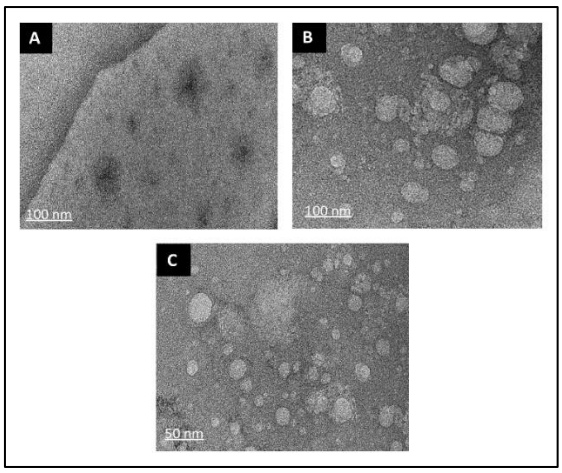


Figure 2: A: TEM of non-drug loaded castor oil-based emulsion. B and C: drug loaded castor oil-based emulsion

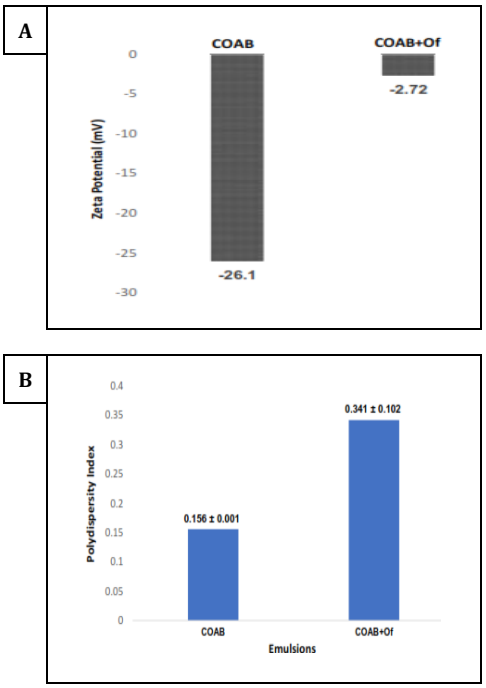


Figure 3: (A) Mean zeta potential (ZP) and (B) Polydispersity index (PDI) measurement of both COAB and COAB+OF

The FTIR analysis works on the principle of bond vibrations between two adjoining atoms as well as determine the functional groups present in a molecule. In this study, FTIR was used to determine the degree of cross-linking to determine the probability of drug-excipient interaction.

