

---

*Section C: Pharmaceutics and Pharmaceutical Manufacturing.*

---

**Research Article**

## **Tailoring of Sodium hypochlorite Lipid-free nanoemulsion: Ex-Vivo Comparative Study with Chlorhexidine HCl Nanoemulsion as an Antibacterial Root Canal Irrigant**

**Rehab Abdelmonem<sup>\*1a</sup>, Mona K. Younis<sup>1b</sup>, Doaa H Hassan<sup>1b</sup>, Mohamed Abdel-Gawad El Sayed Ahmed<sup>1c</sup>,  
Ehab Hassanien<sup>2,3</sup>, Kariem El-Batouty<sup>3</sup>, Alaa Elfaham<sup>3</sup>**

<sup>1a</sup>Department of Industrial Pharmacy, College of Pharmaceutical Sciences & Drug Manufacturing, Misr University for Science and Technology, 6<sup>th</sup> of October City, 12566, Egypt.

<sup>1b</sup>Department of Pharmaceutics, College of Pharmaceutical Sciences & Drug Manufacturing, Misr University for Science and Technology, 6<sup>th</sup> of October City, 12566, Egypt

<sup>1c</sup>Department of Microbiology and Immunology, College of Pharmaceutical Sciences & Drug Manufacturing, Misr University for Science and Technology, 6<sup>th</sup> of October City, 12566, Egypt.

<sup>2</sup>Department of Endodontic Faculty of Dentistry, Galalla University, 15888, Cairo, Egypt.

<sup>3</sup>Department of Endodontic Faculty of Dentistry, Ain Shams University, 11566, Cairo, Egypt.

\*Correspondence: [rehab.abdelmonem@must.edu.eg](mailto:rehab.abdelmonem@must.edu.eg); Tel.: +201222127127

**Received:** 24 January 2024

**Accepted:** 25 January 2024

**Published:** 26 January 2024

### **Editors**

Rofida Albash  
Mahmoud Eltahan

### **Keywords**

Sodium hypochlorite.  
Root canal irrigant.  
Debris surface area.  
Cleansing effect.  
Chlorhexidine Hydrochloride.

### **Abstract**

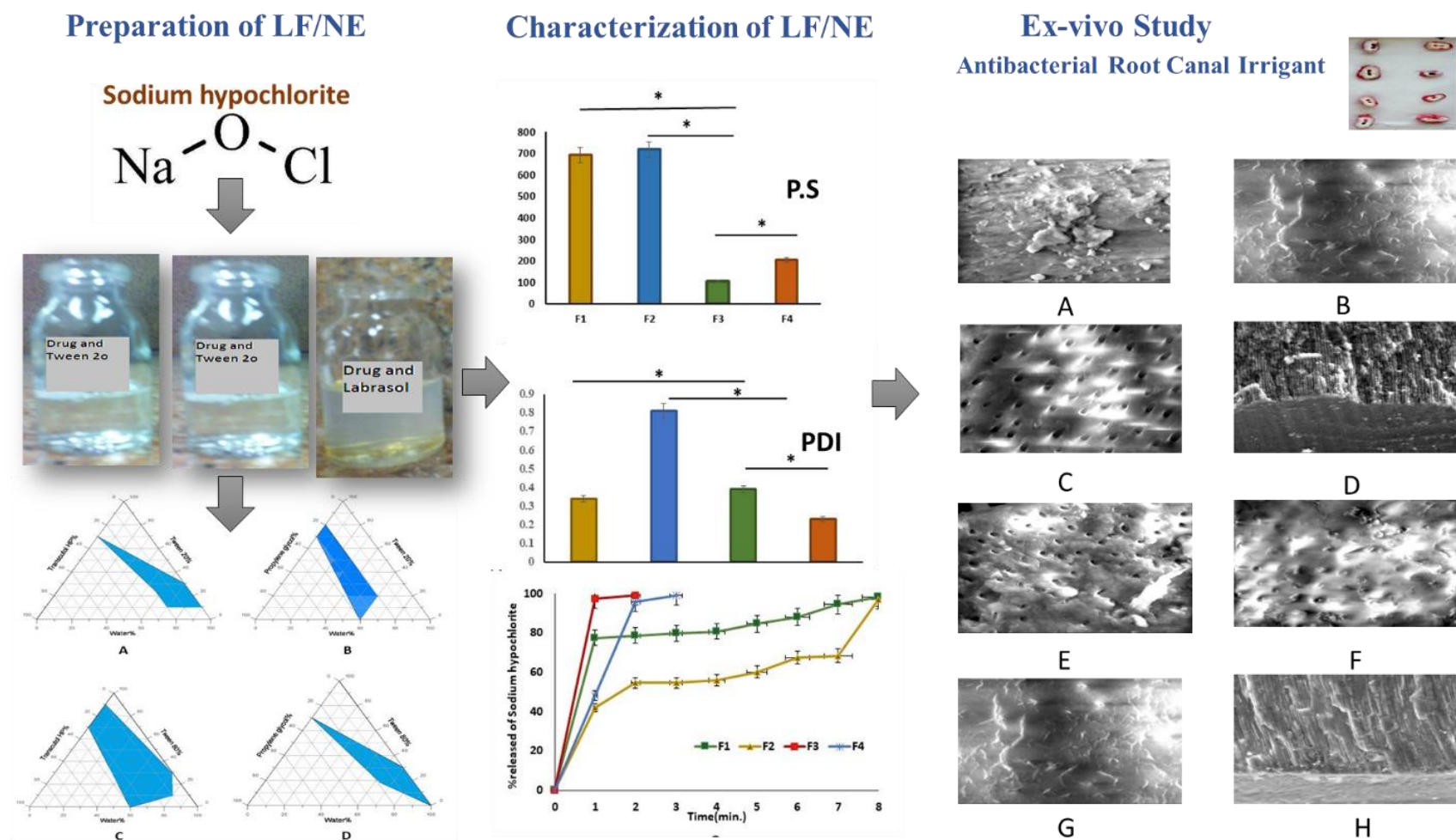
**Introduction and Aim:** Sodium hypochlorite (NaOCl) has proven to be the chosen irrigator in modern practice due to its high tissue solution power, antibacterial, and lubricant properties. This study aimed to use a Lipid-Free Nanoemulsion (LF/NE) to improve the penetration efficiency, antibacterial effect, tissue dissolving, and cleansing ability of NaOCl. Moreover, masking NaOCl taste when used as a root canal irrigant.

