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Original article.

## Formulation and Characterization of a Transfersome-based Oral Care Gel: Development and Delivery of Encapsulated Lidocaine

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### ABSTRACT

**Introduction:** This study reports the formulation and development of oral gel-based transfersome nanoformulations prepared via the thin-film method. Lidocaine was incorporated as the active local anaesthetic agent, and different concentrations of excipients were employed to achieve the optimized gel characteristics.

**Aims:** The aim of this research is to design, develop, and optimize a novel oral care gel in combination with lidocaine-entrapped transfersome carriers intended to promote gingival health, and improve the physicochemical and structural characteristics of the formulation for therapeutic applications in oral care.

**Methods:** Hydrogels with lidocaine were manufactured via sequential phase A–D compounding with and without preservative (phenoxyethanol+ethylhexylglycerin or Plantaserve E), then homogenized via magnetic stirring and high-shear mixing to achieve optimum uniformity. Transfersomes were produced by thin-film hydration using a rotary evaporator. These gel-based formulations were then characterized for their rheological properties, texture analysis, and Fourier transform infrared spectroscopy. Lidocaine entrapment efficiency was quantified using the HPLC technique and antimicrobial activity was tested against *Candida albicans*.

**Results:** The optimized gel incorporated transfersome formulation with lidocaine (NF8) demonstrated near-physiological pH (~6.5) and spreadability of 3.0 cm (the same as the control product, 3.0 cm). The same gel formulation showed a viscosity of ~75,840 cP and ~28,200 cP at 2.5 and 10 rpm. The texture analyzer showed a firmness of  $122.3 \pm 9.1$  g and a shear work of  $61.7 \pm 9.1$  g/sec. Entrapment efficiency of lidocaine in transfersome nanoformulation was found to be 71%, whereas the particle size and zeta potential were found to be 268 nm and -3 mV. Whereas non-sonicated dispersions showed larger transfersome particle size (453 nm) and wide polydispersity (PDI of 0.81).

**Conclusion:** These findings showed that transfersome-based gel has the potential to be used for their oral care application alongside their analgesic, antimicrobial, and anti-inflammatory effects.

**KEYWORDS:** TRANSFERSOME, THIN-FILM METHOD, ORAL GEL, LIDOCAINE.

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## INTRODUCTION

Transfersomes, also known as ultra-deformable vesicles, consist of an inner aqueous core that facilitates the entrapment of hydrophilic drugs, surrounded by concentric bilayers that interact with lipophilic drugs. Due to their elastic nature, transfersomes can deform and squeeze through narrow pores in the skin, even when the pores are significantly smaller than the vesicle size. Transfersomes offer several advantages, including the ability to deliver a wide range of active compounds such as proteins, peptides, insulin, corticosteroids, interferons, anaesthetics, and anticancer drugs. Transfersomes also enhance the sustained release of active ingredients, thereby prolonging their therapeutic effect (1). It is important to note that the novelty of this formulation goes beyond merely incorporating active ingredients, as such compounds are often used in conventional oral care gels. To enhance the innovation and therapeutic potential, advanced formulation strategies have been introduced through nanotechnology. Nanoformulations, which include various drug delivery systems (including liposomes, solid lipid nanoparticles, nanostructured lipid carriers, and niosomes), offer improved bioavailability, targeted delivery, and enhanced stability of active ingredients (2–4). Among these systems, transfersomes represent a particularly promising approach due to their advantages for the development of next-generation oral care formulations (1).

An oral gel can be positioned for several cosmetic and adjunctive purposes. Mucoadhesive gels are designed to hydrate and protect the mucosa, prolong residence time on wet surfaces, and deliver soothing or barrier-forming excipients; these systems exploit the viscoelastic properties of polymers to maintain contact with the oral epithelium. Contemporary reviews of oral/buccal mucosal delivery highlight gels as useful vehicles for local effects (lubrication, protection from mechanical irritation) and, when drug-loaded, for therapeutic indications (e.g., analgesia, antifungal/antimicrobial)—with the latter falling under drug/medicinal regulation (5).

Lidocaine is a tertiary amide base ( $pK_a \approx 7.8$ ). The unionized fraction crosses neuronal membranes; intracellularly, the protonated form binds to the inner vestibule of voltage-gated sodium channels ( $Na_v$ ), preferentially in the open and inactivated states. This state-dependent (use-dependent) block inhibits  $Na^+$  influx, preventing action potential initiation/propagation—first in small, high-frequency nociceptive fibres ( $A\delta$ , C), producing local anaesthesia. Acidic environments (e.g., inflamed tissue) shift lidocaine toward the ionized form, slowing onset; alkalization (buffering) increases the unionized fraction and can hasten onset (6–8).

