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APPLICATION OF MICROFLUIDIC INNOVATION AND NANOENCAPSULATION TO AMPLIFY THE ANTIMICROBIAL EFFECTS OF CIPROFLOXACIN AGAINST FOOD BORN PATHOGEN

Hassan Garba Wafi * and Abdulrahman Ibrahim Kubo

Department of Pure and Applied Chemistry, Adamawa State University, Mubi, PMB, 25 Mubi, Adamawa State, Nigeria.

* Corresponding Author

ORCID Details

Hassan Garba Wafi: <https://orcid.org/0009-0009-2231-1671>

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ABSTRACT

Foodborne illnesses are often caused by microbial contamination during preparation or storage. In this work, stable nanoemulsions of ciprofloxacin were prepared using *Mentha piperita* essential oil (MEO) as a nanocarrier delivery system. Response Surface Methodology was used to optimize the key variables for ciprofloxacin nanoemulsion formulation, including 4.83, 2.83, and 0.14%w/w surfactant, essential oil, and ciprofloxacin, respectively. The stability of MEO/ ciprofloxacin nanoemulsion (MEO/C NE) with a mean droplet size of 75.46 ± 3.2 nm was monitored over 3 months. The antibacterial activity of MEO/C NE and bulk compounds against *E. coli* bacterium was compared using a conventional method and a microfluidic chip. A significant difference in the antibacterial activity was observed by employing a microfluidic chip as compared to the conventional technique, probably due to a high contact surface area between the nanodroplets and bacterial membrane. In the microfluidic chip, the *E. coli* was completely inhibited in 30 min, whereas 3 h was needed for complete inhibition using the conventional method. The results of this study highlight the significance of nanoemulsion delivery systems to improve the antimicrobial activity of ciprofloxacin and also microfluidic technology as a fast and reliable technique for examining antibiotics and nano delivery systems against microorganisms.

Keywords: Microfluidic; Nanoencapsulation; Antimicrobial; Ciprofloxacin; Food Born Pathogen

1 INTRODUCTION

Microbial contaminants that can result in foodborne illnesses are known as foodborne pathogens, including bacteria, viruses, and parasites (Atasever, 2026). The emergence of two or more cases of a similar illness brought on by the consumption of a food is known as a foodborne disease outbreak. *Escherichia coli* (*E. coli*) are among the more frequently reported food pathogens that are found normally in warm-blooded creatures' lower intestines. While most strains of *E. coli* are not harmful, some can result in severe food poisoning (Liu *et al.*, 2026). The bacteria known as Shiga toxin-producing *E. coli* (STEC) is capable of causing serious foodborne illnesses. *E. coli* may evolve in vegetables and other foods and live in the environment for long period of time. Since *E. coli* is the most prevalent Gram negative