**Methods:** NaOCl LF/NE were comprised of a mixture of surfactants (Tween20 and Tween 80) and cosolvents (Transcutol HP and propylene glycol). The desired size range for dispersed globules was achieved using an ultrasonicator. After then, it was left to equilibrate. To designate the best systems, pseudoternary phase diagrams were constructed. The drug content, dispersibility, droplet size, drug release, and antibacterial activity of the formulae were all evaluated. Ex-vivo study was performed for the selected formula. NaOCl LF/NE was compared with two different concentrations levels 0.75 and 1.6% versus 5.25% NaOCl normal particle size and prepared Chlorhexidine Hydrochloride (CHX.HCl) nanoemulsion (in previous work) for their penetration ability as a root canal irrigant, Antibacterial activity cleansing effect, and tissue dissolving were done.

**Results:** formula F3 with a composition of 33% Tween 80 and 33% Transcutol HP was selected. It had a small particle size (106 nm) and a maximum dissolution rate after 2 min. It was proven a thermodynamically physically stable system. The ex-vivo study results showed the deepest penetration with a mean depth of 75.71µm for 1.6% NaOCl LF/NE. The result was higher than the previous work of CHX.HCl as the mean for CHX.HCl nanoemulsion irrigant was 11.29µm.

**Conclusion:** NaOCl LF/NE had better ability in the cleansing of the root canals than both of NaOCl normal particle size and CHX.HCl with high efficacy as an antibacterial agent on *Enterococcus faecalis*.

## Graphical abstract



**Table 1:** Compositions of Sodium hypochlorite LF/NE

Formulae	Drug (1.5 %)	Surfactant (33%)	Cosolvent (33%)	Distilled water (32.5%)
F1	Sodium hypochlorite	Tween 20	Transcuol HP	Water
F2		Tween 20	Propylene glycol	
F3		Tween 80	Transcuol HP	
F4		Tween 80	Propylene glycol	

## Introduction

Utilization of irrigation fluids that have an antibacterial action, mechanical tools, and irrigation help to prevent root canal bacterial infections [1]. The target of root canal treatment is to eliminate virulence factors from this system. [2]. This can be achieved by using mechanical tools combined with chemical methods [3]. For endodontics, sodium hypochlorite (NaOCl) is the most widely utilized irrigation solution [4]. One of NaOCl's limitations is its inability to remove the smear layer [5]. Chlorhexidine hydrochloride is a broad-spectrum antibacterial agent with prolonged action and minimal toxicity. In contrast to NaOCl, the main advantages of CHX.HCl is its lacks foul odour and poor taste [6].

Lipid-free nanoemulsion (LF/NE) are composed of a mixture of surfactant and co-solvent forming micellar solution. The advantage of combining a surfactant and a co-solvent is that the surfactant has a significantly higher good solvent potential (as a micellar solution) than the co-solvent alone [7]. This study highlighted the significance of using nanotechnology to mask the distinct tastes and odours of sodium hypochlorite (NaOCl) and improve its activity as a root canal irrigant. Comparison of NaOCl LF/NE with two different concentrations levels 0.75 and 1.6% versus 5.25% NaOCl normal particle size and prepared in previous studies, the penetrating capability, antibacterial activity, cleaning effect, and tissue dissolving of chlorhexidine hydrochloride (CHX.HCl) nanoemulsion as a root canal irrigant were studied [6].

## Material and Methods

### Materials

Sodium hypochlorite (NaOCl), Propylene Glycol, Tween 80 (Polyoxyethylene (80), Tween 20 (Polyoxyethylene (20) Sorbitan Monolaurate), Sorbitan Monooleate) were provided by Gomhorya Company, Cairo, Egypt. Transcutol HP and Labrasol (Caprylocaproyl Polyoxy-8 Glycerides) were supplied as a gift by Gattefosse, Saint-Priest, France. Single-rooted noncarious human teeth were collected due to periodontal disorders. Oral and Maxillofacial Department, Faculty of Dentistry, Ain Shams University, Cairo, Egypt. E. Faecalis (strain ATCC29212) pure culture grown in brain heart infusion (BHI) broth (RC CLEANER, Iichung Dental Ltd., Seoul, Korea).