## MATERIALS AND METHODS

### Materials

Sodium phosphate monobasic monohydrate (SPMH;  $\geq 98\%$ ) Lidocaine ( $\geq 97.5\%$ ), and sodium hydrogen phosphate dibasic ( $\geq 98\%$ ) were purchased from Thermo Scientific, USA. Sucralose (SCL) was purchased from Bulk™ (Colchester, UK), while xylitol was obtained from Merisant Company 2 Sàrl, UK. Carbomer 940 was supplied by Shelly Pol Interchem, India. Sodium phosphate dibasic dihydrate (SPDD;  $\geq 99\%$ ) and triethanolamine (TEA) ( $\geq 99\%$ ) were purchased from Sigma-Aldrich, UK. Ethylhexylglycerin ((EHG) cosmetic grade) was obtained from Make Your Own Cosmetics, India. Vegetable Glycerin (VG) was purchased from Cosmetics Natural Oils Ltd., UK. Phenoxyethanol (PE) was purchased from Clariant Product, Germany. Plantaserve E (PP, cosmetic preservative) was obtained from Mystic Moments, UK. Tea tree oil (TTO, *Melaleuca alternifolia*) was purchased from Essentially Oils Ltd., UK. Hyaluronic acid (HA) powder was obtained from Micro Ingredients, USA. Sorbitan monooleate (Span 80) was purchased from Thermo Fisher Scientific, USA. 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC, Lipoid PC

14:0/14:0) was obtained from Lipoid, Germany. Acetonitrile (ACN), sodium chloride ( $\geq 99.5\%$ ), and methanol (MeOH) were purchased from Fisher Scientific, UK.

### Preparation of gel formulation

Gel formulations (F1–F5) were prepared in order to get the optimized gel based on employing different ingredients and their concentrations (Table 1). In formulations F1 and F2, ethyl lauroyl arginate (LAE) was incorporated at 0.08 g and 0.10 g to provide antibacterial activity. F1 and F2 exhibited visible white particulate residues; therefore, LAE was replaced in subsequent formulations with tea tree oil as an alternative antimicrobial. Across F1–F5, Carbopol 940 was screened at 0.28, 0.20, 0.23, and 0.24 g. Based on handling and performance, 0.24 g of Carbopol 940 was selected and carried forward for the F5 gel formulation, and hence the selected F5 formulation was incorporated with the transfersome nanoformulation (N) and hence referred to as NF6–NF10.

*Table 1. Formulation design of gels F1–F5 and incorporation of nanoformulations (N) into gel referred to as NF6–NF10 with various excipients and their concentrations (%w/w, for 50%), n = 3.*

| Formulation | HA  | VG | Xylitol | PE  | EHG | SPDD | TEA  | H <sub>2</sub> O | SCL | Carbopol    | LAE         | TTO        | PP (Drops) | NaOH (0.1%) | NaOH (1:9) |
|-------------|-----|----|---------|-----|-----|------|------|------------------|-----|-------------|-------------|------------|------------|-------------|------------|
| F1          | 0.3 | 6  | 4       | 0.4 | 0.2 | 0.15 | 0.23 | 33               | 0.1 | 0.28        | <b>0.08</b> | -          | -          | -           | -          |
| F2          | 0.3 | 6  | 4       | 0.4 | 0.2 | 0.15 | 0.23 | 33               | 0.1 | 0.28        | <b>0.10</b> | -          | -          | -           | -          |
| F3          | 0.3 | 6  | 4       | 0.4 | 0.2 | 0.15 | 0.23 | 33               | 0.1 | 0.20        | -           | <b>0.1</b> | -          | -           | -          |
| F4          | 0.3 | 6  | 4       | 0.4 | 0.2 | 0.15 | 0.23 | 33               | 0.1 | 0.23        | -           | 0.1        | -          | -           | -          |
| F5          | 0.3 | 6  | 4       | 0.4 | 0.2 | 0.15 | 0.23 | 33               | 0.1 | <b>0.24</b> | -           | 0.1        | -          | -           | -          |
| NF6         | 0.3 | 6  | 4       | 0.4 | 0.2 | 0.15 | 0.23 | 33               | 0.1 | 0.24        | -           | 0.1        | -          | 55D         | -          |
| NF7         | 0.3 | 6  | 4       | -   | -   | 0.15 | 0.23 | 33               | 0.1 | 0.24        | -           | 0.1        | 14D        | 55D         | -          |
| NF8         | 0.3 | 6  | 4       | 0.4 | 0.2 | 0.15 | 0.23 | 33               | 0.1 | 0.24        | -           | 0.1        | -          | -           | <b>7D</b>  |
| NF9         | 0.3 | 6  | 4       | -   | -   | 0.15 | 0.23 | 33               | 0.1 | 0.24        | -           | 0.1        | 14D        | -           | 7D         |
| NF10        | 0.3 | 6  | 4       | -   | -   | 0.15 | 0.23 | 33               | 0.1 | 0.24        | -           | 0.1        | -          | -           | -          |