infection in humans, antibiotic resistance in this pathogen is especially concerning. Consequently, it is crucial to use natural compounds to combat antibiotic resistance since they present an acceptable replacement for conventional antibiotics, which are losing their efficacy as a result of the emergence of resistant bacteria. It has been demonstrated that natural compounds possessing antibacterial qualities can offer a viable path forward for the development of novel therapies (Abdallah *et al.*, 2026). The antibacterial effects of natural compounds EOs and the synergistic effect of two or more EO components have been distinguished by several researchers. The antibacterial properties of EOs result from components that act synergistically or additively, acting at multiple sites of action at the cellular level rather than through a single mechanism. Recently, much consideration has been given to the isolation and utilization of new bioactive compounds with antioxidant and or antimicrobial activity from botanical sources. For example, Nostro and Papalia examined and confirmed the antibacterial activity of carvacrol against a variety of food-borne pathogens and microorganisms (Barbieri *et al.*, 2026). As an advanced strategy in this area, our team has already published information on the EOs' enhanced antibacterial activity through the use of nanoemulsification techniques. Nanoemulsions are defined as fine colloidal dispersions of water-in-oil or oil-in-water droplets within the size range of 10–600 nm, used in pharmaceuticals and biomedical industries (Melk *et al.*, 2026). Nanoemulsions are showing promising horizons for the development of novel cosmetics, diagnostics, and pharmaceuticals as well as biotechnology products. The terms submicron emulsion (SME), small emulsion, and ultrafine emulsion are also used synonymously. Nanoemulsions usually consist of heterogeneous mixtures of lipid and aqueous phases wherein stability is achieved through the use of suitable materials known as emulsifiers (Melk *et al.*, 2026). The use of nanoemulsions as delivery systems has been shown to extend the residence time of drugs in the body. Previous studies have shown the use of nanoemulsion technology to improve the bioavailability of lipophilic drugs and antibiotics. Simultaneous administration of existing antibiotics and essential oils (EOs) has been experimentally investigated as an alternative strategy for treating infections caused by drug-resistant bacteria (Jalendiran *et al.*, 2026). Some studies have confirmed the synergistic and/or additive effects of EOs and some antibiotics against bacteria. Indeed, nanoemulsification provides a large contact surface area. This is more intensified when a highly efficient microfluidic chip is employed to attain a high contact area between bioactive compounds and cell membranes. When it comes to detecting antimicrobial activity, microfluidic devices have several advantages over traditional techniques including fast antibacterial compound testing in less than an hour, small quantities of the required reagents and medium and versatile microfluidic designs for special purposes (Liu *et al.*, 2026). Furthermore, microfluidic devices can support modular detection techniques and comprehensive cultivation regimens, enabling more accurate and effective investigation of the bacterial cell membrane interaction with antibacterial agents. This is in contrast to the conventional techniques, which frequently need more intricate handling and longer incubation times. Moreover, visual investigation of the bacterial status upon treatment is possible using microfluidic technology. Incorporating appropriate natural compounds along with an antibiotic such as *ciprofloxacin* in nanoemulsions can be considered a practical solution to increase the contact surfaces and hence the antibacterial activity of antibiotics (Melk *et al.*, 2026). This study aims to investigate the possibility of formulating a stable *Mentha piperita* essential oil/*ciprofloxacin* nanoemulsion (MEO/C NE) for antibacterial activity. At first, the effect of important formulation variables, including surfactant, EO, and *ciprofloxacin* concentrations on the nanoemulsion properties like mean particle size and stability was studied using the Response Surface Methodology (RSM) technique. Then, the application of a microfluidic chip for evaluating the antibacterial activities of nanoemulsions against *E. coli* bacterium was considered and compared to the conventional method. The amounts of potassium, nucleic acid, and protein release were scrutinized to assess the extent of bacterial cell destruction. Recently, much consideration has been given to the isolation and utilization of new bioactive compounds with antioxidant and or antimicrobial activity from botanical sources. As an advanced strategy in this area, this research provides information on the essential oils' enhanced antibacterial activity through the use of nanoemulsification techniques. Previous studies have shown the use of nanoemulsion technology to improve the bioavailability of lipophilic drugs and antibiotics. Simultaneous administration of existing antibiotics and essential oils has been experimentally investigated as an alternative strategy for treating infections caused by drug-resistant bacteria. Some studies have confirmed the synergistic and/or additive effects of essential oils and some antibiotics against bacteria. Indeed, nanoemulsification provides a large contact surface area. This is more intensified when a highly efficient microfluidic chip is employed to attain a high contact area between bioactive compounds and cell membranes. When it comes to detecting antimicrobial activity, microfluidic devices have several advantages over traditional techniques including fast antibacterial compound testing in less than an hour, small quantities of the required reagents and medium and versatile microfluidic designs for special purposes.

2 MATERIALS AND METHODS

2.1 MATERIALS

The study used *E. coli* in Nutrient Broth containing 15% v/v Glycerol supplied as a cryoprotectant. The organisms were kept in freezer stock at – 20 °C. Before starting any experiment, a fresh culture was created on a Nutrient agar plate were phosphate-buffered saline (PBS), neutral red, and non-ionic surfactants Span 80 and Tween 80. *ciprofloxacin* base and *Mentha piperita* EO were used to detect the bacteria. Dynamic Light Scattering (DLS) device and UV-visible spectrophotometer were utilized for further experiments.

2.2 Design of experiment

The implementation of design of experiments (DOE) including orthogonal array design, Box-Behnken design (BBD), and central composite design (CCD) to optimize technical processes and formulations is expanding in research laboratories. Based on some initial testing, the appropriate ranges including surfactant 3.0, 4.0, 5.0 w/w%, essential oil 2.0, 3.0, 4.0 w/w%, and *ciprofloxacin* 0.01, 0.055, 0.10 w/w% will be chosen. 12 experiments will be selected based on BBD, including 5 central point replications to estimate reasonably the experimental error. It should be noted that each characterization test of formulations will be repeated three times and the average values will be reported. The quadratic regression model will be executed to predict the relationship between the nanoemulsion droplet size and variables as demonstrated in Eq. (1):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \dots \text{Equa.... (1)}$$

Where Y denotes the droplet size (nm); β_0 is the intercept coefficient, β_1 , β_2 and β_3 stands with the main effect coefficients, β_{11} , β_{22} , and β_{33} are the squared coefficients, and β_{12} , β_{13} , and β_{23} show the interaction coefficients. Design-Expert Software (State-Ease Inc., version 7.0.0) will be used to analyze the experimental data and evaluate the predicted responses.

