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Investigation and comparison of the performance of various throat spray devices using different types of nanoformulations with encapsulated lidocaine as a local anaesthetic

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ABSTRACT

Lidocaine is most often employed as a local anaesthetic *via* the parenteral route, whereas advanced drug delivery systems are a key concept using needle-free formulations for improved drug stability, deposition, and sustained release. Three delivery systems (liposomes, ethanol-based proliposomes, and proniosomes) were prepared with and without cholesterol and delivered *via* four commercially available throat spray devices (referred to as A, B, C, and D) for their performance and deposition. Formulations without cholesterol demonstrated higher drug entrapment and release. Upon analysis, spray device A demonstrated lower numbers of actuations for priming and tailing-off phases and higher numbers of full actuations; thus, it delivered a lower number of total actuations. Therefore, each shot weight delivered a larger amount of formulation (189 mg; an average of all formulations) using spray device A than counterpart devices. Spray device A showed significantly superior plume geometry (including plume angle (59°), plume width (14 cm), and total plume length (54 cm) (an average of all three formulations)). Furthermore, spray device A showed a round-shaped spray pattern with an ovality ratio of 1.05 when compared to the crescent, oval, and irregular/star-shaped patterns and ovality ratios of 1.19, 1.07, and 1.16 by devices B, C, and D, respectively. In addition, spray device A exhibited longer phases (i.e., formation, evolution, and dissipation) and higher mass output, drug output, drug deposition, and aerosol output rate. Thus, spray device A and formulations without cholesterol were identified as the best combination for their superior performance and targeted drug delivery.

1. Introduction

Pain management is one of the major therapeutic goals in intensive care units. A gold standard for pain relief is the use of opioids. However, a number of unwanted and potentially severe side effects are associated with their systemic administration, including drowsiness, sedation, neutrophil dysfunction, nausea, vomiting, and respiratory depression [1]. Therefore, lidocaine is used as a local anaesthetic by blocking the transmission of painful stimuli to the brain *via* inhibiting the influx of sodium ions. Sodium ions are responsible for the initiation and conduction of nerve (i.e., nociceptor nerve fibre) impulses that are associated with pain; hence, reversible blocking of nerve conduction provides local pain relief for both acute and chronic pain [2]. Moreover, in order to improve local anaesthetic's pharmacokinetic properties, enhance pharmacological activities, and reduce toxicity, various researchers have formulated local anaesthetics in various forms, like oral dissolving films [3], creams [4], ointments [5], patches [6], polymeric nanoparticles [7], viscous solutions [8], implants [9], and injectables [10]. However, the majority of the available forms of local anaesthetics have a shorter duration of action, and therefore frequent administrations

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are required in order to achieve long-term pain relief, which consequently greatly affects patient compliance and increases side effects. A sustained release formulation employing a local anaesthetic would be a great option that can significantly improve the issues related to the previous formulations [11]. Furthermore, a sustained release formulation would reduce the risk associated with high and multiple dose applications irrespective of the route of administration [12,13].

There are few local anaesthetic licensed formulations that produce delayed effects, therefore minimizing frequent administration. Methods for adopting such effects were gained through the incorporation of vasoconstrictors (i.e., preventing leakage of local anaesthetic to systemic circulation) or through the introduction of novel lipid-based formulations (i.e., DepoFoam) [14–19]. The novel vesicular formulations such as liposomes, ethanol-based proliposomes, and proniosomes provide several advantages as drug delivery carriers. These novel vesicular formulations are also intended to be sustained release delivery systems and hence a better approach to enhance and improve patient compliance and achieve the desired effect. These vesicular delivery systems have the advantage and ability of delivering hydrophilic or lipophilic active pharmaceutical ingredients in the central core and concentric bilayers. Furthermore, this is the first study to employ a combination of novel vesicular formulations (liposomes, ethanol-based proliposomes, and proniosomes) with spray devices, in which both the formulations and the devices were thoroughly characterized and analysed. These formulations are composed of biocompatible and biodegradable lipids that self-assemble into vesicle form and work as delivery agents [20,21]. Vesicle formulations tend to leak drugs, and therefore the incorporation of cholesterol makes these vesicles more rigid and less prone to drug leakage. These delivery systems gained attention since they can entrap both small and large molecular weight agents and possess the ability to be delivered *via* many routes of administration (i.e., transdermal, buccal, or parenteral) [22,23].

Previous research has indicated that spray devices are often used as local anaesthetics that significantly decrease pain perception during burn dressing changes [24], before injections or arterial lines [6,25], and as a general topical use [26]. Local anaesthetics can be used to avoid throat pain prior to and post-gastroscopy and endoscopy, as well as pain caused by viral and bacterial infections [27–29]. However, the majority of local anaesthetics have a very short life [30]; that is why the entrapment of local anaesthetic in vesicular formulation as a drug carrier is important to give a sustained release of the drug for a longer time and to discontinue its frequent administration. Furthermore, this is the first study to employ a combination of novel vesicular formulations (liposomes, ethanol-based proliposomes, and proniosomes) with spray devices, in which both the formulations and the devices were thoroughly characterized and analysed.

One particular aspect of throat spray devices is that there is very limited literature around spray pattern (i.e., ovality ratio, shape pattern) and plume geometry (i.e., plume angle, plume width, plume height, and different phases of plume generation). Similarly, none of the formulations was investigated using these spray devices for deposition, shot weight, and dose accuracy. Here, in the current study, three vesicular formulations (liposomes, liposomes generated from ethanol-based proliposomes, and niosomes hydrated from proniosomes) were characterized using four commercially available throat spray devices. These formulations and spray devices were extensively investigated for size, zeta potential, morphology, entrapment efficiency, dose accuracy, shot weight, *in-vitro* release, drug deposition, spray pattern, plume geometry, aerosol mass, drug output, and output rate. The main benefit of this investigation could be a potential future clinical use of lidocaine in vesicle-based formulations using an appropriate throat spray device, with better outcomes resulting in pain management and quick pain recovery with sustained release of the drug from nanoformulations. Additionally, increased pain comfort can significantly reduce lengthy stays in hospitals, and easy use of appropriate devices would be able to provide a higher degree of patient satisfaction and healthcare.

2. Materials and methods

2.1. Materials

Soya phosphatidylcholine (SPC, lipoid S-100) was supplied by Lipoid GmbH, Ludwigshafen, Germany. Lidocaine was purchased from Acros Organic, New Jersey, USA. Bromophenol blue dye, cholesterol, sorbitan monostearate (Span 80), and sucrose were purchased from Sigma Aldrich, Gillingham, UK. Di-potassium hydrogen orthophosphate anhydrous (potassium phosphate) was obtained from BDH Chemicals, UK. Dialysis membrane, phosphotungstic acid, absolute ethanol, methanol, and acetonitrile (HPLC grade) were purchased from Fischer Scientific Ltd., Loughborough, UK. Four throat spray devices were bought from Boots, UK.