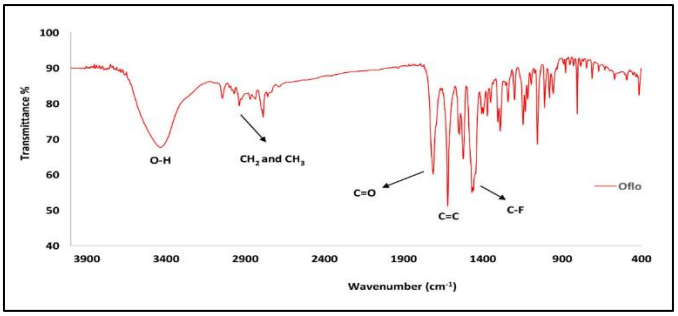


Figure 4: FTIR spectrum of ofloxacin

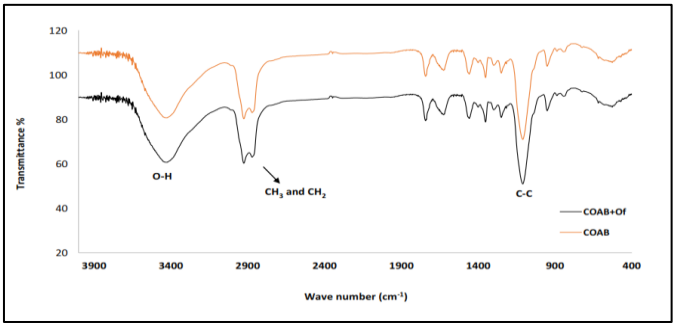


Figure 5: FTIR spectrum of COAB+OF and COAB

It is expedient to develop a suitable HPLC protocol for pharmacokinetic study. This will be used to determine the absorption of the drug encapsulated by castor oil-based nanoemulsion and the concentration of the absorbed drug in the plasma of Wistar rats. Figure 7 shows the calibration curve of the working solution and the chromatogram showing the absorption maxima of OF. The chromatogram as shown in Figure 7a exhibited a maximum peak of OF at a retention time range of 2.29 - 2.50 min. While Figure 7b shows the calibration curve of the working standard with a linear equation of y=103.8x and a regression (R^2) of 0.999.

The antimicrobial activities of the drug, emulsion and the drug-loaded emulsion are shown in Tables 1 and 2 at varying concentrations of 500, 250, 125, 62.50, and 35.25 mg/ml on *Pseudomonas aeruginosa* and *Pseudomonas aeruginosa* (ATCC 15442**)**.

Table 1: The antimicrobial activities showing the zone of inhibition (ZI) of drug-loaded and non-drug loaded emulsion on *Pseudomonas aeruginosa*

Conc. (mg/mL)	OF (mm)	COAB (mm)	COAB+OF (mm)
500	22.5±0.5	1.0±0.0	18.3±0.5
250	18.3±0.6	0.5±0.5	15.1±0.3
125	15.4±0.5	0.0±0.0	3.1±1.0
62.50	12.2±1.0	0.0±0.0	0.0±0.0
35.25	7.2±1.0	0.0±0.0	0.0±0.0

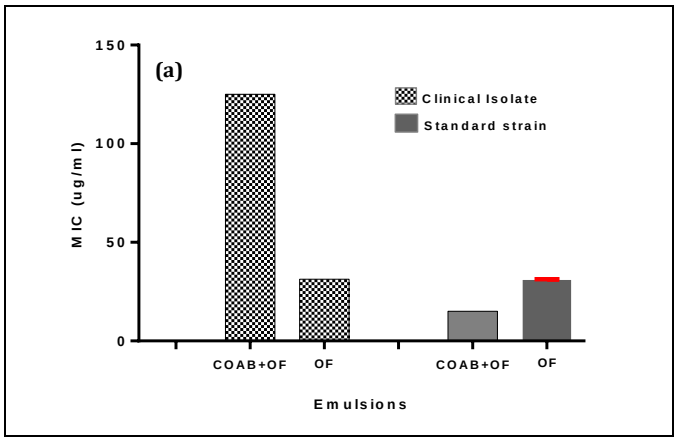
Data are the means of three replicates (n = 3) ± SD

Table 2: The antimicrobial activities exhibiting the zone of inhibition (ZI) of drug-loaded and non-drug loaded emulsion on *Pseudomonas aeruginosa* (ATCC 15442)

Conc. (mg/mL)	OF (mm)	COAB (mm)	COAB+OF (mm)
500	27.0±0.20	6.9±0.12	28.3±0.6
250	23.0±0.1	5.0±0.10	25.7±0.6
125	11.1±0.2	2.8±0.29	15.1±0.1
62.50	8.0±0.2	0.0±0.00	12.8±0.3
35.25	5.9±0.2	0.0±0.00	9.9±0.4

Data are the means of three replicates (n = 3) ± SD

The MIC and MBC on *Pseudomonas aeruginosa* and *Pseudomonas aeruginosa* (ATCC 15442**)** were shown in Figures 6a and 6b respectively in which a value of 250 and 31.25 µg/mL were recorded for COAB+OF and OF respectively on the hospital isolate. As well as 15.07 and 31.25 µg/mL on the standard. In the case of MBC, 250 and 125 µg/ml was recorded for COAB+OF and OF on the clinical isolate, while 62.50 and 125 µg/mL was recorded for COAB+OF and OF respectively on the standard strains.