2.3 Nanoemulsion preparation

ciprofloxacin -loaded nanoemulsions will be prepared by dissolving *ciprofloxacin* in the EO and Span and then mixing in deionized water containing a non-ionic surfactant. A solution of Tween 80 in deionized water (the aqueous phase) will be added to the mixture of *ciprofloxacin*, EO, and Span80 (the oil phase). A high-speed homogenizer will be used to prepare the nanoemulsion samples. The whole mixture will be homogenized under the stirring rate of 4,000 rpm for 20 min. The mean particle diameter of the nanoemulsions stored at -4°C will be scanned over 3 months to determine the stability of the nanoemulsion samples (Moradi Alvand *et al.*, 2025).

2.4 Microfluidic system

The microchannel made of PDMS will be created using the microlithography method. The PDMS liquid and PDMS curing agent will be then carefully blended well in a 10:1 ratio and poured onto the SU-8 molds. PDMS began to separate from the molds as it will be curing at 90°C for 30 min. In the end, the oxygen plasma procedure will be used to bind the two PDMS layers for 1 min at 8 mbar and 40 W. The inlets and outlets of the microchannels will be created using a biopsy punch with a 1.25 mm width (Moradi Alvand *et al.*, 2025).

2.5 E. Coli bacterium cultivation and preparation

A colony will be removed and added to sterilize (MHB) broth medium and incubated under aerobic conditions at 37°C and 200 rpm, followed by centrifugation for 10 min at 4000 rpm at 4°C . 1 mL of neutral red solution (0.04 mg in 100 mL) will be added to the obtained extract followed by incubation in the shaker for 10 min at 37°C . After centrifuging the resultant solution will be centrifuge for 10 minutes at 4000 rpm at 4°C , 10 mL of PBS buffer will be slowly added. The obtained solution has been injected into a microfluidic chip. In the quantitative tests, the bacterial suspension will be injected directly into the device (Abbas *et al.*, 2024).

2.6 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

To determine the MIC, a continuous two-fold dilution of samples at concentrations of 10 to 0.005 mg/mL will be prepared in sterile 96-well plates as described by the Clinical and Laboratory Standards Institute (CLSI). The MIC value will be the concentration that completely inhibited the growth of bacteria (the first clear well). Using the microtiter broth dilution technique, the minimum bactericidal concentration (MBC) will be recorded as the minimum sample concentration of 99.9% bacterial death after culturing at 37°C for 24 h²². Each experiment will be repeated at least three times (Tethool *et al.*, 2026).

2.7 Cell membrane integrity

The integrity of the cell membrane will be evaluated by monitoring the discharge of intracellular materials including nucleotides and proteins at 260 and 280 nm, respectively (Kumar *et al.*, 2026).

2.8 Time kill assay

A time-kill assay will be performed to confirm the antibacterial effects of *ciprofloxacin*, MEO, and MEO/C NE bacterial growth inhibitory. The samples will be collected at four intervals (1, 2, 3, and 4 h) during the time-kill assay. The original bacterium solution will be further diluted with a sodium chloride solution (0.085%W/V) to facilitate the counting of colony units. Subsequently, 100 μL specimens will be extracted from the dilutions. To facilitate the counting of colony units, a predefined amount of outflow will be sampled in the microfluidic chip at the predetermined residence time. The

colony counting unit underwent the same procedure. Following a 24-hour incubation period at 37 °C, the initial bacterial solution will be serially diluted using a sodium chloride solution (0.085%W/V) before the plate colony count carried out (Rosenberg *et al.*, 2026).

3 RESULTS AND DISCUSSIONS

3.1 Optimization of nanoemulsion formulation

A subclass of RSM, Box Behnken Design (BBD), was used to determine the most appropriate nanoemulsion droplet size targeting the highest stability and biological response. The droplet size of the nanoemulsion was calculated using the DLS procedure for the combination of the independent parameters, which includes the percentages of surfactant (A), EO (B), and (C) *ciprofloxacin*. The mean droplet size was calculated as the average of three measurements. The BBD comprises a total of 17 proposed experimental runs, including five repeatable center points.