2.2. Preparation of liposome formulations via thin-film method

SPC, lidocaine, and cholesterol (with or without) were used in a 2:1:1 M ratio (310.40:77.33:9.28 w/w ratio) and referred to as a lipid phase. All ingredients of the lipid phase were accurately weighed in a round-bottom flask (RBF) and dissolved in absolute ethanol (10 ml). The organic solvent (i.e., ethanol) was completely removed with the help of a rotary evaporator (Büchi Rotavapor R-215, Büchi, Switzerland) under a negative pressure of a vacuum pump for 1 h. The rotation speed of the rotary evaporator was adjusted to 185 rpm, and the temperature condition of the water bath was set to 45 °C. After complete evaporation of ethanol, a thin-film layer formed in the RBF was hydrated with deionized water (10 ml), followed by manual shaking. The lipid suspension was left for 1 h annealing at room temperature to generate liposomes. This ensured that the final vesicles were free of ethanol before further analysis.

2.3. Preparation of ethanol-based proliposome formulations

A lipid phase containing SPC, lidocaine, and with or without cholesterol was used as a 2:1:1 M ratio (310.40:77.33:9.28 w/w ratio). All ingredients were weighed in a glass vial (50 ml), only a very small volume of absolute ethanol (76 μl) was pipetted into the glass vial to dissolve the ingredients, and the glass vial was placed in a water bath for 5 min previously adjusted to 70 °C. This was followed by the addition of deionized water (1 ml) into the lipid phase, and vortex (Labnet International, Edison, NJ, USA) mixing was conducted for 2 min for primary hydration. For secondary hydration, deionized water (9 ml) was added to the same vial, and vortex mixing was done for 2 min. The lipid suspension was left for 1 h annealing at room temperature to generate liposomes.

2.4. Preparation of proniosome formulations

A lipid phase comprising Span 80, lidocaine, and with or without cholesterol was used as a 2:1:1 M ratio (177.44:77.33:9.28 w/w ratio). Sucrose carrier (2640.50 mg) was incorporated in a 10:1 w/w (carbohydrate carrier to the lipid phase ratio) and placed in a 100 ml RBF. The lipid phase was dissolved in absolute ethanol (10 ml) and transferred into the RBF containing sucrose carriers. The RBF was then attached to a rotary evaporator under a vacuum pump (reduced pressure). The temperature of the water bath was set to 45 °C with a rotation speed of 185 rpm. After 1 h, a thin-film layer was formed over sucrose particles in an RBF when ethanol was completely evaporated *via* rotary evaporator. Finally, deionized water (10 ml) was used to generate niosomes from sucrose-based proniosomes after 1 h annealing time at room temperature.

2.5. Particle size reduction

Probe sonication (Qsonica Probe Sonicator, Q125, Newtown, CT,

USA) was employed to reduce vesicle size. Initially, the tip of the probe sonication was washed and cleaned with deionized water, followed by drying. Formulation suspension (10 ml) in a 50 ml glass vial was placed under a probe sonicator using 40 % intensity (i.e., 50 W) for 10 min. To avoid overheating of the formulation suspension, an interval of 2 min of sonication followed by 1 min of rest cycle was conducted for all formulations. It is important to know that any ethanol that remained in the formulations was eliminated during the particle size reduction step, as probe sonication was applied for 10 min, leading to its evaporation.

2.6. Particle size and zeta potential analysis

Formulation suspension containing vesicles was measured *via* dynamic light scattering (DLS) using Zetasizer Nano Series (Malvern Zetasizer Nano Series, Malvern, Worcestershire, UK). DLS measured the particle's Brownian motion, which was then related to their size. This was performed by adding a formulation suspension (1 ml) with the help of a Gilson pipette into a disposable cuvette cell (Fisher Scientific Ltd, UK) for size analysis. Size and size distribution were referred to as Zaverage and polydispersity index (PDI), respectively.

Zeta potential of formulations was analysed by Laser Doppler Velocimetry (LDV) using Zetasizer Nano Series. LDV measures the electrophoretic mobility of particles (to determine the velocity of particles in an electric field). The formulation suspension was transferred into a polystyrene zeta cell with the help of a Gilson pipette to measure the zeta potential of the individual formulation. Moreover, these characterization studies (i.e., particle size, PDI, and zeta potential) were only performed before aerosolization performance.

2.7. Morphology study via transmission electron microscopy (TEM)

Surface morphology analysis using TEM was carried out for liposomes, ethanol-based proliposomes, and proniosomes only when the formulations were freshly prepared. A drop of formulation suspension and a drop of negative stain (1 % of phosphotungstic acid) were mixed together and placed over a 400-mesh carbon-coated copper grid (TAAB Laboratories Equipment Ltd., UK). Post-drying, samples were viewed and photographed using various magnifications *via* transmission electron microscope (TEM) (Morgagni 268D, EFI, MegaView, Brno, Czech Republic).

2.8. Determination of entrapment efficiency of lidocaine

To separate the entrapped drug in vesicles from the unentrapped drug (i.e., free drug), formulation suspension (0.5 ml) was placed into a Millipore filter (3.5 kDa, Amicon® Ultra, Merck Millipore Ltd, County Cork, Ireland), and bench centrifugation (pectrafuge 24D, Labnet International, Edison, NJ, USA) was conducted at 15,100 rcf for 30 min. After centrifugation, a clear filtrate (unentrapped drug, allowing only free lidocaine to pass through the filter) was collected and analysed *via* HPLC (Agilent 1200 Series Instrument, Waldborn, Germany). To determine the total drug, the formulation (1 ml) was dissolved and diluted with methanol until the solution was clear, followed by HPLC analysis (Eq. (1)).

$$\text{Entrapment efficiency } (\%) = \frac{\text{Total drug} - \text{Unentrapped drug}}{\text{Total drug}} \text{ x } 100 \quad \text{(Eq. 1)}$$

An HPLC study was performed using a C18 column (250 mm × 4.6 mm, 5 μm) (Phenomenex, Torrance, CA, USA) with a detection wavelength set at 254 nm. Acetonitrile and water (80:20 % v/v) were used as a mobile phase with a flow rate of 1 ml/min, and the temperature was set at 30 °C, with an injection volume of 10 μl. A calibration curve of lidocaine (0.1–2 mg/ml) was constructed for the identification of unknown lidocaine concentration.

2.9. Determination of dose accuracy and shot weight of formulations

In this study, four commercially available throat spray devices were used (each one has a different design and volume capacity and was purchased over the counter) and referred to as spray devices A, B, C, and D. All four spray devices were emptied of the original formulations and thoroughly washed with a mixture of water and ethanol and left to dry overnight at 30 °C before using them for further studies.