To prepare the formulation, Phase A (water-based) was first created by transferring Carbopol 940 (0.24 g) to a 100 mL beaker containing 23 g of distilled water. The dispersion was mixed using a magnetic stirrer (VELP Scientifica, Italy) at 500 rpm for 15 min, then covered with Parafilm and left to stand for 1 hour. Next, Phase B (pre-dispersion of hyaluronic acid) was prepared by adding hyaluronic acid (0.30 g) to 3 g of glycerin in a 100 mL beaker. Mixing was carried out using a magnetic stirrer at 200 rpm for 30 min, after which the mixture was covered and left to rest for 1 hour. In separate weighing boats, Phase C (active and sweetener phase), xylitol (4 g), sucralose (0.10 g), and SPDD (0.15 g) were weighed. In a 100 mL beaker, distilled water (8 g) and glycerine (2 g) were mixed via magnetic stirrer at 300 rpm for 30 min. However, xylitol was added after 1 min, SPDD was added at 11 min, and finally sucralose was added by 25 min (during the total 30 min mixture time). Phase D (Preservatives) was prepared in a 100 mL beaker; 2 g of distilled water and 1 g of glycerine were combined and placed on a magnetic stirrer. Tea tree oil (0.10 g or ~3 drops) was added first, followed by phenoxyethanol (0.40 g or ~9 drops), ethylhexylglycerin (0.20 g or ~5 drops), and 7 drops of sodium hydroxide. The mixture was stirred at 300 rpm for ~1 min to complete Phase D. Finally, all phases were combined using an IKA T18 digital ULTRA-TURRAX (IKA®, Germany),

at 3000 rpm. Phase C (actives) was added to the hydrated Phase A (Carbopol dispersion) and mixed for approximately 1 min. Phase B was then incorporated and mixed for another minute, followed by the addition of Phase D under the same conditions for 1 min. Finally, triethanolamine (TEA) (0.23 g) was added, and the batch was mixed for an additional 2 min. The resulting gel was allowed to stand for 12 hours.

### **pH, spreadability and viscosity measurement**

pH was measured using a calibrated pH meter (buffers pH 4.0/7.0/10.0). Spreadability was determined by the parallel-plate method: 0.5 g gel was compressed with a 500 g weight for 5 min, and the final diameter was recorded (9). Rotational viscosity was measured using a Brookfield DVE LV viscometer (spindle S64) at 2.5, 5, and 10 rpm; both viscosity (cP) and torque (%) were determined (10). Both these methods were repeated for all F1-NF10 formulations.

### **Texture analyzer**

The 'Gel spreading' test was performed using the TTC Spreadability Rig HDP/SR. The gel sample was placed, avoiding air entering and providing a smooth upper surface, in a cone-shaped receiver. The probe, which is also cone-shaped, was preliminarily installed above the surface of the gel. Parameters were chosen, including a movement speed of 3 mm/s, and the distance (depth of probe insertion into the gel) was 23 mm. Three replicate analyses were performed at room temperature for each sample, providing the same conditions for each measurement. The same method was repeated for all formulations (F1-NF10).

### **Preparation of transfersome nanoformulation (N)**

Transfersomes were prepared by thin-film hydration. For each batch (NF5–NF10), DMPC (0.37 g) and Span 80 (0.12 g) (lipid: surfactant 75:25, w/w) together with lidocaine (0.15 g) were dissolved in absolute ethanol (10 mL) in a 100 mL round-bottom flask. The solvent was removed on a rotary evaporator (RE100-Pro, DLAB, USA) at 45 °C and 125 rpm under reduced pressure for 10 min to produce a uniform lipid film. The film was hydrated with deionized water (20 mL), followed by incremental additions to a final volume of 50 mL with continuous swirling. The dispersion was allowed to anneal at ambient temperature for 15–20 min before subsequent characterization or incorporation into gels (11). Probe sonication (Qsonica probe sonicator, UK) was used to reduce the particle size of the transfersome vesicles, with a total duration of 9 minutes (three cycles of 2 min of sonication followed by 1 min of rest) at 60% amplitude intensity.

### **Size, polydispersity index, and zeta potential analysis**

Particle size, polydispersity index (PDI), and zeta potential were determined by dynamic and electrophoretic light scattering using a Zetasizer Nano (Malvern Instruments Ltd, Malvern, UK). Each nanoformulation (N) was diluted 1:3 (v/v) with deionized water immediately before measurement. Diluted samples were transferred to disposable polystyrene cuvettes for size/PDI and to a folded capillary cell for zeta potential. Three measurements were recorded per batch at ambient temperature before and after probe sonication (12).

### **Entrapment efficiency of lidocaine via HPLC**

Lidocaine was quantified by HPLC using a C18 column (4.6 × 150 mm, 5 µm) with an isocratic mobile phase of acetonitrile: phosphate-buffered saline (0.1 M, pH 7.4) at 70:30 (v/v), a flow rate of 1.0 mL/min, at a column temperature of 30 °C, an injection volume of 10 µL, and UV detection at 255 nm. A stock solution of lidocaine was prepared and serially diluted to generate working standards (linearity was established within the range of 0.1–2.0 mg/mL). Peak area (AUC) was plotted against concentration and fitted by least-squares linear regression ( $R^2$  reported) (12–14).