$$\text{Droplet size of nanoemulsion (actual)} = -104.94593 + 67.45111 \times \text{Surfactant} + 30.91194 \times \text{essential oil} + 17.63519 \times \text{ciprofloxacin} - 5.92500 \times \text{surfactant} \times \text{essential oil} + 5.38889 \times \text{surfactant} \times \text{ciprofloxacin} + 11.55556 \times \text{essential oil} \times \text{ciprofloxacin} - 7.23000 \times \text{surfactant}^2 + 0.64500 \times \text{essential oil}^2 - 41.25926 \times \text{ciprofloxacin}^2 \quad \dots (3)$$

$$\text{Droplet size of nanoemulsion (Coded)} = \text{Mean droplet size} = +104.76 - 5.20 \times A + 17.44 \times B + 12.81 \times C - 5.93 \times A \times B + 2.43 \times A \times C - 7.23 \times A^2 + 0.65 \times B^2 - 8.36 \times C^2 \quad \dots (4)$$

Table 1 displays the ANOVA statistical analysis for the regression model. From Table 1, the low *p*-value ($p < 0.0002$) and/or high *F*-value ($F = 24.24$) *p*-confirm the statistical significance of the predicted model. Also, the Lack of Fit index is not significant (0.1309) relative to the pure error indicating the model fits well and the independent variable has considerable effects on the response. The other statistics of the model like the coefficient of determination was 0.9689, Adjusted $R^2 = 0.9289$, adequate precision = 17.766, and predicted $R^2 = 0.6274$, standard deviation = 4.68, coefficient of variation (C.V%) = 4.79, suggesting a strong connection between experimental and projected values. As a result, the resulting model is sufficient to draw a response surface curve and predict stability conditions for maximizing the stability values.

Table 1: Analysis of variance (ANOVA) results for the response surface quadratic model.

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-Value
Model	3676.74	8	430.75	24.24	0.0002 <i>significant</i>
A-Surfactant	316.32	1	316.32	9.88	0.0163
B-Essential Oil	3432.53	1	2432.53	111.09	<0.0001
C- <i>Ciprofloxacin</i>	1213.28	1	1313.28	59.98	0.0001
AB	140.42	1	140.42	6.41	0.0391
AC	24.52	1	23.52	1.07	0.3345
BC	208.16	1	108.16	4.94	0.0617
A ²	120.10	1	220.10	10.05	<0.0157
B ²	2.75	1	1.75	0.080	0.7855
C ²	393.92	1	293.92	13.42	<0.0080
Residual	253.28	6	21.90		
Lack of fit	210.63	3	36.88	3.46	0.1309 not significant
Pure error	32.65	3	10.66		
Cor Total	5930.02	16			

3.2 Interactive effect of droplet size of NE formulation factors

The ultimate droplet size is significantly influenced by the concentration of the constituents that make up the nanoemulsion. High surfactant concentrations have been suggested to raise the risk of toxicity in food. Herein, the EO, *ciprofloxacin*, and surfactant percentages were altered to scrutinize the suitable NEs-based *ciprofloxacin* droplet size

(Massoud *et al.*, 2026). The quadratic polynomial models' 3D response surface contour plots, which depict the relationships between interacting independent and dependent variables. The mutual effect of surfactant and essential oil percentages on the droplet size of the MEO/C NE was investigated, a significant interaction between the surfactant and essential oil variables is considered. At the lower constant value of surfactant (3 to 4 w/w%) increasing the essential oil leads to a significant increase in the droplet size, while when surfactant is fixed at a higher value (4.5 to 5 w/w%) there is no remarkable change in mean droplet size. In addition to reducing the droplet size, increasing the essential oil concentration is the main goal of this study. Concentrations can be moderated to see the noticeable effects of essential oils. In this case, the droplet size will also be moderate. The ideal situation occurs when the essential oil and surfactant amounts are in the middle of the curve because a lower surfactant proportion is advantageous. These findings corroborate those of who proposed that an increase in surfactant % causes a decrease in the droplet size of cinnamon oil nanoemulsion. As observed, with the simultaneous increase in *ciprofloxacin* concentration at constant surfactant concentration, the droplet size increased significantly, which could refer to the *ciprofloxacin* droplet size (Massoud *et al.*, 2026). Also, increasing the surfactant concentration to a specific amount leads to a larger droplet size until a peak is reached, then with more increasing concentration the droplet size can be reduced. Surfactants typically lower the free energy required for the generation of nanoemulsions. By lowering the surface tension at the water-oil interface, surfactants which are made up of both hydrophilic and hydrophobic components interlink the space between the aqueous and oil phases. This implies that a decrease in the nanoemulsion droplet size could result from raising the Tween 80 content.