### Methods

#### Pseudoternary phase diagram

Surfactants namely Tween 20, Tween 80, and Labrasol, co-surfactants like Transcutol and Propylene Glycol, and NaOCl are mixed. First, the NaOCl was combined with a surfactant and co-surfactant ( $S_{mix}$ ) mixture in the varying proportion of 1:1, 2:1, and 3:1. Then water was added dropwise during an aqueous titration [8]. The results were plotted on the Golden Software Grapher program (Version 8.1.388) detect the emulsification regions

#### Preparation of LF/NE

Based on pseudoternary phase diagram results certain selected ratios of Tween20 and Tween 80, were mixed with Transutol HP and Propylene Glycol and sonicated (Ultrasonic Lc 60 H Sonicator, Elma, Germany) for LF/NE preparation. It is then left to equilibrate [9]. [Table \(1\)](#)

#### Characterization of the Prepared LF/NE

##### Dispersibility Test

Dispersibility tests were performed using the United States Pharmacopeia (USP) dissolution apparatus (Pharma Test, Hainburg, Germany). 1 mL of each formula was added to 500 mL of water at  $37 \pm 0.5^\circ \text{C}$ . A standard stainless-steel dissolution paddle at 50 rpm was used to gently stir the mixture. Visual examination was done to determine the type of emulsion that developed. The formulation is classified as clear, translucent with a bluish tone, milky, or turbid. [10, 11].

##### Determination of Drug Content

3 mL of the formulation was diluted with 50 mL of water. 10 mL of 6 N acetic acid and 2 g of potassium iodide were added to the mixture. The liberated iodine was titrated with 0.1 N sodium thiosulfate VS, and 3 mL of starch TS was added near the endpoint. A blank analysis was carried out. Each 1 mL of (0.1 N) sodium thiosulfate is equivalent to 3.722 mg of NaOCl [12].

##### Droplet Size Analysis

The average droplet size and polydispersity index for the prepared formulations were determined using the Malvern Zetasizer 2000 (Malvern Instruments Ltd., Malvern, UK). The measurements were carried out after dilution [13].

##### In-vitro Release Study

The United States Pharmacopeia (USP) apparatus type II (paddle) was used to perform the in-vitro dissolution test at 50 rpm (Pharma Test, Hainburg, Germany). Using 500 mL of distilled water at  $37 \pm 0.5^\circ \text{C}$  as a dissolution media. 5 mL of the tested systems were added into the dissolution

medium at time 0. At various periods, 3 mL of dissolution media was withdrawn and replaced with fresh medium at the time intervals of 1, 2, 3, 4, 5, 6, 7 and 8 min. A UV spectrophotometer was used to measure the analysed aliquots at  $\lambda_{\text{max}}$  291.3 nm [14].

### Stability Studies of Prepared LF/NE

#### Drug Precipitation and Phase Separation

Phase separation was checked after 48 hours of storage of a 2 mL sample of each formulation at room temperature. Additionally, each formula was diluted with distilled water to 10 mL and 100 mL and kept at 25°C for 24 hours before being tested for phase separation [15, 16].

#### Refrigeration Thaw Cycle

2 mL of each formula was kept at 2°C for 24 hours before being taken out and kept at 25°C and 40°C. The samples were then tested for drug precipitation and phase separation [15, 16].

#### Centrifugation

5 mL sample was centrifuged at 4,000 rpm for 5 minutes (Microcentrifuge Shanghai Surgical Instrument Factory, model 800, China). Phase separation and drug precipitation were then observed in the samples [15, 16].

#### Ex-vivo Study of LF/NE Selected Formula.

For root canal irrigation, NaOCl 0.75% (F3a) and NaOCl 1.6% (F3b) prepared in the best formula (F3) were utilized. All the tests were approved by the ethical committee at the Egyptian University Ain Shams.

#### Evaluation of Irrigant Penetration Ability and Cleansing Effect:

Thirty non-carious single-rooted human teeth with mature apices were used. Before using the extracted teeth. Patients signed consent letters of informed permission. The exterior root surfaces were debrided using a curette. The surfaces of the teeth were then disinfected with 0.5% NaOCl for 24 hours before being preserved in normal saline until they were needed. The crowns were extracted, and the tooth length was 16 mm from the root apex to the coronal edge [17]. Depending on the irrigation solution, the teeth were divided into three groups:

Group (A) [n=12] F3 which divided into two subgroups:

(I) [n=6] 5mL (F3a).

(II) [n=6] 5mL (F3b).

Group (B) [n=12] NaOCl normal particle size which divided into two subgroups:

Subgroup (I) [n=6] 5mL 2.5% NaOCl normal particle size.

Subgroup (II) [n=6] 5mL 5.25% NaOCl normal particle size.

Each subgroup was divided according to the method of activation of the irrigant

- Passive ultrasonic irrigation (PUI) [n=3] (Satelec P5, France, Irrigation tip size 25). According to the previous

study on CHX, the optimum activation method was PUI [6] so, it was used in this study.

- No activation [n=3].

- Control groups [n=6]:

- Positive control: [n=3] 5mL 5.25% NaOCl normal particle size (No activation).

- Negative control: [n=3] 5 mL saline (No activation).