Prior to the use of spray devices, a number of priming actuations are required to remove/purge the air off the device in order to achieve full actuation for a consistent dose release [31]. Suspensions (5 ml) of liposomes, liposomes generated from ethanol-based proliposomes, and niosomes hydrated from proniosomes were transferred into the spray devices. The number of actuations required to prime the spray devices, and the number of full actuations delivered by these devices were determined. When the volume in the devices became low, degradation/inconsistency in the accuracy of the devices was observed as a non-uniform dose was emitted, known as tailing-off; spraying was continued until complete cessation was achieved (where no further formulation was able to aerosolize upon actuation; also known as dryness). The total number of actuations, including priming, full actuation, and tailing-off, was determined for all devices with different formulations. Moreover, the shot weight or spray weight from a single actuation was also determined for the number of actuations from each device using all three formulations (liposome, ethanol-based proliposomes, and proniosomes). This was attained by recording the weight of each spray device before and after each actuation for each formulation [32].

2.10. Characterization of spray pattern and plume geometry

Spray pattern and plume geometry were assessed in order to characterize the generation of spray cloud from the spray devices [33]. For the spray pattern, the impaction technique was used, where the spray from the device was fired horizontally on a thin-layer chromatographic (TLC) sheet. Spray pattern was characterized at specific distances (i.e., 3, 12.5, and 30 cm) from the tip of the device to determine the shape of aerosol delivered from the device upon actuation. It is noteworthy that both 3 cm and 12.5 cm are the most relevant distances to evaluate devices' spray patterns, and 30 cm has no physiological relevancy. A distance of 30 cm was only selected to see the appearance and design of the spray pattern at a longer distance. A bromophenol blue dye was incorporated in formulations in order to visualize the splatter pattern easily on the TLC sheet. The resultant images of the spray pattern on the TLC were then analysed for maximum and minimum diameter (i.e., Dmax and Dmin) and the ovality ratio (Dmax to Dmin).

Plume geometry describes the shape of the discharged or generated plume cloud parallel to the axis after actuation from the device. A high-speed digital camera (Cannon LTD., Tokyo, Japan) was used by firing or actuating the spray horizontally against a dark background to record the spray cloud generation. The emitted plumes or spray clouds from spray devices were captured as a movie clip (25 frames per second). The recorded videos or clips were transferred and processed on a computer.

Additionally, various phases of spray cloud development, including the formation phase (early stage, where pressure and flow rate through the device were low), the evolution phase (fully developed stage, once the correct atomization was reached), and the dissipation phase (last stage, when the plume spray started to separate from the device tip, i.e., the tailing-off stage), were recorded for their time duration. According to the recommendation of the Centre for Drug Evaluation and Research (CDER), image analysis can be done at a time delay corresponding to the fully developed phase of the plume while the plume was still touching the tip of the nozzle [31,34].

The fully developed plumes from videos were printed and characterized for plume angle and plume width, whereas the equivalent ruler marks on the background were used to calculate the plume height. The plume angle (measured from the vertex of the spray cone, which is

Table 1
Particle size of vesicles, polydispersity index (PDI), zeta potential, and drug entrapment of liposomes, liposomes generated from ethanol-based proliposomes, and niosomes generated from proniosomes with and without cholesterol formulations.

Formulations	Particle size (nm)	PDI	Zeta potential (mV)	Drug entrapment (%)
Liposome (cholesterol)	201.83 ± 3.16	0.36 ± 0.05	−1.18 ± 0.62	33.72 ± 5.46
Liposome (without cholesterol)	176.2.68 ± 4.55 *	0.30 ± 0.05	0.98 ± 0.75	67.72 ± 5.73 *
Ethanol-based proliposome (cholesterol)	198.64 ± 4.98	0.26 ± 0.06	−1.48 ± 0.66	51.30 ± 7.76
Ethanol-based proliposome (without cholesterol)	171.4 ± 3.56 *	0.29 ± 0.04	0.85 ± 0.71	71.77 ± 2.14 *
Proniosome (cholesterol)	415.93 ± 4.58	0.28 ± 0.06	−1.42 ± 0.53	39.25 ± 8.15
Proniosome (without cholesterol)	387.40 ± 5.71 *	0.27 ± 0.05	−1.21 ± 0.49	69.84 ± 3.05 *

Data are mean ± SD, n = 3; $p > 0.05$ for PDI and zeta potential; *$p < 0.05$ for particle size without cholesterol compared to with cholesterol; *$p < 0.05$ for entrapment efficiency without cholesterol compared to with cholesterol.

located at or close to the tip (i.e., using a height of 3 cm)), plume width (maximum width of the plume at a specific distance, i.e., 3 cm from the apex of the spray cone), and plume height (from the apex of the spray cone to the maximum distance covered) were determined after actuation of the different spray devices using a protractor and ruler.

2.11. In-vitro study performance using two-stage impinger

The performance of formulation deposition *via* spray devices was determined *in-vitro* using two-stage impinge (TSI) (Copley Scientific Ltd, UK). This apparatus comprises two stages, the upper stage (stage 1) representing the upper airways (i.e., covering areas from mouth to trachea) and the lower stage (stage 2) representing the lower airways. Deionized water was placed in stage 1 (7 ml) and stage 2 (30 ml) as a collection medium. According to the British Pharmacopoeia (BP), when using a two-stage impinger for inhaled products, the flow rate is 60 L/min in order to simulate physiological conditions [35]. Aerosols/-droplets that are collected in the throat of the upper stage are referred to as nonrepairable. The cut-off diameter between the upper and lower stages of the TSI is 6.4 μm [36], which means droplets bigger than this range will deposit in the upper stage and smaller in the lower stage. Formulation (3 ml) was placed in spray devices, and spray/aerosol generated from these devices was directed towards the mouthpiece of TSI. This experiment was repeated for all four spray devices and all three formulations in triplicate.