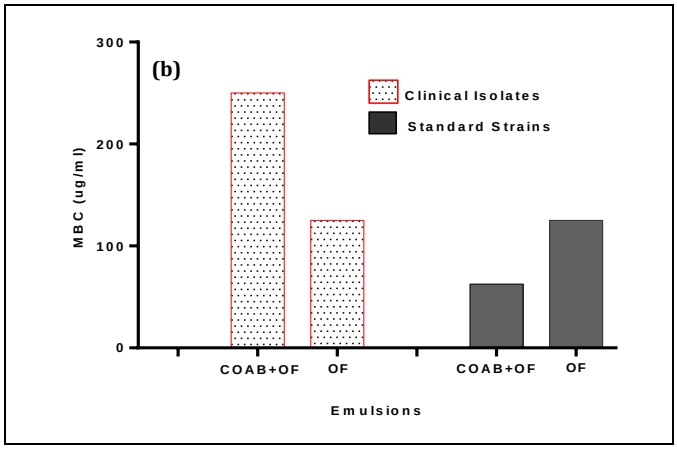
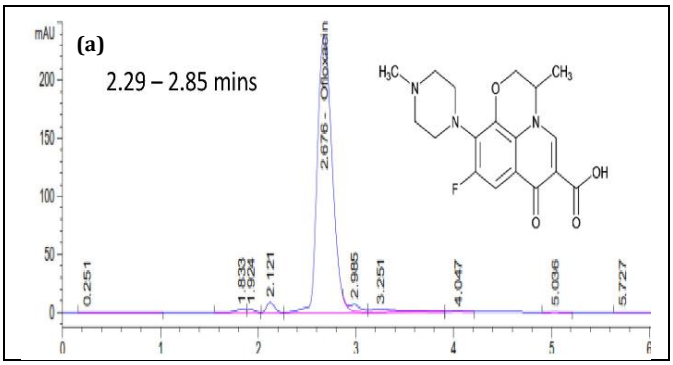


Figure 6: A: Minimum inhibitory concentration (MIC) and B: Minimum bactericidal concentration (MBC) drug-loaded and non-drug loaded emulsion on the microorganisms



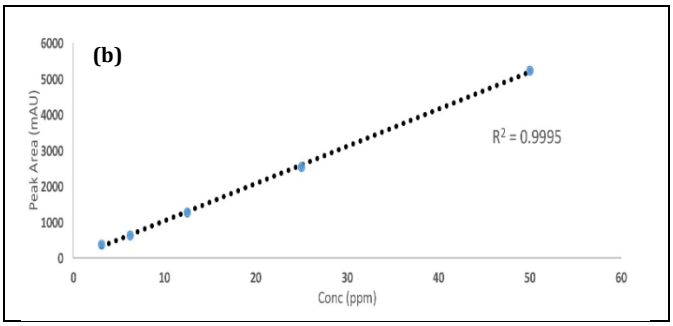


Figure 7: (A) The chromatogram of OF standard and (B) Calibration curve of the working standard

Pharmacokinetics provides the complete pathway of absorption, distribution, bio-transformation, and elimination of drugs in animals and humans (Guerini et al., 2017). Absorption indicates the passage of drug molecules from the administration site to the blood. While distribution explains the passage of drug molecules from the blood to tissues respectively. Figure 8 shows the concentration-time study of OF carried by COAB in the plasma of Wister rats.