Mechanical preparation was performed to a master apical file size using a standardized methodology. All samples were longitudinally sectioned into two halves using a hammer and chisels [18, 19]. Then, all samples were analysed using a scanning electron microscope (SEM)

#### Study of Antibacterial Effect:

A total of 60 single-rooted, non-carious human teeth with developed apices were chosen. The upper and lower incisors and mandibular premolars were included in the teeth. The teeth were treated the same as the previous test [17, 20]. Using a standardized technique, a master apical file size of 45 was mechanically prepared. The canals were washed for one minute with 2mL of 17% EDTA to eliminate the smear layer. Each sample's whole root surface, including the apical foramen, received two layers of nail paint. To facilitate handling and labelling, the teeth were next positioned vertically in dental stone blocks [20-22]

Samples were then autoclaved for 20 minutes at 121°C and 15lb pressure. Samples were transferred and processed using sterile instruments under aseptic conditions. Using a pure *E. faecalis* culture, root canals were infected. All teeth prepared canals were treated with an inoculum of pure *E. faecalis* suspension. Then, the blocks were placed inside sterile beakers and incubated for 24 hours at 37°C [22-24]. The teeth were divided into the following groups according to the irrigation solution:

Group (A) [n=24] F3.

Subgroup (I) [n=12] 5mL of the prepared (F3a).

Subgroup (II) [n=12] 5mL the prepared (F3b).

Group (B) [n=24] NaOCl irrigation solution of normal particle size.

Subgroup (I) [n=12] 5mL 2.5% NaOCl irrigation solution of normal particle size.

Subgroup (II) [n=12] the samples were with 5mL 5.25% irrigation solution of normal particle size.

Each subgroup was divided according to the method of activation of the irrigant:

Passive ultrasonic irrigation (PUI) [n=6] (Satelec P5, France, Irrigation tip size 25).

No activation [n=6].

Group (C) [12]:

Positive Control: [n=6] the contaminated root canals were irrigated with 5mL of normal saline.



**Table 2:** Drug content of prepared Sodium hypochlorite LF/NE

Formulae	Drug content (Mean±SD) (%)
F1	98.38±0.31
F2	94.84±0.86
F3	99.69±0.21
F4	98.51±0.78

Negative Control: [n=6] suspension injections were not given to the specimens, that is, the root canals were not contaminated and kept sterile as a negative control to ensure s sterility and reliability of procedures. Each sample contained 5mL of test irrigator. 1 mL of sterile normal saline was then used to irrigate each sample.

#### Evaluation of Bacterial Growth:

Using sterile paper with a point size of 35, root canal samples were obtained. After inserting the paper points into the canals until they reached the working length, they were kept in situ for 10 seconds. The colony-forming units (CFU) per plate were then counted after being transferred to agar plates. The plates were examined visually for bacterial growth after a 24-hour incubation period at 37°C. Clear plates are sterilized. Plates that were blurred were considered positive growth. For each plate, the average number of CFUs was counted, and the total number of CFUs was calculated. Pour plates and diluted ones were both utilized to count high CFUs and low CFUs, respectively [25].

#### Viable Count

The sample is applied to a melted agar medium before it solidifies. Colonies are evenly distributed across the medium after the desired sample dilution is plated. Serial dilutions will be needed if the sample contains more cells than the agar plate can hold, with a statistically significant range of 30 to 300 CFU. On the day of the experiment, the agar medium was autoclaved, and a tube containing 15 mL of melted agar material was prepared. *E. faecalis* ATCC29212 bacteria were used in the sample, which was made by mixing colony cells with sterile saline. After that, a 10 µL bacterial sample was diluted in saline to McFarland 0.5, which equals 108 CFU/mL. A 100-mL graduated cylinder was filled to the top with 1% sulfuric acid and 0.6 mL of a 1% solution of barium chloride dihydrate to create a turbidity standard. The sample was poured into the middle of the empty Petri dish once the lid was opened. Once the agar had fully solidified, the plate was inverted and left to incubate for 24 hours at 37 °C [20].

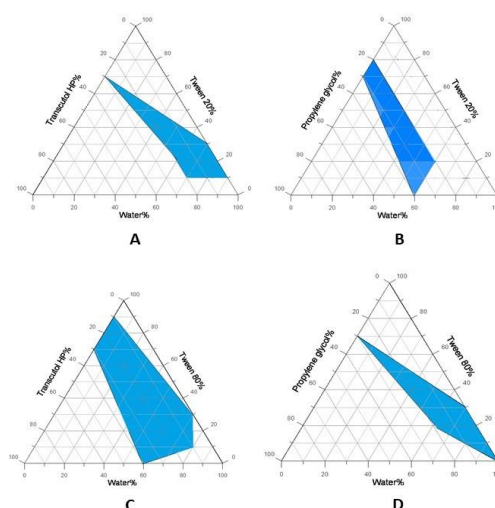
#### Statistical Methods

The statistical analysis was performed using IBM® SPSS® Statistical Version 17 for Windows [IBM Corporation, NY, USA, SPSS, Inc., an IBM Company].

#### Results

##### Preparation of LF/NE formulae

Trials were made to mix Labrasol with different ratios with Tween 80 and Tween 20 with co-surfactants, but all formulas produce a milky appearance during the water titration test, so a formula that contains Labrasol were excluded from further evaluation. The other systems were plotted on the Grapher program (Version 8.1.388) to determine the emulsification regions. Formulae in this study were selected from high emulsification region on pseudoternary phase diagram. This may be attributed to that NaOCl is miscible with the used tested vehicles (Fig.1).