For “unentrapped” drug, 0.5 mL of transfersome dispersion was placed in a 3 kDa centrifugal filter unit (Amicon Ultra) and centrifuged at 3,250 rpm for 15 min at room temperature; the filtrate (free drug) was then diluted with methanol and analyzed using the developed HPLC method. For the “total” drug, a 0.5 mL aliquot of the same dispersion was dissolved in 4.5 mL of methanol to form a clear homogenous solution. From this clear solution, 2.5 mL was combined with 2.5 mL of mobile phase, and then the final clear solution was quantified by HPLC with the help of the below equation (11,12,15).

$$\text{Entrapment Efficiency (\%)} = \left( \frac{\text{Total drug loading} - \text{Unentrapped drug}}{\text{Total drug loading}} \right) \times 100$$

### Combination of transfersome nanoformulations (N) in gel formulation

After the preparation of the gel formulation, 33 g of distilled water was used (F1-F5). This distilled water was replaced with transfersome (N) suspension (NF6-NF10). It is important to know that only in the preparation of phase A was the transfersome nanoformulation diluted; this means that the transfersome formulation was 3-fold diluted with distilled water (23 mL in total for phase A). By doing dilution, this corresponded to 7.66 mL of transfersome formulation and 15.33 mL of deionized water. For the rest of the preparation process there was no further dilution.

### Microbiology study

Plates were incubated at the organism-appropriate temperature for 18–48 h to allow diffusion and growth. Antimicrobial activity was assessed by the agar diffusion method. Fresh cultures of *Candida albicans* were incubated at 30 °C, then spread uniformly over agar plates to form confluent lawns using sterile cotton swabs. Sterile wells were created in the agar using a cork borer, and the test preparations were dispensed into the wells without causing overflow. After incubation, the antimicrobial effect was evaluated by measuring inhibition zone diameters (mm) around each well (16).

### Fourier transform infrared spectroscopy (FTIR)

Molecular interactions were examined by ATR-FTIR using a Cary 630 spectrometer equipped with a diamond ATR accessory (Agilent Technologies, USA). Samples were placed directly on the crystal and compressed with the built-in pressure tower to ensure uniform contact. Spectra were collected at ambient temperature over a range of 4000–650 cm<sup>-1</sup>, at 8 cm<sup>-1</sup> resolution with 16 scans per spectrum. Peak positions were obtained and processed in MicroLab Pharma software (Agilent). The crystal was cleaned with solvent and lint-free tissue between runs (17).

## RESULTS AND DISCUSSION

### pH analysis

Upon analysis it was found that the pH of NF6-NF10 was between the range of 6.5–6.8 (Table 2). The recorded pH was closely aligned with that of Control B (pH 6.5) and within the normal range of the oral cavity (salivary pH: 6.7–7.4) (18). These results are similar to the previous literature, which found a pH between 6.68 and 6.80 (19).

Table 2. Physicochemical and textural properties of controls and gel formulations. Data are mean  $\pm$  SD,  $n = 3$ .

| Formulations         | pH        | Spreadability (cm) | Viscosity (cP) and torque (%) |          |          | Texture Analyser |                       |
|----------------------|-----------|--------------------|-------------------------------|----------|----------|------------------|-----------------------|
|                      |           |                    | 2.5 rpm                       | 5 rpm    | 10 rpm   | Firmness (g)     | Work of shear (g.sec) |
| Control A (face gel) | 6 ± 1     | 3 ± 0.5            | 105100 cp                     | 61920 cp | 37320 cp | 113.91 ± 20.08   | 33.49 ± 14.12         |
|                      |           |                    | 43.8%                         | 51.6%    | 62.2%    |                  |                       |
| Control B (oral gel) | 6.5 ± 1.5 | 3 ± 0.3            | 70560 cp                      | 57240 cp | 41760 cp | -                |                       |
|                      |           |                    | 29.4%                         | 47.7%    | 69.6%    |                  |                       |
| F1                   | 7.5 ± 1   | 2.3 ± 0.2          | 62880cp                       | 40800cp  | 27000    | -                |                       |
|                      |           |                    | 26.2%                         | 34.0%    | 45.0%    |                  |                       |
| F2                   | 7.7 ± 0.5 | 3 ± 0.3            | 30240 cp                      | 19560 cp | 13200 cp | -                |                       |
|                      |           |                    | 12.6%                         | 16.3%    | 22.0%    |                  |                       |
| F3                   | 7.7 ± 1   | 2 ± 0.3            | 36240 cp                      | 21960 cp | 14520 cp | 87.13 ± 4.01     | 39.43 ± 4.07          |
|                      |           |                    | 15.1%                         | 18.3%    | 24.2%    |                  |                       |
| F4                   | 7.7 ± 0.5 | 2.3 ± 0.2          | 64080 cp                      | 39720 cp | 25500 cp | 132.85 ± 0.36    | 78.91 ± 2.74          |
|                      |           |                    | 26.7%                         | 33.1%    | 42.5%    |                  |                       |
| F5                   | 5.5 ± 1   | 2.3 ± 0.1          | 73200 cp                      | 43920 cp | 27000 cp | 127.78 ± 9.81    | 72.80 ± 11.61         |
|                      |           |                    | 30.5%                         | 36.6%    | 45.0%    |                  |                       |
| NF6                  | 6.5 ± 1   | 2 ± 0.2            | 64080 cp                      | 38640 cp | 23520 cp | -                |                       |
|                      |           |                    | 26.7%                         | 32.2%    | 39.2%    |                  |                       |
| NF7                  | 6.5 ± 0.5 | 2.5 ± 0.1          | 80640 cp                      | 44880 cp | 26880 cp | -                |                       |
|                      |           |                    | 33.6%                         | 37.4%    | 44.8%    |                  |                       |
| NF8                  | 6.5 ± 0.5 | 3 ± 0.2            | 75840 cp                      | 45840 cp | 28200 cp | 122.25 ± 9.09    | 61.73 ± 9.12          |
|                      |           |                    | 31.6%                         | 38.2%    | 47%      |                  |                       |
| NF9                  | 6.8 ± 1   | 3 ± 0.2            | 79440cp                       | 48600cp  | 29820cp  | 130.48 ± 6.81    | 75.46 ± 9.32          |
|                      |           |                    | 33.1%                         | 40.5%    | 49.8%    |                  |                       |
| NF10                 | 6.5 ± 0.5 | 2.8 ± 0.3          | 74160cp                       | 43920cp  | 26340cp  | 133.35 ± 3.78    | 87.23 ± 10.71         |
|                      |           |                    | 30.9%                         | 36.6%    | 43.9%    |                  |                       |