2.12. Performance of throat spray devices

In order to investigate the performance of spray devices, several endpoints of the total actuations (i.e., priming, full actuation, and tailing-off) were determined. Post total actuation, the amount of drug was also analysed by collecting samples from each compartment of TSI (i.e., upper stage and lower stage), and the dead volume in the spray devices (a fraction of formulations that was unable to aerosolize and remained in the devices) using HPLC. Moreover, the performance of spray devices was also evaluated in terms of mass output, aerosol output rate, and drug output. Mass output was determined by weighing the spray device before actuation and again after complete or total actuations (i.e., when droplet formation ceased and no further formulation was released). The difference in weight before and after actuation represented the mass output, which was calculated using Eq. (2).

$$\text{Mass output } (\%) = \frac{\text{Weight of aerosolized or actuated formulation}}{\text{Weight of formulation present in spray device before aerosolization or actuation}} \text{x } 100 \quad \text{(Eq. 2)}$$

Aerosol output rate was gravimetrically analysed by determining the volume/amount of formulation generated per actuation (after complete or total actuations), as shown in Eq. (3).

$$\text{Aerosol output rate}\left(\frac{\text{mg}}{\text{actuation}}\right) = \frac{\text{Weight of aerosololized or actuated formulation}}{\text{Number of actuations}} \quad \text{(Eq. 3)}$$

After complete actuations, the amount of drug deposited in the upper and lower stages of TSI, as well as the formulation that remained in the spray device as a residual or dead volume, was analysed *via* HPLC (Section 2.8). The recovered dose (RD) of the drug is the total amount of drug identified in the upper stage, lower stage, and reservoir of the spray device. Whereas the amount of drug deposited in the two stages of TSI represents the drug output, which is calculated as the percentage of RD and represented as Eq. (4).

$$\text{Drug output } (\%) = \frac{\text{Drug delivered to TSI}}{\text{Recovered dose}} \text{ x } 100 \quad \text{(Eq. 4)}$$

All experiments were performed in triplicate under room temperature conditions. All actuations were done manually by the author using all four spray devices.

2.13. In-vitro sustained release of lidocaine from formulations

A sustained release study of lidocaine was carried out using the dialysis bag method at 37 °C. Formulations (7 ml, containing 54 mg of lidocaine) were introduced into the dialysis membrane (a cut-off MWCO; 3.5 kDa), and both ends of the membrane bag were tied with the help of thread. Similarly, free lidocaine drug was also used as a control (54 mg) sample. Dialysis membrane-containing formulations were placed in a 250 ml glass beaker already filled with 143 ml of deionized water, and the membranes were stirred in the medium with the help of a magnetic stirrer adjusted to 250 rpm. Aliquots (0.5 ml) were withdrawn from the medium with the help of a Gilson pipette at predefined time intervals (i.e., 1, 2, 3, 4, 5, 6, 22, 23, and 24 h) and replaced with deionized water (0.5 ml). The withdrawn aliquots were analysed using HPLC.

2.14. Statistical analysis

Statistical analysis was performed on the data obtained using one-way analysis of variance (ANOVA) and the student t-test, as appropriate, *via* SPSS. Values were expressed as mean ± standard deviations, and a p-value less than 0.05 indicated that the difference between the groups was statistically significant. All experiments were performed in triplicate.

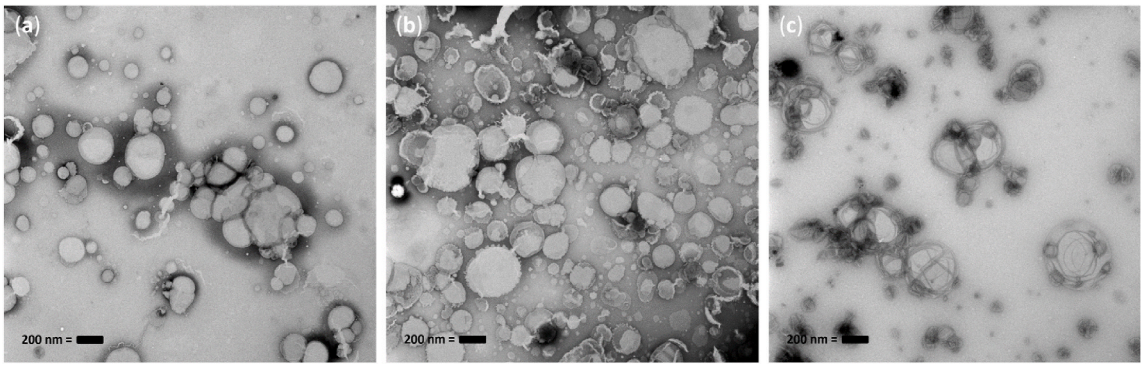

Fig. 1. Transmission electron microscopy (TEM) images of all three formulations prepared are (a) liposomes, (b) liposomes generated from ethanol-based proliposomes, and (c) niosomes hydrated from proniosomes. These images are typical of three different batches.