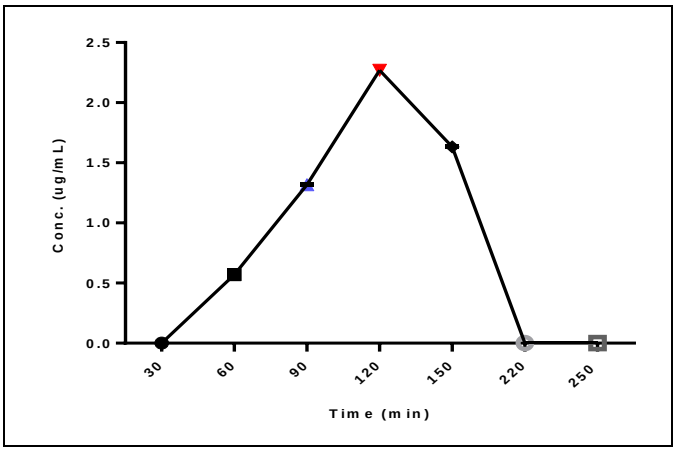


Figure 8: The concentration-time chart of COAB+OF

4. Discussion

The PDI and ZP values are considered to be an important parameter for the application of colloids in pharmaceutics and cosmetology. These values assist in determining and forecasting the longevity of an emulsion by reducing the interfacial tension. Meanwhile, both COAB+OF and COAB show a characteristic negative charge. This is as a result of PEGylation, due to the presence of PEG which is a negatively charged stabilizing component that makes up the emulsion system (Brigger et al., 2012). This fosters rapid opsonization, phagocytosis of nanomaterials in the blood stream as well as massive removal by fixed macrophage (Thakur et al., 2013). It is a useful parameter in nanomedicine as it improves the pharmacokinetic properties of nano-systems in the body. This however accords COAB the propensity to serve as an excellent drug delivery medium by encapsulating, rapid cellular uptake, and ease of release of biomolecules. The Cryo-TEM micrographs showed a spherical morphology of both COAB and COAB+OF with a mean particle size of 166.8 and 177.5 nm respectively. This result is corroborated by the work of Jiang et al. where it was reported that spherical shaped nanoparticles were taken up significantly by cells at 375-500 % compared to their rod-shaped

counterparts (Jiang et al., 2008). Furthermore, a researcher reported an encapsulation process and spherical shaped ciprofloxacin loaded *Hura crepitan* based nanoemulsion with a dispersed phase diameter of 94 nm (Bamisaye et al., 2018).

The drug excipient interaction study using FTIR was carried out in order to ascertain the wholeness of the drug in the emulsion phase which was shown in Figures 4 and 5. It should be noted that the intensity is measured as Transmittance (%) and this shows that, the higher the intensity value, the lower the frequency of absorption of such moiety.

The COAB recorded an absorption band around 3423.76 cm^{-1} wavenumber which suggests the presence of O-H. Prominent absorption bands around 2924 and 2872 cm^{-1} which is suggestive of – CH_3 – symmetric and asymmetric vibrations. And an absorption band of 950.94 cm^{-1} was observed which is indicative of *trans* C –H out of plane bends.

Since the degrees of unsaturation of both surfactants and poly or mono unsaturated fatty acid chain would have been reduced due to the rearrangements of bonds for micelles formation. This resulted to the high intensity absorption band of –C-C- observed in the emulsion phase. The superimposition of COAB and COAB+OF spectrum (Figure 5) shows an encapsulation of the drug without drug-excipient interactions.