### Spreadability studies

Spreadability is an important characteristic of a gel, as it reflects the behaviour of the gel when applied to the gums. Spreadability is an essential requirement for consistent and easy application of the topical gel. Spreadability also affects the therapeutic efficacy of the drug. It facilitates smooth application of gel onto the gums and enhances patient acceptance (9). Table 2 demonstrates the spread diameters of the tested gels ranged from 2.0 cm (F3, NF6) to 3.0 cm (F2, NF8, NF9), with the two commercial controls also at 3.0 cm. Measurements followed the standard parallel-plate protocol (0.5 g on a 1 cm circle, second glass plate, 500 g load, 5 min), which is widely reported for semisolid gels. Although prepared and tested under comparable conditions, notable differences in spreadability were observed among the NF series. NF8 and NF9 (3.0 cm) demonstrated the best spreadability (based on Controls A and B), whereas NF6 (2.0 cm) and NF7 (2.5 cm) showed less favourable results. Based on the literature review, the diameters of the spread circles ranged from 3 cm seen with the Pluronic-based gel, and 5 cm was observed with Carbopol and HPMC gel (20). Therefore, based on the value generated from controls A and B, as well as literature, formulation NF8 demonstrated an ideal outcome.

### Viscosity of gel formulations

The viscosity data of all samples exhibited pseudoplastic (shear-thinning) behaviour, with viscosity decreasing substantially from 2.5 to 10 rpm (e.g., NF8: from 75,840 to 28,200 cP; and NF9: 79,440 to 29,820 cP; which is about a 63% reduction), which is characteristic of carbomer/HA gels and facilitates ease of application under shear (Table 2).

### Texture analyzer of gels

Firmness in the texture analyzer defines the resistance of the product to deformation under an applied force. When specific pressure is applied, according to firmness, it is possible to measure how hard or soft that product feels. The work of shear is the amount of energy required to deform a material by applying a force that causes shear deformation. According to the available data (Table 2), NF9 required higher maximum force (firmness) than the market gel (130.477 g vs. 113.912 g) and more work to shear/spread (75.461 g·sec vs. 33.486 g·s). This means NF9 is firmer and less readily spreadable than the market gel. The market product has lower firmness compared to NF9; therefore, it was softer and more fluid. This data showed that NF9 was thicker and denser. The marketed control product exhibited a lower work of shear compared to the softer NF9, indicating that NF9 is more resistant to deformation and therefore requires more energy to shear. According to Table 2, F4 (firmness: 132.848, work of shear: 78.914), F5 (firmness: 127.781, work of shear: 72.797), NF8 (firmness: 122.251, work of shear: 61.732), and NF10 demonstrated behaviour similar to that of NF9 and the marketed gel. The desirability of these results depends on the intended application: oral gels designed to remain in place on moist mucosal surfaces typically aim for moderate firmness to resist wash-off, rather than prioritizing maximum spreadability.