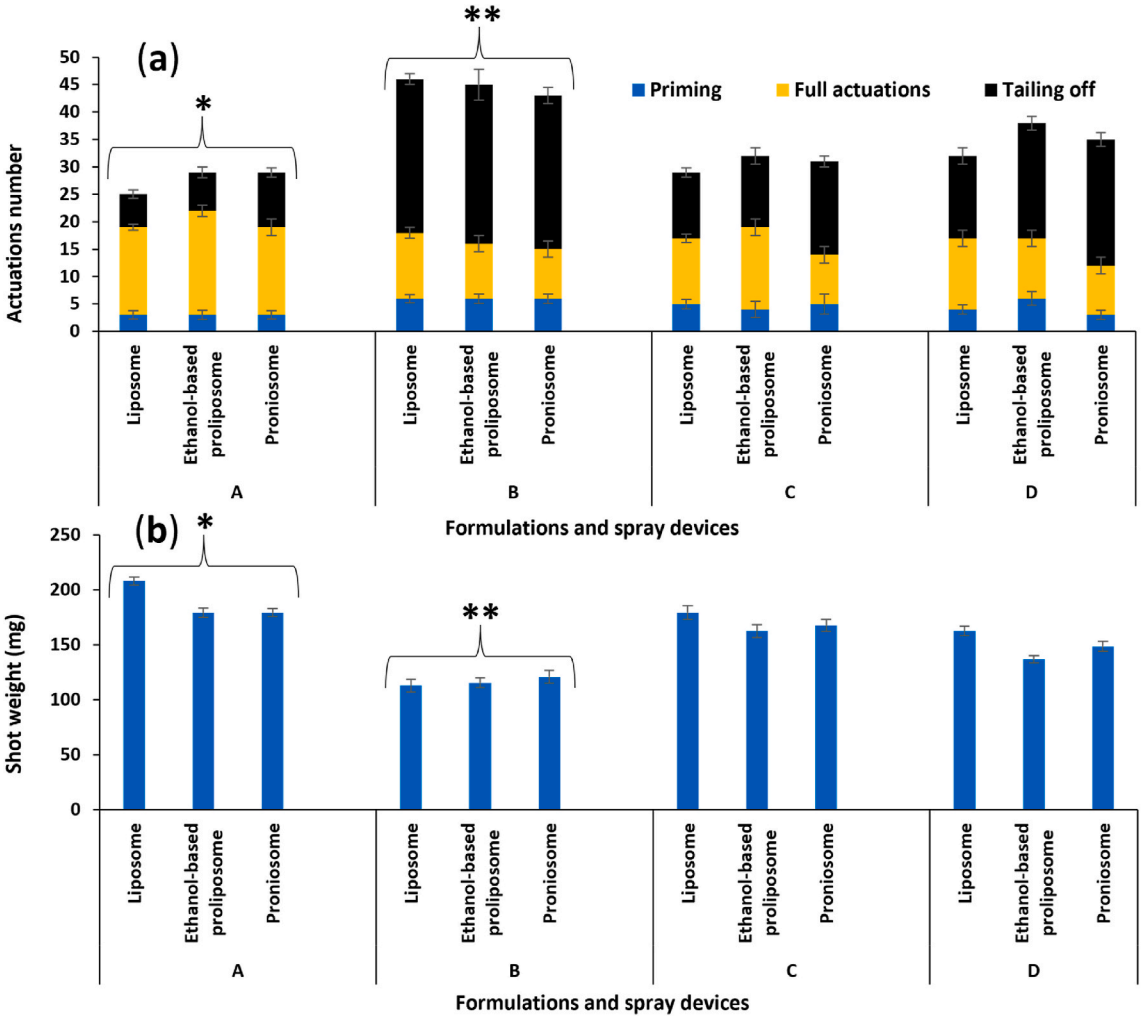

Fig. 2. Liposomes, ethanol-based proliposomes, and proniosome formulations *via* four throat spray devices, i.e., A, B, C, and D, were used for the determination of (a) number of actuations required for priming, full actuation, and tailing-off phases, and (b) shot weight per actuation. Data are mean ± SD, n = 3. (a) *p < 0.05 for device A full actuations compared to devices B, C, and D; **p < 0.05 for device B total actuations compared to devices A, C, and D. (b) *p < 0.05 for device A shot weight compared to device B, C, and D. **p < 0.05 for device B shot weight compared to device A, C and D.

3. Results and discussion

3.1. Particle size, zeta potential, and entrapment efficiency of lidocaine-loaded formulations

Upon analysis, a significantly ($p < 0.05$) smaller particle size was found for liposomes, liposomes generated from ethanol-based proliposomes, and niosomes hydrated from proniosomes prepared without cholesterol when compared to formulations with cholesterol (Table 1). It is important to know that cholesterol presence in the formulations may enhance vesicle rigidity and make them less prone to drug leakage. Cholesterol is a lipophilic molecule and therefore has a high affinity for

Table 2
Plume geometry (including plume angle, plume width, and total plume length) generated from all four throat spray devices (i.e., A, B, C, and D) at a distance of 3 cm using liposome, ethanol-based proliposome, and proniosome formulations. Data are mean ± SD, n = 3.

Spray devices	Liposomes			Ethanol-based proliposome			Proniosome		
	Plume angle (at 3 cm)	Width (at 3 cm)	Total plume length (cm)	Plume angle (at 3 cm)	Width (at 3 cm)	Total plume length (cm)	Plume angle (at 3 cm)	Width (at 3 cm)	Total plume length (cm)
A	58.63 ± 1.52	14.22 ± 1.30	54.98 ± 4.24	57.84 ± 2.68	13.76 ± 1.54	53.65 ± 4.47	62.06 ± 2.51	14.77 ± 1.64	52.33 ± 4.39
B	35.36 ± 1.23	8.16 ± 1.50	34.13 ± 3.20	35.36 ± 1.06	8.07 ± 1.62	32.27 ± 2.21	36.89 ± 1.35	8.89 ± 1.67	33.52 ± 2.08
C	54.45 ± 1.32	11.50 ± 1.02	45.44 ± 3.35	52.52 ± 1.86	11.49 ± 1.58	44.06 ± 3.80	55.51 ± 1.93	12.13 ± 1.74	42.37 ± 2.55
D	44.37 ± 1.61	9.47 ± 1.46	33.97 ± 2.06	42.55 ± 1.19	9.42 ± 1.51	33.76 ± 2.11	46.14 ± 1.78	10.06 ± 1.68	33.28 ± 2.21

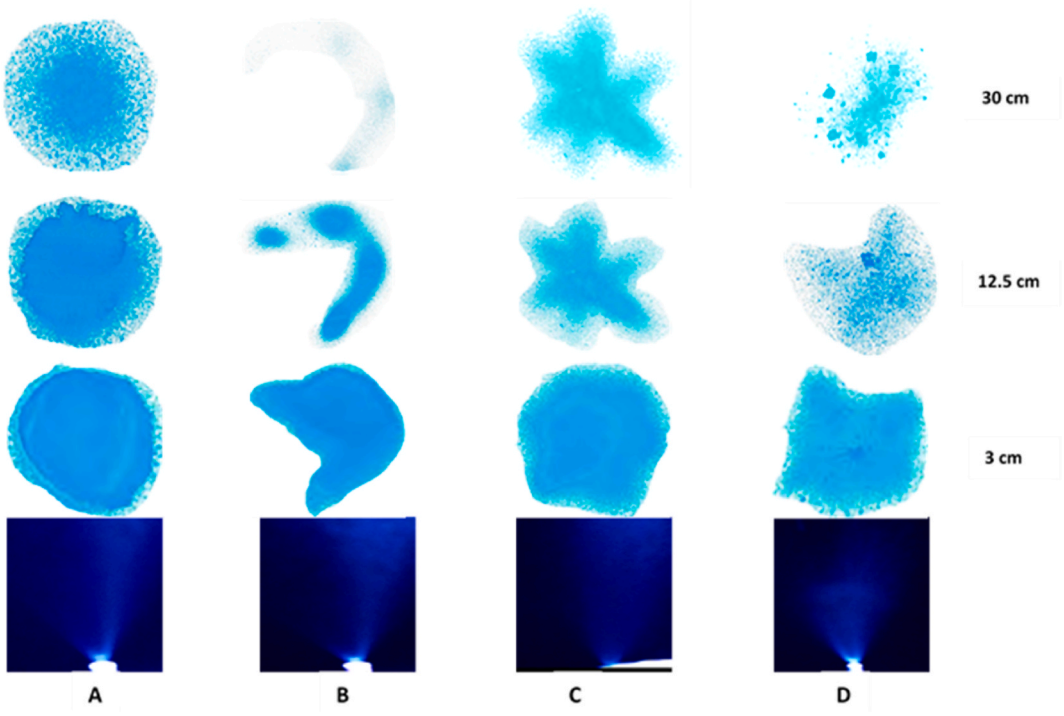

Fig. 3. Images of spray patterns generated by all four throat spray devices (i.e., A, B, C, and D) using liposome formulations. Images of spray patterns deposited on a TLC sheet using distances of 3, 12.5, and 30 cm. These images are typical of three such different experiments.

the hydrocarbon chains of the phospholipid or surfactant. Moreover, cholesterol is a large molecule (with a molecular weight of 521.24 g/mol), and its accommodation in the bilayer of vesicles may occupy a large space, resulting in large vesicle formation. Similar results of significantly large vesicle size due to the incorporation of cholesterol were reported by Shaker et al. [37] and Khan et al. [38] when compared to formulations without cholesterol [39]. Furthermore, previous research also exhibited that as the concentration of cholesterol increases, the size of vesicles also increases [40–42]. Both PDI (referred to as size distribution) and zeta potential values exhibited no significant difference among formulations (when prepared with or without cholesterol) (Table 1).