**Fig. 1** Pseudoternary phase diagrams

#### Characterization of LF/NE formulae

##### Dispersibility Test

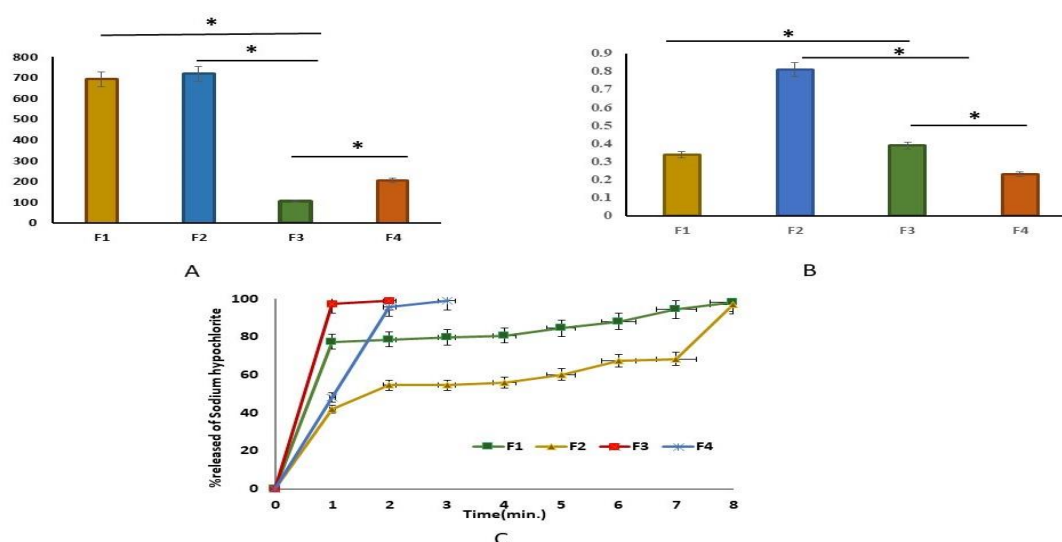
All the prepared formulae that appeared to be clear were included for further evaluation research.

##### Drug Content

The prepared formulae showed drug content ranged between 94.84-99.69% Table (2).

##### Droplet Size Analysis

The droplet size of the prepared formulae F1, F2, F3, and F4 were 693±94.5, 719±97.6, 106±1.7, and 205±12.5 nm respectively, with polydispersity index 0.34, 0.81, 0.39, and 0.23. (Fig. 2)



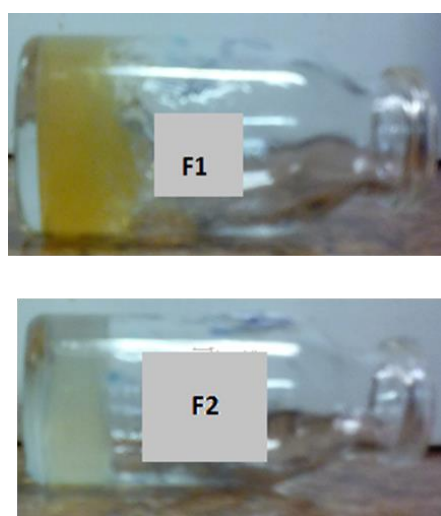
**Fig. 2** Characterization of prepared LF/NE formulae (A) average droplet size, (B) polydispersity index (PDI), (C) *In-vitro* drug release.

### In-vitro Release Study

The percentage of NaOCl released from the formulae showed in (Fig.2). The complete drug release for prepared NaOCl formulae ranged between 2 to 8 minutes. It was observed the formula F3 showed a higher dissolution rate than other formulas,

### Stability Studies

All formulations were put through intensive thermodynamic testing using centrifugation, freezing, and thawing cycles. F3 and F4 passed the thermodynamic stability tests with no signs of phase separation. F1 and F2, which contain Tween 20 and the co-surfactants propylene glycol and Transcutol HP, respectively, showed gelling appearances (Fig.3)



**Fig.3** Thermodynamic Stability Results Showed Gelling of F1 and F2.

### Ex-vivo Study of LF/NE Selected Formula.

The concentration of the components under investigation was standardized at the beginning of the study to resemble that of a commercially available irrigant. The viscosity of the LF/NE (F3) was too high to use for irrigation. Serial dilutions were performed until the highest concentration that could be used for irrigation was reached. F3b was diluted to 1.6% w/v to give the higher concentration and F3a was diluted to 0.75% w/v giving the lower concentration. Reducing the concentration is better for biocompatibility and decreasing the cytotoxicity to the apical and periodontal tissues.

There was a statistically significant ( $P < 0.001$ ) difference between the mean depths of irrigant penetration in DT at different irrigant concentrations. F3b was statistically highly significant ( $P < 0.001$ ) recording the deepest penetration with a mean depth of  $75.71\mu\text{m}$  and SD 0.007. NaOCl normal particle size 5.25% and F3a were statistically ( $P > 0.05$ ) non-significant, which means that the higher concentration of F3b is more effective. The result was higher than the previous work of CHX as the mean for CHX nanoemulsion irrigant was  $11.29\mu\text{m}$ . the statistical analysis was significantly ( $P > 0.05$ ) different with a P-value of 0.23576. (Fig.4).

The effect of LF/NE irrigating solutions on *E. faecalis* was investigated by incubating the bacteria in tooth specimens and then calculating Colony Forming Units (CFU). It was observed that conventional irrigants into DT, they are unable to entirely remove *E. faecalis*. While, The tissue dissolving and debris removal of LF/NE irrigants were much more than the normal particle size irrigants (Table 3).