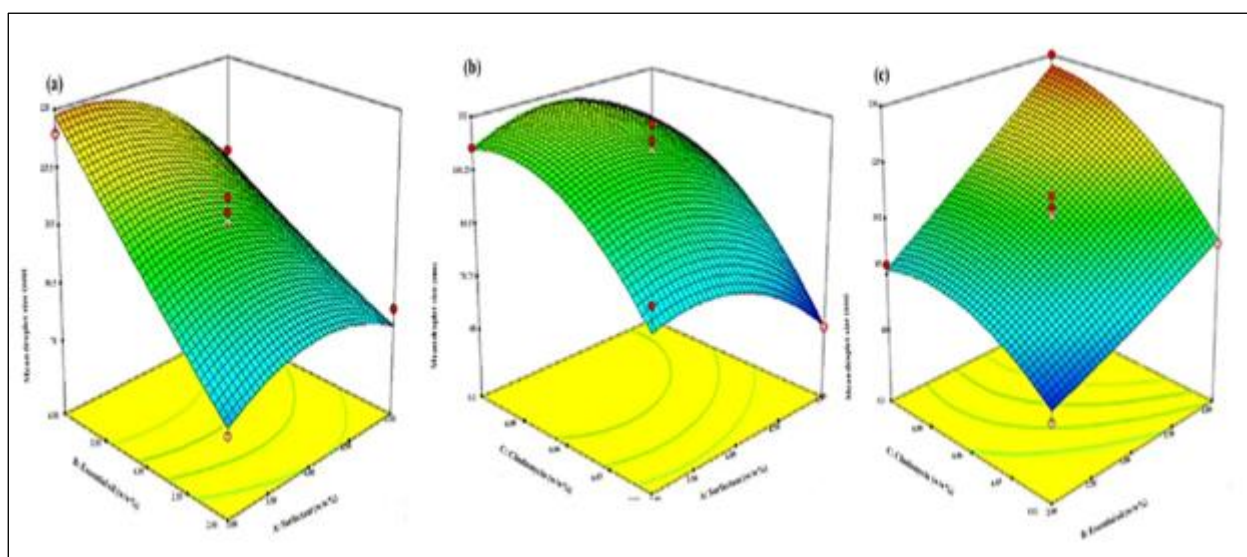


Figure 2: Response surface plots for the effect of (a) surfactant and essential oil, (b) surfactant and *ciprofloxacin*, (c) *ciprofloxacin* and essential oil on the droplet size.

3.3 Optimization of the droplet size of nanoformulation

The regression model was employed to attain the optimal values for the selected factors. Three various proposed cases by DOE software are re-performed to verify the accuracy of the fitted correlation. It was shown that both experimental and predicted values are in close agreement, approving that the fitted model is reliable. The DLS plots associated with the mean particle diameter of the nanoemulsions are 75.46 ± 3.2 nm, 73.62 ± 4.4 nm, and 99.35 ± 2.6 nm. To assess the encapsulation efficacy percentage (EE%) of *ciprofloxacin* in nanoparticles, a colorimetric method using ferric acid ion as a colorimetric agent has been utilized. Briefly, stock solutions of *ciprofloxacin* and ferric ion were prepared, as described in supporting information. Then, to plot a calibration curve, *ciprofloxacin*-ferric ion standards ranging from 10 to 250 $\mu\text{g/mL}$ were prepared, and the UV absorption in 590 was evaluated. The prepared nanoemulsion was separated into two distinct phases (organic and aqueous). By adding the ferric chloride solution to the organic phase and upon color change, the absorption was evaluated at 590 nm and the concentration was calculated. The encapsulation EE% was calculated resulting in 59.63% W/W. After three months of storage at 4 °C, it was demonstrated that the mean particle diameter had not significantly changed, indicating the long-term storage stability. Following three months of storage, the produced nanoemulsions' polydispersity index (PDI) was determined to be 0.185, 0.183, and 0.210. The zeta potential of the prepared nanoemulsions (No 1, 2, and 3) was calculated -16, -15.4, and -15.3 mV respectively.