The invasion mechanism of most bacteria pathogens is by breaching the endothelia and epithelia barrier. This however enables them to have their way into the host's body system thereby causing disease (Ajay et al., 2013). *Pseudomonas aeruginosa*, a gram negative rod-shaped bacteria is not vindicated. Because, it has been earlier reported that this microorganism is one of the leading causes of hospital acquired pneumonia. *Pseudomonas aeruginosa* makes use of the type III secretion system effector to intoxicate pulmonary epithelia cells. Its rod like structure extends through the inner and outer membrane thereby providing a pathway through which protein can be secreted from the bacterium into the host cells. Furthermore, ofloxacin is an antibiotic which belongs to the family of fluoroquinolones. It functions by interfering with the breakage-rejoining step of DNA gyrase. The DNA gyrase is an enzyme that is responsible for the replication of bacteria cell. It carries out activities by harnessing the energy released from ATP hydrolysis thereby affecting the replication of the cell which results to cell death (Hauser, 2009; Drlica et al., 1997). However, due to the complex nature of *Pseudomonas aeruginosa* and its resistance to fluoroquinolones, it is therefore expedient to modify and improve its therapeutic ability (Mishra et al., 2014; Lanini et al., 2011). This necessitates the use of a colloidal system; nanoemulsion. Its particle size, shape, and charge are essential features that makes it fit for biological applications. However, based on the aforementioned properties, the encapsulation of drug in a colloidal system has the tendency to improve its antibiotic potency. In this study, *Pseudomonas. aeruginosa* and *Pseudomonas aeruginosa* (ATCC 15442) were studied against OF, COAB, and COAB+OF at concentrations of 500, 250, 125, 62.50, and 32.25 mg/mL. The use of Castor oil this studies to serve as the organic phase component is dues its vast array of applications in food and drug industries owing to its therapeutic abilities (Figueiredo et al., 2007). The results of antimicrobial activities of OF, COAB, and COAB+OF shows their bio-potency on both standard and clinical strains. COAB recorded a mean ZI value of 6.9±0.12, 5.0±0.10, and 2.8±0.29 mm at 500, 250, and 125 mg/mL respectively on the standard strain. While the hospital isolate showed a resistance, with a ZI value of 1.0±0.0, 0.5±0.5, and 0.0±0.00 mm. Upon comparing the ZI value of OF with COAB+OF on both bacterial strains, it could be observed that the hospital isolate showed a mild resistance to the drug-loaded emulsion. COAB+OF recorded a ZI value of 18.3±47 mm compared to OF with 22.5±0.5 mm at 500 mg/mL. While on the standard strain, at 500 mg/mL, COAB+OF recorded a ZI value of 28.3±0.6 mm against 27.0±0.20 mm of OF. The observed ZI value in this study decreased as the concentration of test samples decreased. This is in accordance with the earlier reported results of the antimicrobial activity of plant extracts and antimicrobial mechanism of silver nanoparticle on *Pseudomonas aeruginosa* (Pandey and Singh, 2011). However, mild reduction in the potency of the COAB+OF on the clinical isolate of *Pseudomonas aeruginosa* may be due to continual metamorphic ability of the pathogen. The MBC and MIC results provide useful information on the minimum concentration of any antibiotics that can kill and inhibit the growth of microorganisms. Figures 6a and b show that COAB+OF was efficient at 250 µg/ml on the hospital isolate than the standard strain at 15.07 µg/mL. This could be due to the controlled release of active ingredient by COAB+OF. Similarly, from MBC results, 250 µg/mL was recorded for COAB+OF and 125 µg/mL for OF on the clinical isolate. While 62.50 and 125 µg/mL was recorded for COAB+OF and OF on the standard strains, respectively.

The gastrointestinal enzymatic degradation resulting in reduced bioavailability of hydrophobic drugs is of great concern in drug delivery. This affects the potency of antibiotics. To determine the potential of COAB as a drug carrying agent, both the calibration curve of the working standard and chromatogram of OF were determined, as shown in Figures 7a and 7b. The correlation coefficient for the standard curve was found to be closer to 1 (0.9995). The generated regression equation was y = 103.89x, which was used to calculate the concentration of OF in the plasma of rats. A pharmacokinetic study was shown by the concentration-time graph of COAB+OF in Figure 8. This shows that as time increases, the concentration of drug in the plasma increases steadily until an absorption maxima with a mean concentration value of 2.2703 µg/mL at 120 min. This suggests that the drug absorption was at its peak in the body of the rat. However, a reduction in the concentration of the drug was observed at 150 min which decreases to zero at 180 and 210 min which suggests the metabolism and excretion of the drug from the body of the rat. This, however, shows that the emulsion has the potential to serve as a drug carrier system.

5. Conclusion

The findings of this study show that castor oil-based emulsion system assists in the antimicrobial potency of encapsulated OF without any observed cross-linking thereby suggesting there is no drug-excipient interaction, with a droplet size in the nanometer range, a good metastability as a result of the low PDI value coupled with an excellent encapsulating potential and reasonable surface charge that facilitates opsonization and controlled delivery of OF. This, however, suggests that castor oil is a suitable organic phase in the production of nanoemulsion as well as serve as a potential carrier for oral drug delivery.

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