### Discussion

NaOCl is miscible with all tested vehicles except Labrasol. All formulae that contain Labrasol in different percentages give a milky appearance this may be due to the

**Table 3:** The Number of Viable Bacterial Colonies.

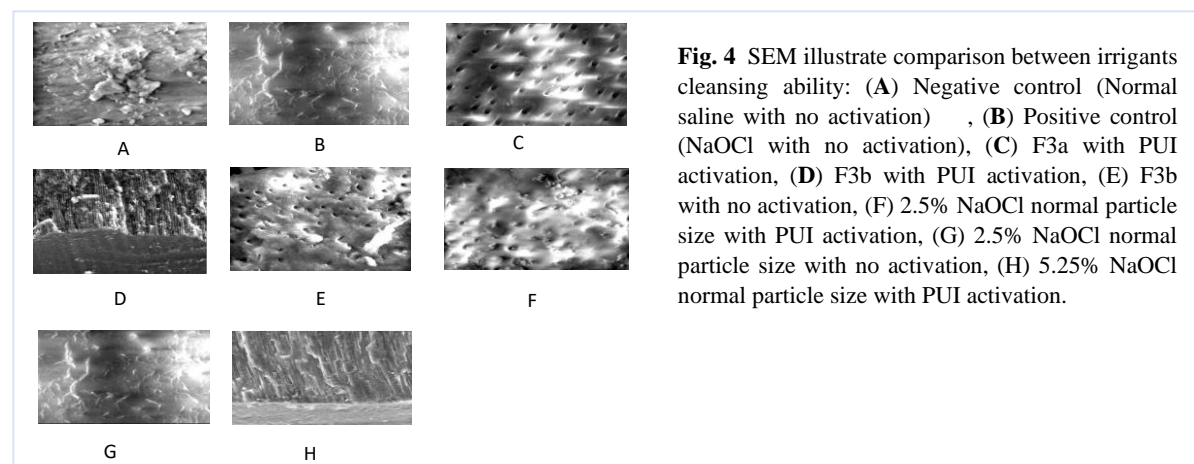
Positive Control	Bacteria only on Agar → ++++ve		Bacteria only in the canals → ++ve	
	F3		Normal Particle Size	
Irrigation Concentrations	F3a	F3b	NaOCl I 2.5%	NaOCl II 5.25%
Negative Control	-ve	-ve	-ve	-ve
Activation Methods showing number of viable colonies per plate				
Cfu/ml	21000	9200	-	-
PUI	140	-ve	-ve	-ve
Cfu/ml	14000		-	-
Without Activation showing number of viable colonies per plate				
No Activation	164	50	-ve	-ve
Cfu/ml	16400	5000	-	-

immiscibility of Labrasol with NaOCl. The ratio of surfactant and cosurfactant of more than 3:1 was not tested to avoid increasing the viscosity of preparation to ease its rheology during dental irrigation. Also, It was noted that formulae F3 and F4 have smaller particle sizes than other formulae. This could be due to the presence of Tween80, which has better emulsifying and solubilizing properties for sodium hypochlorite due to its high HLP value [26, 27]. LF/NE do not contain natural lipids and represent the most hydrophilic formulations. These types produce very fine dispersions and have greater drug payloads. This may be due to the increased drug solubility in the co-solvent and surfactants. As a result, the drug showed greater absorption and is released more quickly [28]. Formula F3 which has the smallest particle size (106 nm) showed a higher dissolution rate than other formulas, indicating that particle size and hence surface area of drug particles have a greater impact on dissolution rate. This is due to the fact that reducing the size below 1µm increases the solvation pressure, enhancing solubility and causing the solute-

when diluted [31]. Thermodynamic stability and phase separation studies were carried out to test formulae's ability to withstand stressful conditions. Formulae containing Tween 20 showed gelling appearance which may be a sign that they are unstable due to droplet aggregation which confirm the high capacity of Tween 80 as stabilizer to decrease surface free energy of droplets and prevents its aggregation in formulae F3 and F4 [32, 33].

F3 was chosen as the best formula since it has the smallest droplet size (106 nm), a rapid dissolution rate after 2 minutes, as well as being thermodynamically/physically stable system.

Ex-vivo Study was designed to compare the antibacterial performance of the new irrigation F3 to commercially available irrigation of normal-size NaOCl as an initial study in the evaluation of a new endodontic irrigant. The microorganism studied in this investigation was *E. faecalis*. *E. faecalis* was selected because it is one of the most frequently isolated species in persistent root canal



**Fig. 4** SEM illustrate comparison between irrigants cleansing ability: (A) Negative control (Normal saline with no activation), (B) Positive control (NaOCl with no activation), (C) F3a with PUI activation, (D) F3b with PUI activation, (E) F3b with no activation, (F) 2.5% NaOCl normal particle size with PUI activation, (G) 2.5% NaOCl normal particle size with no activation, (H) 5.25% NaOCl normal particle size with PUI activation.

solute interaction to be disrupted, which greatly facilitates the solubilisation process [29, 30]. The prepared formulation must maintain its stability over various temperatures and maintain its ability to solubilize even

infections. Additionally, *E. faecalis* may survive in treated root canals for a very long period with harsh conditions and a lack of nutrition. Extremely high and low pH values,

as well as extremely high temperatures, are not barriers to *E. faecalis* survival [2, 34]

The effect of LF/NE irrigating solutions on *E. faecalis* was investigated by incubating the bacteria in tooth specimens and then calculating Colony Forming Units (CFU). Because of the low penetration depth of conventional irrigants into DT, they are unable to entirely remove *E. faecalis* [35].