3.4 Effect of different formulated samples on bacterial inhibition

The MIC and MBC tests for antibacterial activities of prepared samples (Table 3) were evaluated against the *E. coli* bacterium. The MIC values for (MEO/C NE1), (MEO/C NE2), (MEO/C NE 3) exhibited that the optimized formulations are in the same order of magnitude, 0.0195, 0.0390 and 0.0195 mg/mL Comparing MEO NE indicates the effect of nanoemulsification to improve antibacterial activity. However, as can be observed, these values are considerably lower compared to the pure MEO and *ciprofloxacin*, indicating an enhanced inhibitory effect, which can be ascribed to the impact of the nanoemulsion system as well as active compounds. Other biological studies were conducted after the compound (MEO/C NE 1) was determined to be the best experimental formulation based on the obtained data, which showed no discernible variation in the MIC of the optimal points. Other reports, confirm that the antibacterial activity is related to the impact of physicochemical characteristics of essential oil NEs and particle size. It has also been reported that as the droplet size decreases, the electrostatic interaction with the bacterial membrane increases, thereby producing stronger antibacterial potency. From Table 3, similar trends were observed for MBC values using different test samples. Similar results were reported for ginger oil emulsions and, lemongrass oil emulsions. By binding to the lipid and protein components of the bacterial membrane, nanoemulsions penetrate cell walls and cytoplasmic membranes, resulting in cytolytic and intracellular toxic effects. In other words, the possible mechanism underlying with antibacterial compounds of EO is that they react with the phospholipid components of the cell wall converting them into other compounds such as glycerol and phosphoric acid. Due to this conversion, phospholipid layer can no longer maintain the shape of the cell membrane, consequently, leakage occurs in the cell membrane of the bacteria.

Table 2: The number of optimal points employed for validation in nanoemulsion system.

S/N	Surfactant (w/w%) A	Essential Oil (w/w%) B	Ciprofloxacin (w/w%) C	Predicted Droplet size (nm)	Reported droplet size (nm)	Zeta Potential (mV)	Desirability
1	4.83	2.83	0.14	73.53	75.46±3.2	-16	1.000
2	4.63	2.07	0.12	69.51	73.62±4.4	-15.4	1.000
3	4.43	3.48	0.29	96.18	99.35±2.6	-15.3	1.000

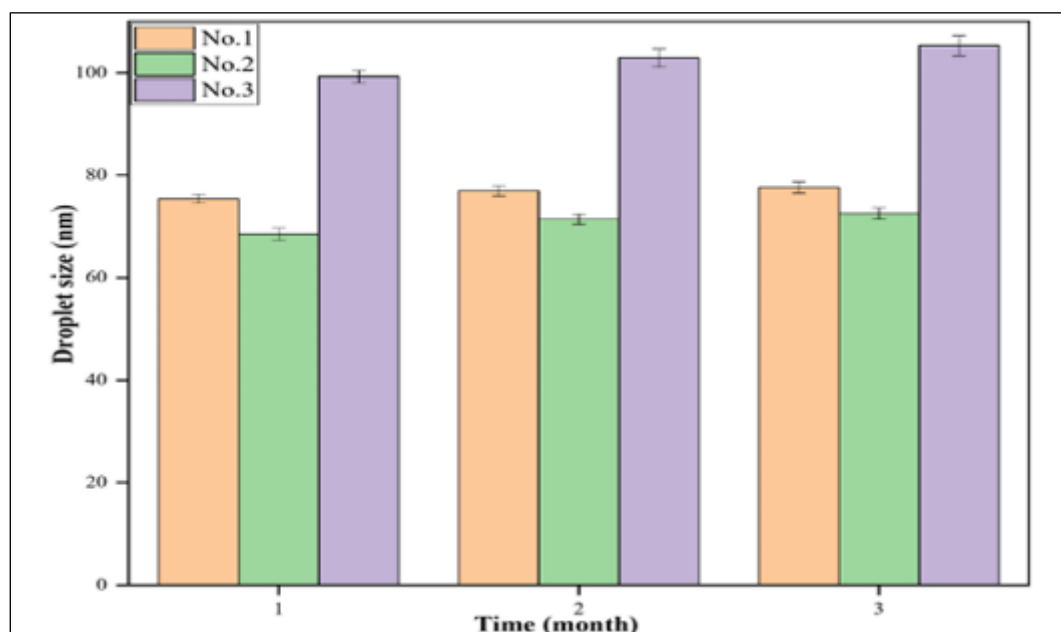


Figure 3: Optimum points stability evaluation after 3 months using size changes

It can be seen morphologically in the ghost cell shape of *E. coli* bacteria as a consequence of this reaction which gives rise to shrinking in the cell membrane. This process somewhere is also called spheroplast. In visual inspection, it should be noted that some of the lysed cells are partially visible whereas the others are visible in full. Figure 5 also presents the variations in concentration of *E. coli* bacterium (OD600) for different sample formulations at different residences times 5, 10, 15, 20, 25, and 30 min in the microfluidic chip, From Fig. 5, the (MEO/C NE) have a high potential to inhibit the growth of bacterial cells compared to *ciprofloxacin* and MEO alone, and after 30 min of incubation within the microfluidic chip at a concentration of 62.0 µg/mL, nearly all bacteria were suppressed. Table 3, is worth noting that the bacteria were killed in 24 h in the conventional method (Table 3), however, based on the results obtained from