### Particle size, polydispersity index, zeta potential, and entrapment efficiency of transfersome nanoformulations

Transfersome nanoformulations showed mean diameters of 453 nm and polydispersity index (PDI) from 0.80 prior to probe sonication (Figure 1a and b). The sonication process demonstrated a significant reduction in particle size as well as polydispersity among vesicles. This reduction in particle size and PDI is directly related to the sound waves produced by probe sonication, which break the particles, and hence homogenous dispersion was achieved. Similar results were also observed by Khan et al., (21,22). Moreover, zeta potential analysis did not show a change in their values before and after probe sonication. This also suggested that probe sonication has no effect on the surface morphology of transfersome particles (Figure 1c). The low magnitude is expected for vesicles composed of zwitterionic DMPC and non-ionic Span 80 (stabilizes the vesicles sterically rather than electrostatically, which explains the relatively low magnitude of zeta potential) at pH 7.4; the surface charge arises mainly from head-group ionization and adsorbed ions rather than ionic surfactants. Systems containing ionic surfactants typically exhibit much higher negative zeta potentials; the absence of such components here explains the relatively small values. These results are analogous to previous results in the literature (23,24). Upon investigating the entrapment efficiency of lidocaine in transfersome vesicles, no significant difference was found between formulations before and after probe sonication (Figure 1d). However, there is a lower entrapment efficiency after probe sonication, which may be attributed to the particle size reduction process, and hence, during particle breakage, the entrapped drug might leak.

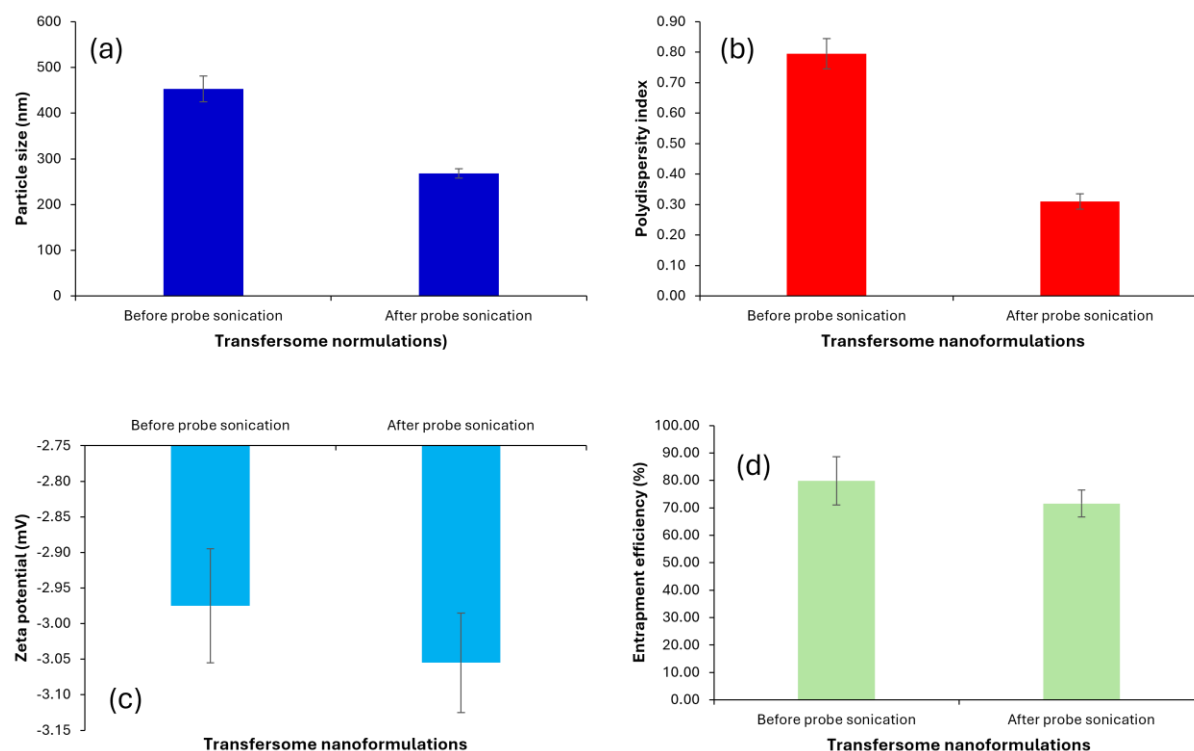


Figure 1. Particle size, polydispersity index (PDI), zeta potential and entrapment efficiency of transfersome formulations encapsulation lidocaine as an active ingredient. Data are mean  $\pm$  SD,  $n = 3$ .

### Microbiology studies

For microbial analysis, six wells were made in each agar plate, containing the following samples: water (used as a control), NF10, a combination of preservatives (phenoxyethanol and ethylhexylglycerin, referred to as 'oldp'), a single preservative (Plantaserve E, referred to as 'newp'), NF8, and NF9. Zones of inhibition were measured as the diameter of the clear halo around each well (Table 3 and Figure 2).

Table 3. Illustrating the zone of inhibition for *Candida albicans* (microorganism). Data are mean  $\pm$  SD,  $n = 3$ .

| Microorganism           | Zone of inhibition diameter (cm): water | Zone of inhibition diameter (cm): NF10 | Zone of inhibition diameter (mm): oldp | Zone of inhibition diameter (mm): newp | Zone of inhibition diameter (mm): NF8 | Zone of inhibition diameter (mm): NF9 |
|-------------------------|-----------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|---------------------------------------|---------------------------------------|
| <i>Candida albicans</i> | -                                       | -                                      | 30 $\pm$ 2                             | 30 $\pm$ 3                             | 20 $\pm$ 4                            | 20 $\pm$ 3                            |

For general observations across organisms, both water and NF10 (the gel without preservative) produced no measurable inhibition zones (0 mm), confirming that the gel base itself is not antimicrobial. Both preservatives—'oldp' and 'newp'—when tested alone, produced clear inhibition zones of 30 mm. When either preservative was incorporated into the gel (NF8 or NF9), distinct but slightly smaller zones of 20 mm were observed. This indicates that the preservatives retained their antimicrobial activity within the gel formulations, as shown in Table 3 and Figure 2.