NaOCl's taste and odour are two of its most unpleasant drawbacks. The use of the micellar solution of the reagent masked the disgusting taste and odour [36]. LF/NE of NaOCl is a tasteless and odourless clear irrigating solution. It was found that the higher concentration of nanosized delivery formulations (F3a and F3b) had a deeper penetration ability in DT due to the smaller particle size. The tissue dissolving and debris removal of LF/NE irrigants were much more than the normal particle size irrigants. F3b was statistically highly significant recording the deepest penetration and it was higher than the previous work of CHX. (Fig.4).

When NaOCl was exposed to sunlight, the chlorine content decreased quickly. Heated NaOCl lost around 5% of its potency in just six hours [37]. The ultrasonically activated irrigant facilitates root canal cleaning and disinfection. The efficacy of ultrasonic instrumentation is due to the interaction of ultrasonic energy and the irrigating solution. Transient cavitation occurs when ultrasonic energy creates a bubble that expands to a certain size before the collapse. This collapse creates a vacuum pressure that kills microorganisms and cleans channel irregularities. When using conventional techniques like irrigation alone, such as on rough or difficult-to-reach surfaces, the cavitation implosion works successfully [38]. When F3b was activated by PUI, complete bacterial elimination was achieved and the deepest penetration and better cleansing effect was found when PUI was used.

## Conclusion

The root canal cleaning performance of NaOCl LF/NE was superior to NaOCl normal particle size, and it was also more effective as an antibacterial agent. CHX nanoemulsion was an effective antibacterial agent but its cleansing ability was not as effective as the NaOCl LF/NE. We recommend that NaOCl LF/NE is a promising irrigant that should replace the currently used irrigants.

## Acknowledgment(s)

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

[1] Borzini L, Condò R, De Dominicis P, Casaglia A, Cerroni L. Root Canal Irrigation: Chemical Agents and Plant Extracts Against *Enterococcus faecalis*. *Open Dent J*. 2016;10:692-703.

[2] Narayanan LL, Vaishnavi C. Endodontic microbiology. *J Conserv Dent*. 2010;13(4):233-9.

[3] Nogo-Živanović D, Kanjevac T, Bjelović L, Ristić V, Tanasković I. The effect of final irrigation with MTAD, QMix, and EDTA on smear layer removal and mineral content of root canal dentin. *Microsc Res Tech*. 2019;82(6):923-30.

[4] Elmessiry A, Darrag AM, Ghoneim WM. Effect of different irrigation solutions and techniques on push-out bond strength of resin-based sealer. *Tanta Dental Journal*. 2019;16:46 - 54.

[5] Mohammadi Z, Shalavi S, Moeintaghavi A, Jafarzadeh H. A Review Over Benefits and Drawbacks of Combining Sodium Hypochlorite with Other Endodontic Materials. *Open Dent J*. 2017;11:661-9.

[6] Abdelmonem R, Younis MK, Hassan DH, El-Sayed Ahmed MAE, Hassanein E, El-Batouty K, et al. Formulation and characterization of chlorhexidine HCl nanoemulsion as a promising antibacterial root canal irrigant: in-vitro and ex-vivo studies. *Int J Nanomedicine*. 2019;14:4697-708.

[7] Khani S, Keyhanfar F, Amani A. Design and evaluation of oral nanoemulsion drug delivery system of mebupine. *Drug Deliv*. 2016;23(6):2035-43.

[8] Khani S, Keyhanfar F, Amani A. Design and evaluation of oral nanoemulsion drug delivery system of mebupine. *Drug Deliv*. 2016;23(6):2035-43.

[9] Ahmad N, Ahmad R, Al-Qudaihi A, Alaseel SE, Fita IZ, Khalid MS, et al. Preparation of a novel curcumin nanoemulsion by ultrasonication and its comparative effects in wound healing and the treatment of inflammation. *RSC Advances*. 2019;9(35):20192-206.

[10] P. S. Rajinikanth, Yeoh Suyu, Sanjay Garg. Development and in vitro Characterization of Self-nanoemulsifying Drug Delivery Systems of Valsartan. *International Journal of Medical, Medicine and Health Sciences*. 2012Dec28;6.0(12).

[11] Kotta S, Khan AW, Ansari SH, Sharma RK, Ali J. Formulation of nanoemulsion: a comparison between phase inversion composition method and high-pressure homogenization method. *Drug Deliv*. 2015;22(4):455-66.

[12] Jackson DS, Crockett DF, Wolnik KA. The indirect detection of bleach (sodium hypochlorite) in beverages as evidence of product tampering. *J Forensic Sci*. 2006;51(4):827-31.

[13] Roselan MA, Ashari SE, Faujan NH, Mohd Faudzi SM, Mohamad R. An Improved Nanoemulsion Formulation Containing Kojic Monooleate: Optimization, Characterization and In Vitro Studies. *Molecules*. 2020;25(11).

[14] Singh G, Pai RS. Optimized self-nanoemulsifying drug delivery system of atazanavir with enhanced oral bioavailability: in vitro/in vivo characterization. *Expert Opin Drug Deliv*. 2014;11(7):1023-32.