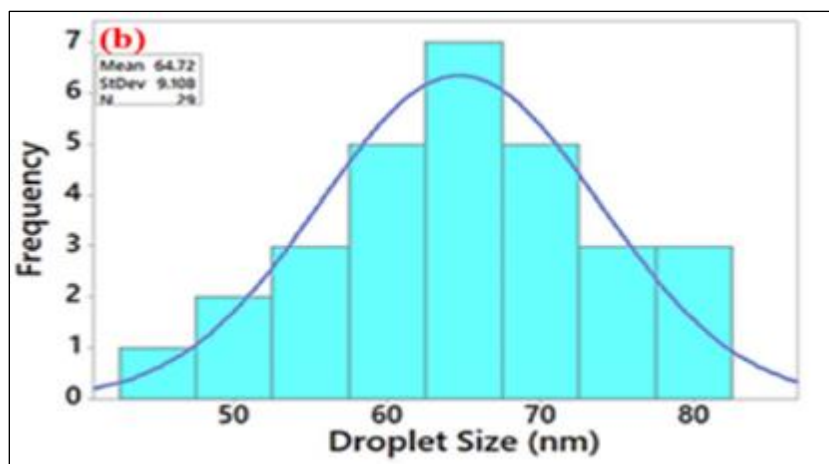


Figure 4: Number frequency histograms showing particle size distribution of optimum nanoemulsion in linear scale

Table 3: MIC and MBC (mg/mL), values of drug, EOs and nanoemulsions Against E-Coli

Compounds	MIC	MBC
MEO	3.75	7.50
CIPROFLOXACIN	0.625	0.625
MEO NE	0.80	1.60
MEO/C NEI	0.0195	0.0195
MEO NE NE 2	0.0390	0.0780
MEO NE NE 3	0.0195	0.0780

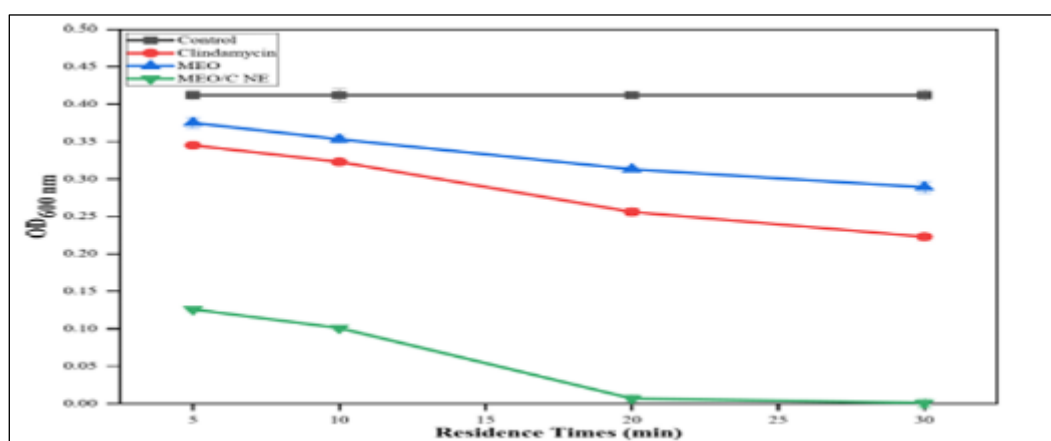


Figure 5: Bacteria viability for various formulations at different residence time in the presence of E. coli, bacterium.

Figure 5, it is obvious that after 30 min of incubation, the bacteria are killed demonstrating a more effective way for bactericidal effect. Further insight into the bactericidal properties of emulsions was gained by intracellular component release assays. Since disruption of cell membrane integrity is always associated with the leakage of intracellular substances, the amount of intracellular leakage into the medium has been monitored. By measuring the release of proteins and nucleic acids after a 30-minute residence period in the microfluidic chip and using the standard approach (MIC concentration), the antibacterial activity of samples against the E. coli strain was evaluated. It is evident from the results that the (MEO/C NE) has higher absorption values as compared to the MEO and ciprofloxacin alone. The highest leakage in terms of OD_{260 nm} and OD_{280 nm} for E. coli were recorded as 0.815 and 0.866. The obtained results show that when comparing the microfluidic chip to traditional approaches, there are noticeable differences, possibly due to

the high surface area to volume ratio effect, which leads to the generation of surface forces such as surface tension (a dominant force in the microfluidic chip).