The main reason for inclusion or exclusion of cholesterol was to investigate its impact on particle size and entrapment efficiency. For the selection of the best formulation, particle size and entrapment efficiency are very critical parameters. The smaller particle size fits better in the small droplet, with minimal fragmentation upon actuation from the spray devices, which may help with higher deposition of vesicles in the throat. Moreover, the optimum formulation should have the ability to encapsulate a higher amount of drug. Thus, the best formulation should be smaller in size with higher encapsulation efficiency. It is noteworthy that cholesterol also increases the hydrophobicity of particle surfaces, which may result in aggregation of particles and hence increase their size. Upon using low-angle x-ray diffraction spacing, the thickness of the phospholipid layer membrane showed an increase of 3 Å when cholesterol was incorporated [43]. This increase in thickness of membrane by

Table 3
Spray pattern generated (at a distance of 3 cm) from all four throat spray devices (i.e., A, B, C, and D) using liposome, ethanol-based proliposome, and proniosome formulations. Data are mean ± SD, n = 3.

Spray devices	Liposomes			Ethanol-based proliposome			Proniosome		
	D_{min} (cm)	D_{max} (cm)	Ovality	D_{min} (cm)	D_{max} (cm)	Ovality	D_{min} (cm)	D_{max} (cm)	Ovality
A	4.20 ± 0.12	4.43 ± 0.08	1.05 ± 0.04	4.13 ± 0.09	4.33 ± 0.10	1.05 ± 0.06	4.32 ± 0.20	4.53 ± 0.04	1.05 ± 0.02
B	4.22 ± 0.22	5.03 ± 0.12	1.19 ± 0.05	4.13 ± 0.13	4.87 ± 0.11	1.18 ± 0.05	4.51 ± 0.09	5.34 ± 0.11	1.18 ± 0.04
C	4.77 ± 0.06	5.10 ± 0.10	1.07 ± 0.03	4.61 ± 0.08	4.98 ± 0.07	1.08 ± 0.04	4.89 ± 0.16	5.25 ± 0.07	1.07 ± 0.04
D	4.33 ± 0.11	5.01 ± 0.18	1.16 ± 0.07	4.33 ± 0.15	4.98 ± 0.05	1.15 ± 0.03	4.51 ± 0.12	5.22 ± 0.10	1.16 ± 0.03

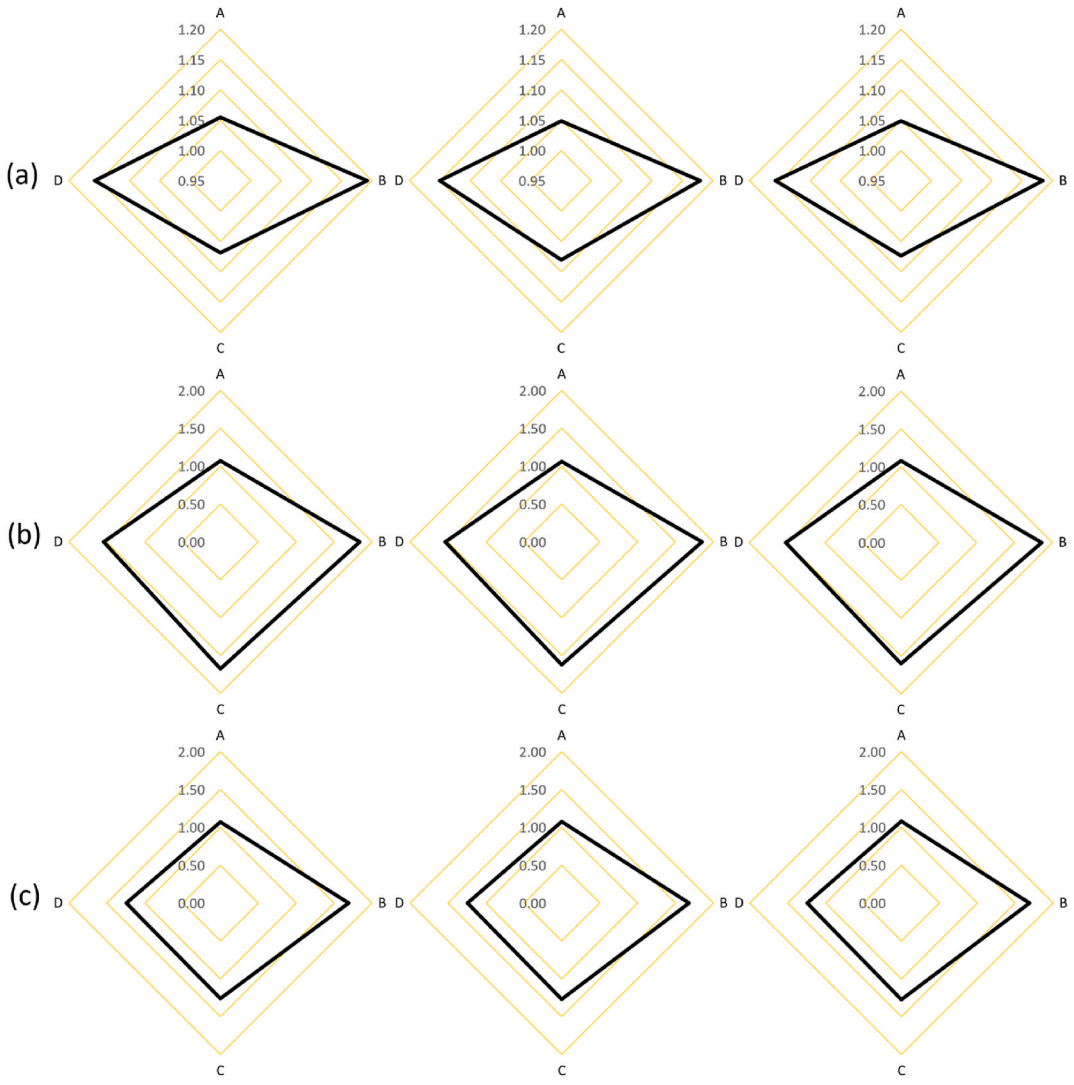

Fig. 4. Ovality ratio from spray pattern generated by all four throat spray devices, i.e., A, B, C, and D (representing four corners of each individual image), using various distances from top to bottom, i.e., for (a) 3 cm, (b) 12.5 cm, and (c) 30 cm, for three formulations from left to right (liposome, ethanol-based proliposome, and proniosome). These images are typical of three such different experiments.

cholesterol may reduce the area per molecule of phospholipid in the plane of the membrane and therefore increase particle size.