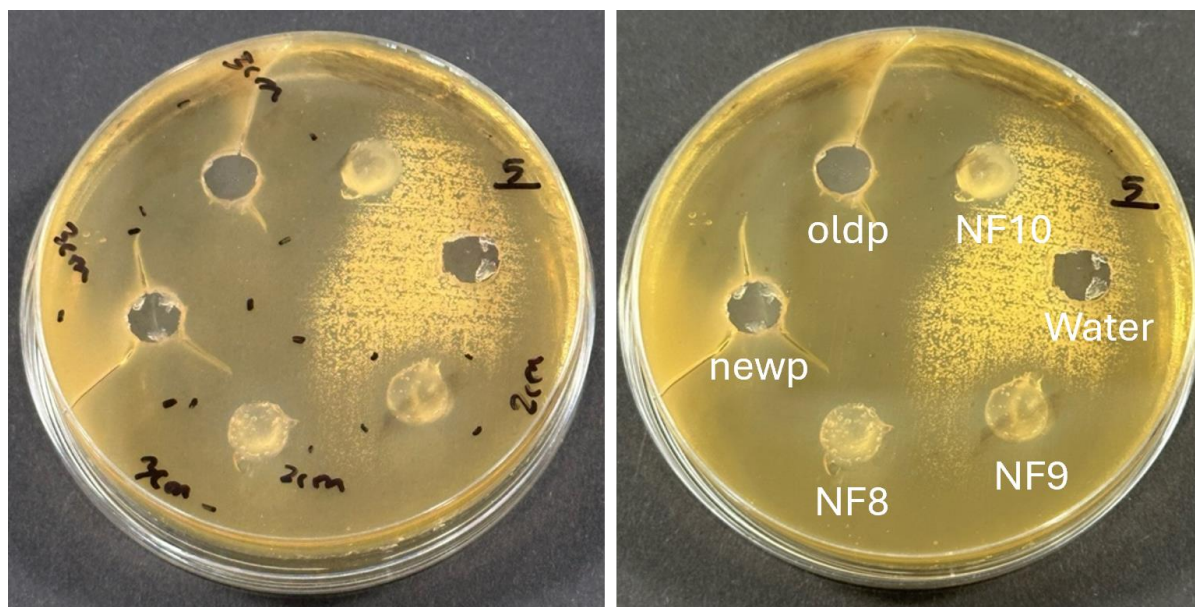


Figure 2. Agar plate showing the zone of inhibition for water (used as a control), NF10, a combination of preservatives (phenoxyethanol and ethylhexylglycerin, referred to as 'oldp'), a single preservative (Plantaserve E, referred to as 'newp'), NF8, and NF9. These images are typical of three such different experiments.

### FTIR analysis

Upon investigation, bands around  $3400\text{ cm}^{-1}$  and  $1640\text{ cm}^{-1}$  are assigned to the water (O–H) (25). Based on FTIR results (Figure 3), and literature review, lidocaine free base has bands around  $3290\text{ cm}^{-1}$  (N–H Stretch),  $1670\text{ cm}^{-1}$  (Amide I, C=O) and  $1495\text{ cm}^{-1}$  (Amide II, C–N). Moreover, the bands for lidocaine free base did not appear. Lidocaine's amide I (C=O) and N–H stretch were masked by bands of water. The comparatively low fraction of lidocaine relative to water (a strongly IR-absorbing excipient) and encapsulation of lidocaine in transfersomes caused a broadening/shift of drug bands and further reduced their apparent intensity (26,27). Based on Figure 3, the band at  $\sim 1710\text{ cm}^{-1}$  is due to the free carbonyl group. When neutralizer TEA was added, the percentage of hydrogen-bonded carboxyl groups increased, and the band at  $1653\text{ cm}^{-1}$  became more dominant (in NF8, NF9, NF10) compared with the band at  $1710\text{ cm}^{-1}$  (28).