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3.5 Time kill studies

Dynamic time-kill measurements were conducted to evaluate the bactericidal properties of active compounds (MEO and *ciprofloxacin*) and nanoemulsion (MEO/C NE) toward the growth of *E. coli* in the conventional method and microfluidic chip. As can be seen from Figure 6, the pure active compounds have decreasing trends towards bacterial inhibition, although they show less inhibitory effects than the (MEO/C NE)). It must be noted that the different physicochemical properties associated with MEO and *ciprofloxacin* result in different action. According to Figure 6, similar trends in CFU reduction were observed by employing a microfluidic chip. However, there was a considerable difference between residence time in which 30 min is needed using a microchip rather than the conventional method (3 h) for complete bacterial death. Other researchers also suggested that decreasing the particle size leads to an improvement in the antibacterial activity of nano-sized compounds, penetrating the microbial cell wall. The authors suggested that the smaller droplet diameters of the nanoemulsions, led to an increase in the bacterial cell surface collision, thus enhancing the antibacterial effect. It has been also showed that the droplet size had a correlation to the antimicrobial efficacy of nanoemulsions.

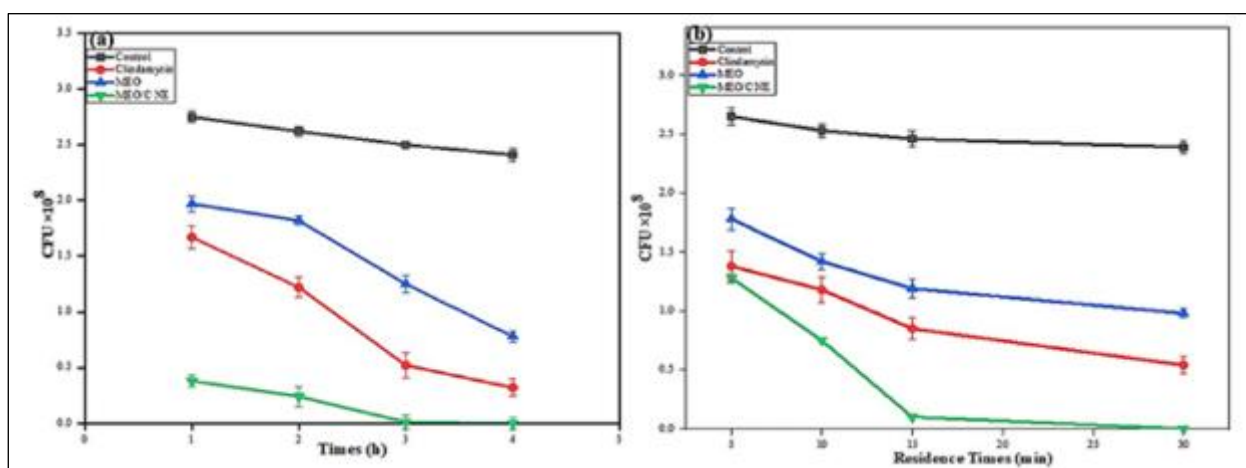


Figure 6: Time killing versus residence time for *E. coli* after treatment: using (a) the conventional method and (b) the microfluidic chip at 62.0 $\mu\text{g/mL}$ at MIC concentration, both significantly ($p < 0.05$) reduced the number of bacterial colonies compared to the control group

4 CONCLUSION

Natural compounds in conjunction with novel approaches like nanotechnology and microfluidic devices can provide new approaches to combat bacterial resistance. The increase in contact surface by nanoemulsion and microfluidic chips as well as the use of active compounds are critical parameters for inhibiting bacterial activity. Using conventional and highly efficient microfluidic techniques, the interaction of optimized MEO/C NE formulations with *E. coli* bacteria was studied. Natural active compounds interact with bacterial phospholipid bilayer membranes, resulting in cell lysis. MEO/C NE and natural bioactive compound demonstrated highly improved performance for bacterial cell destruction and active penetration than MEO and *ciprofloxacin* alone. Remarkably, a significantly greater amount ($P < 0.05$) of internal substances was released from bacterial cell membranes upon nanoemulsion treatment compared to the pure active compounds. Employing microfluidic chips showed superior efficiency in terms of low amount of sample, shorter

residence time, and higher release of cytoplasmic materials compared to the conventional method. It was found that providing more efficient contact between the two phases will result in more destruction of the bacterial cell membrane. The current study highlights the potential to enhance the natural compounds containing nanoemulsions antibacterial activity by integrating microfluidic chips as an optimistic, ingenious and economical technology

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declared that there is no conflict of interest

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