Lidocaine entrapment efficiency was significantly higher ($p < 0.05$) without cholesterol incorporation in liposome (67.72 %), ethanol-based proliposome (71.77 %), and proniosome (69.84 %) than in formulations with cholesterol presence (Table 1). It was demonstrated by the previous studies that both drugs and cholesterol compete for their accommodation in the bilayers [44–46], when the drugs are lipophilic or amphiphilic, as they entrap within the phospholipid bilayers (between hydrocarbon chains of bilayers). Hydrophilic drugs are generally encapsulated within the central aqueous core, or between bilayers (between head groups of bilayers), or on the surface of the vesicles; hence, they do not compete with cholesterol in the bilayers. The presence of cholesterol makes vesicles more rigid and decreases their fluidity [47], while simultaneously occupying more space (due to the large size of the molecule) in the bilayers. Which may be attributed to the larger particle size, which provides limited spaces for lidocaine accommodation due to the steric hindrance and packing of steroid (i.e., cholesterol) between phospholipids. Such packing makes the bilayers tighter and reduces the internal aqueous volume (i.e., less water holds less hydrophilic drug), resulting in less capacity to hold hydrophilic drugs and therefore lower entrapment efficiency. Moreover, cholesterol makes the bilayers less leaky, which may prevent the hydrophilic drug from diffusing into the inner aqueous core during formation. These results were also substantiated by the study conducted by Deniz et al. [48], which exhibited significantly higher drug entrapment when cholesterol was excluded as compared to formulations with cholesterol incorporation. Briuglia et al. [49] also showed that cholesterol presence or high concentration significantly decreases drug entrapment when compared to formulations without cholesterol. Thus, based on the conducted physicochemical properties, formulations without cholesterol demonstrated significantly ($p < 0.05$) smaller particle size and higher drug entrapment, making them ideal candidates for further analysis.

3.2. *Surface morphology via TEM*

TEM was employed to visualize and investigate all three formulations (without cholesterol incorporation), i.e., liposomes, liposomes generated from ethanol-based proliposomes, and niosomes generated from proniosomes, using various magnifications. Upon examination, the morphology of these formulations showed that multilamellar vesicles (MLVs) were successfully achieved (Fig. 1).

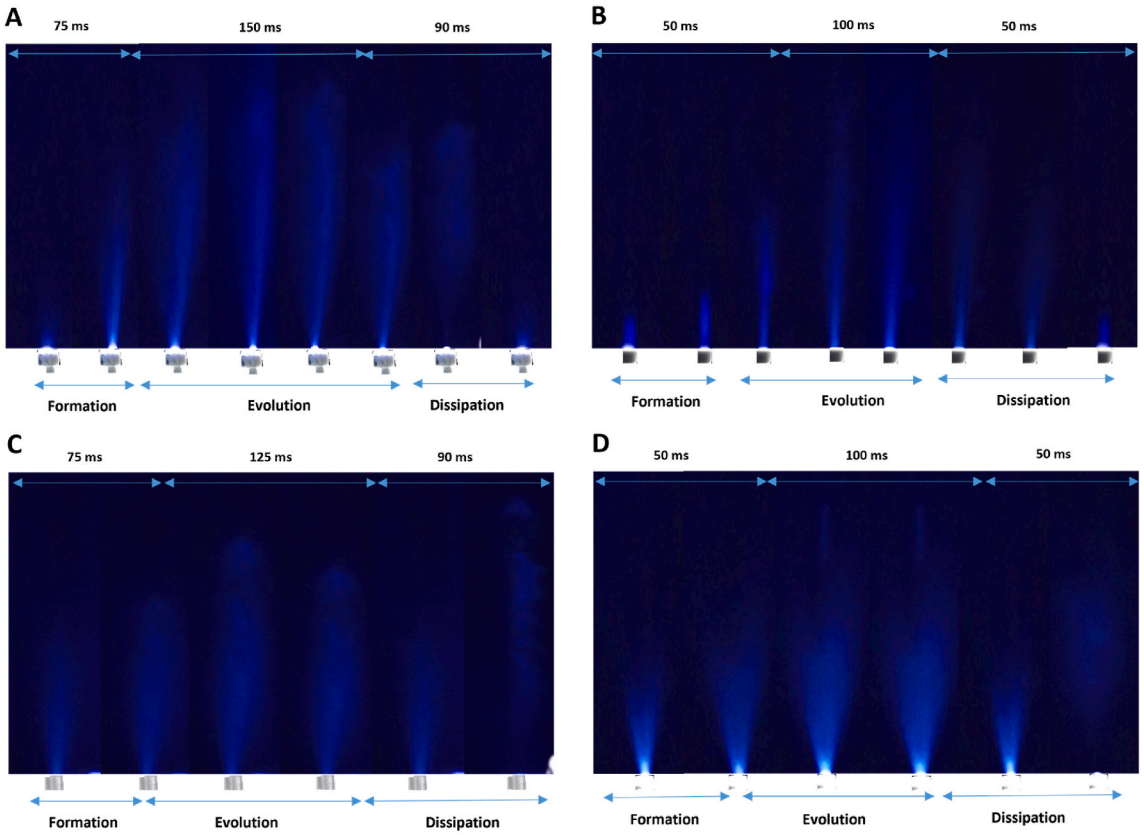

Fig. 5. The generation of plumes from throat spray devices A, B, C, and D showed different phases (i.e., formation, evolution, and dissipation phases) of the spray cloud monitored by videography (using time duration *via* 25 frames per second) using liposome formulations. These images are typical of three such different experiments.

3.3. Dose accuracy and shot weight measurement

The effect and delivery of formulations from the spray devices are mainly based on the numbers of actuations required to prime, full actuation, and tailing-off phases (the numbers of actuations for all three phases are known as total actuations).