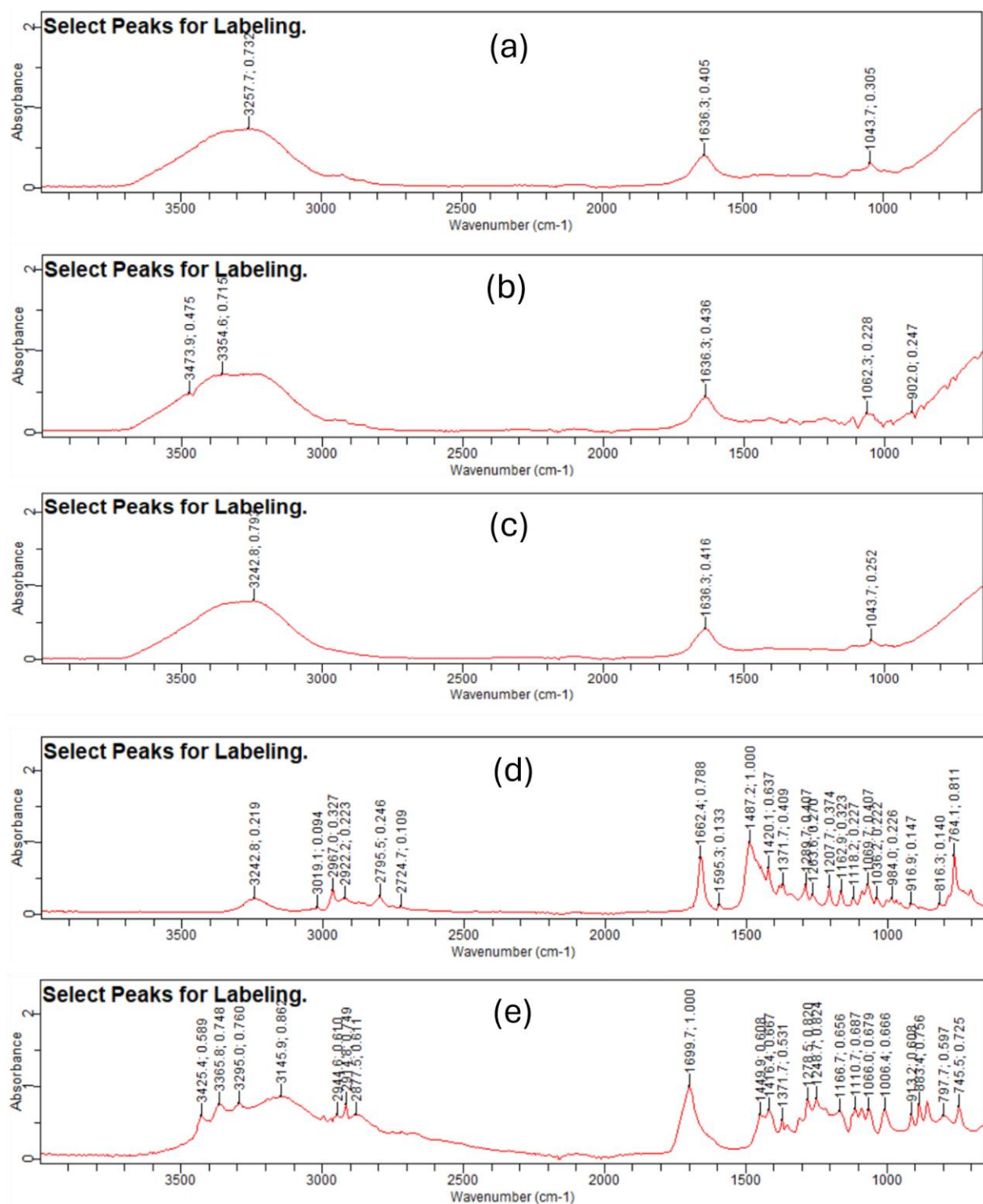


Figure 3. FTIR spectra for gel-based transfersome nanoformulations (a) NF8, (b) NF9, (c) NF10, (d) lidocaine, and (e) Carbapol showing the characteristic absorption peaks of each formulation, drug, and ingredient. The spectra were analyzed to evaluate functional group interactions and confirm the chemical compositions. These images are typical of three such different experiments.

## Microscopy

OPTIKA B-190TB microscope, Italy was used at two magnifications of 10x and 40x to investigate the transfersome nanoformulation. On 10x, initially small circles were found; however, upon looking via 40x, a transfersome particle was captured (Figure 4). Which also demonstrated that no needle-like crystals or dense irregular precipitates are evident in the fields examined.



*Figure 4. Optika microscope result of transfersome formulation in magnification of 40x. These images are typical of three such different batches.*

## CONCLUSIONS

Carbopol gels incorporating lidocaine-loaded transfersomes were developed and characterized. Formulations NF8 and NF9 achieved a saliva-like pH, good spreadability, and marked shear-thinning behaviour. Texture analysis showed NF8 with moderate firmness and favourable work of shear, while NF9 displayed firmer features consistent with mucosal retention but with some trade-off in ease of spread. Probe sonication reduced vesicle size and PDI significantly than when freshly prepared. Agar-well diffusion indicated preservative activity in preserved gels, whereas the base gel alone was inactive. Hence, gel-incorporated transfersome nanoformulation demonstrated a novel approach to potential employment as a local anaesthetic for pain relief and therefore improve patient compliance.

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## Conflict of interest

The authors declare no conflict of interest.

## Authors contributions

CRedit: MJS: Conducting experiments and writing the manuscript, CH: Writing and editing the manuscript and providing scientific discussions, IK: Writing manuscript and planning experiments.

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