- [15] Kandav G, Singh S. Review of Nanoemulsion Formulation and Characterization Techniques. *Indian Journal of Pharmaceutical Sciences*. 2018;80.
- [16] Azeem A, Rizwan M, Ahmad FJ, Iqbal Z, Khar RK, Aqil M, et al. Nanoemulsion components screening and selection: a technical note. *AAPS PharmSciTech*. 2009;10(1):69-76.
- [17] Berber VB, Gomes BP, Sena NT, Vianna ME, Ferraz CC, Zaia AA, et al. Efficacy of various concentrations of NaOCl and instrumentation techniques in reducing *Enterococcus faecalis* within root canals and dentinal tubules. *Int Endod J*. 2006;39(1):10-7.
- [18] Palazzi F, Blasi A, Mohammadi Z, Del Fabbro M, Estrela C. Penetration of Sodium Hypochlorite Modified with Surfactants into Root Canal Dentin. *Braz Dent J*. 2016;27(2):208-16.
- [19] Bumb SS, Bhaskar DJ, Agali CR, Punia H, Gupta V, Singh V, et al. Assessment of Photodynamic Therapy (PDT) in Disinfection of Deeper Dentinal Tubules in a Root Canal System: An In Vitro Study. *J Clin Diagn Res*. 2014;8(11):Zc67-71.
- [20] Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis*. *Int Endod J*. 2001;34(6):424-8.
- [21] Siqueira JF, Jr., Machado AG, Silveira RM, Lopes HP, de Uzeda M. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of *Enterococcus faecalis* from the root canal, in vitro. *Int Endod J*. 1997;30(4):279-82.
- [22] Agrawal V, Rao MR, Dhingra K, Gopal VR, Mohapatra A, Mohapatra A. An in vitro comparison of antimicrobial efficacy of three root canal irrigants-BioPure MTAD, 2% chlorhexidine gluconate and 5.25% sodium hypochlorite as a final rinse against *E. faecalis*. *J Contemp Dent Pract*. 2013;14(5):842-7.
- [23] Rajakumaran A, Ganesh A. Comparative Evaluation of Depth of Penetration of Root Canal Irrigant After Using Manual, Passive Ultrasonic, and Diode Laser-Assisted Irrigant Activation Technique. *J Pharm Bioallied Sci*. 2019;11(Suppl 2):S216-s20.
- [24] Bago I, Plečko V, Gabrić Pandurić D, Schauerperl Z, Baraba A, Anić I. Antimicrobial efficacy of a high-power diode laser, photo-activated disinfection, conventional and sonic activated irrigation during root canal treatment. *Int Endod J*. 2013;46(4):339-47.
- [25] Frough-Reyhani M, Ghasemi N, Soroush-Barhaghi M, Amini M, Gholizadeh Y. Antimicrobial efficacy of different concentration of sodium hypochlorite on the biofilm of *Enterococcus faecalis* at different stages of development. *J Clin Exp Dent*. 2016;8(5):e480-e4.
- [26] Brandelero RPH, Yamashita F, Grossmann MVE. The effect of surfactant Tween 80 on the hydrophilicity, water vapor permeation, and the mechanical properties of cassava starch and poly(butylene adipate-co-terephthalate) (PBAT) blend films. *Carbohydrate Polymers*. 2010;82(4):1102-9.
- [27] Chai JL, Liu N, Bai TT, Zhang HM, Liu NN, Wang D. Compositions and Physicochemical Properties of Tween Type Surfactants-Based Microemulsions. *Journal of Dispersion Science and Technology*. 2014;35(3):441-7.
- [28] Gao Z-g, Choi HG, Shin H-JY, Park KM, Lim SJ, Hwang K-j, et al. Physicochemical characterization and evaluation of a microemulsion system for oral delivery of cyclosporin A. *International Journal of Pharmaceutics*. 1998;161:75-86.
- [29] Khadka P, Ro J, Kim H, Kim I, Kim JT, Kim H-I, et al. Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability. *Asian Journal of Pharmaceutical Sciences*. 2014;9:304-16.
- [30] Braibanti M, Kim HS, Şenbil N, Pagenkopp MJ, Mason TG, Scheffold F. The liquid-glass-jamming transition in disordered ionic nanoemulsions. *Sci Rep*. 2017;7(1):13879.
- [31] Patel H, Patel P, Patel M, Mehta D, Misan C. Development and characterization of liquid and solid self-microemulsifying drug delivery system of Tacrolimus. *Asian Journal of Pharmaceutics*. 2012;6:204.
- [32] Mason TG, Wilking JN, Meleson K, Chang CB, Graves SM. Nanoemulsions: formation, structure, and physical properties. *Journal of Physics: Condensed Matter*. 2006;18:R635 - R66.
- [33] Costa P, Sousa Lobo JM. Modeling and comparison of dissolution profiles. *Eur J Pharm Sci*. 2001;13(2):123-33.
- [34] Li Q, Zhang Q, Zou X, Yue L. Evaluation of four final irrigation protocols for cleaning root canal walls. *International Journal of Oral Science*. 2020;12(1):29.
- [35] Rajeshwari, Kamath P, Kundabala M, Shenoy S, Hegde V, Thukral N. An evaluation of horizontal depth of penetration of various irrigants into the dentinal tubules when used alone and in combination with diode laser: An: in vitro: study. *Journal of Interdisciplinary Dentistry*. 2014;4(3).
- [36] George S, Kishen A, Song KP. The role of environmental changes on monospecies biofilm formation on root canal wall by *Enterococcus faecalis*. *J Endod*. 2005;31(12):867-72.
- [37] Clarkson RM, Moule AJ, Podlich HM. The shelf-life of sodium hypochlorite irrigating solutions. *Aust Dent J*. 2001;46(4):269-76.
- [38] Tashkandi N, Alghamdi F. Effect of Chemical Debridement and Irrigant Activation on Endodontic Treatment Outcomes: An Updated Overview. *Cureus*. 2022;14(1):e21525.