Upon using spray devices (A, B, C, and D), no significant difference ($p > 0.05$) was observed for priming between all three formulations (i.e., liposomes, liposomes generated from ethanol-based proliposomes, and niosomes hydrated from proniosomes) (Fig. 2a). All four throat spray devices required 3–6 actuations to prime the devices. The number of full actuations was significantly different between the spray devices. Spray device A delivered a significantly higher ($p < 0.05$) number of full actuations of 16, 19, and 16 for liposomes, vesicles generated from ethanol-based proliposomes, and proniosomes, respectively, when compared to throat spray devices B, C, and D (Fig. 2a). This difference between aerosolization of formulations may be attributed to the design and metering valve of spray devices, regardless of formulation type. For confirmation, a shot weight of each actuation was measured, where a lower number of total actuations by throat spray device A (27, an average of all three formulations) exhibited a larger shot weight (188 mg, an average of all three formulations). Whereas a higher number of total actuations by throat device B (44, an average of all three formulations) demonstrated smaller shot weight (116 mg) (Fig. 2a and b). Moreover, significantly lower ($p < 0.05$) tailing-off actuations were also recorded for device A and higher ($p < 0.05$) for device B. The higher tailing-off phase (i.e., high number of actuations) may be associated with the performance of spray devices; despite a large volume presence, spray devices were unable to deliver full actuations. This also demonstrates that devices with higher full actuation and lower priming and tailing-off phases are superior in terms of consistent dose delivery. Based on the performance of spray devices, a trend of a higher number of full actuations and larger shot weight was found for Device A, followed by Device C, Device D, and Device B (Fig. 2a and b). Furthermore, based on the average of all three formulations (liposomes, ethanol-based proliposomes, and proniosomes), spray devices A, B, C, and D delivered 188, 116, 169, and 149 mg of shot weight per actuation.

3.4. Plume geometry and spray pattern analysis

The aerosol cloud generation from the throat spray devices offers two important factors (i.e., plume geometry and spray pattern) to evaluate their performance. Moreover, these factors also help to predict the possibility of drug deposition in the throat. Images of plume geometry and spray pattern were investigated using various distances from the apex of spray devices (i.e., 3, 12.5, and 30 cm). A distance of 3 and 12.5 cm generally represents formulation deposition in the posterior oral cavity, which refers to the spray reaching the back of the oral cavity and is considered effective throat-targeted deposition.

Upon analysis of plume geometry at 3 cm, the following trend for larger plume angles was found for throat spray devices: Device A > Device C > Device D > Device B, irrespective of formulation type (Table 2). It was also identified that plume width (from the same distance) is dependent upon the plume angle, demonstrating a greater plume width for spray device A, followed by C, D, and B, respectively (Table 2). Furthermore, the total plume length is directly proportional to the plume width and plume angle, and therefore a similar trend of total plume length (i.e., longer) was demonstrated (Table 2). The generation of different plume angles from spray devices may have great implications on the site of deposition in the oral cavity. A narrow plume angle (<60°) is generally considered to be desirable in order to deposit formulation into the posterior oral cavity and throat, whereas a wider

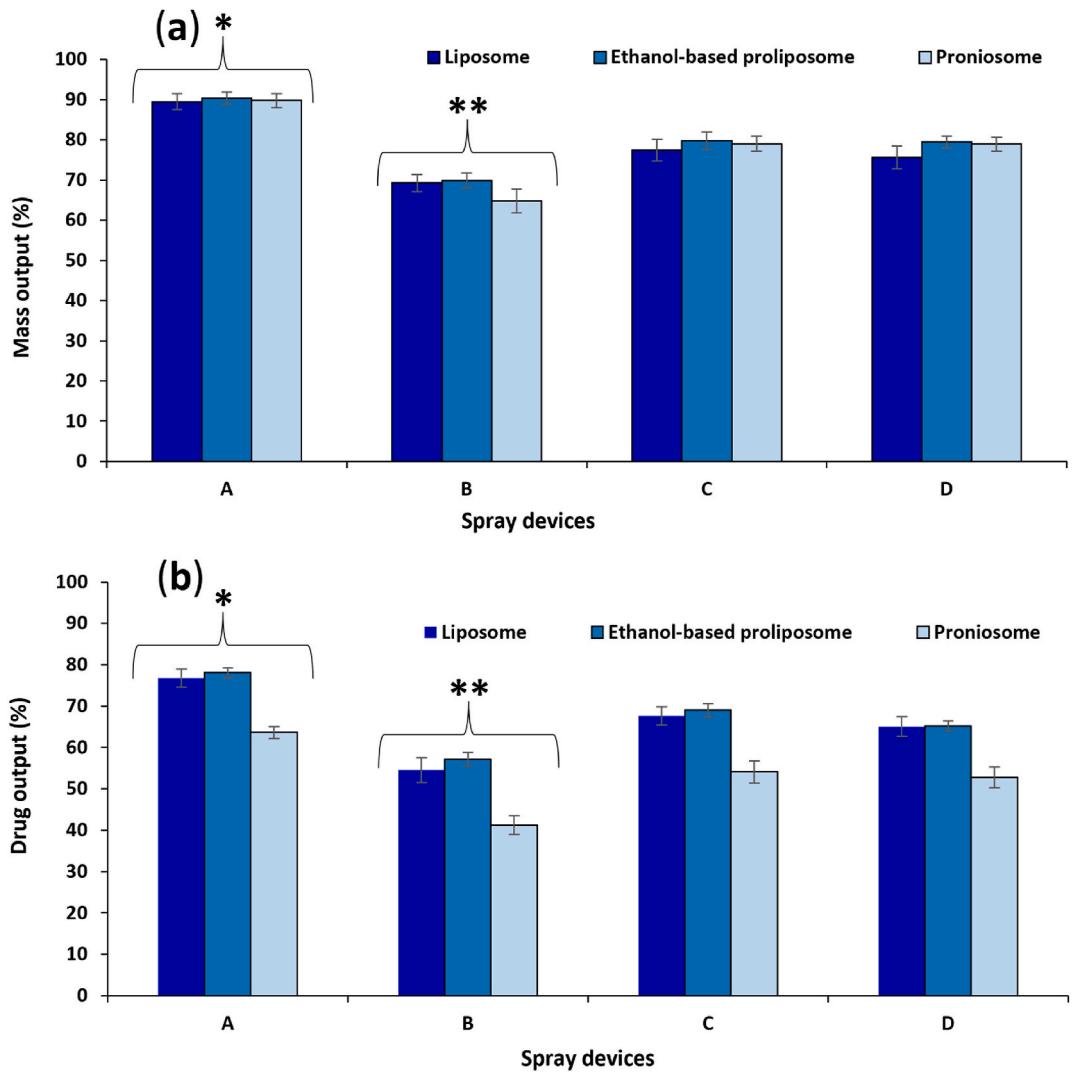

Fig. 6. Liposome, ethanol-based proliposome, and proniosome formulations were aerosolized/actuated *via* four throat spray devices (i.e., A, B, C, and D) for the determination of (a) mass output, and (b) drug output. Data are mean ± SD, n = 3. (a) *p < 0.05 for spray device A mass output compared to other spray devices; **p < 0.05 for spray device B mass output compared to other spray devices. (b) *p < 0.05 for spray device A drug output compared to other spray devices; **p < 0.05 for spray device B drug output compared to other spray devices.

plume angle of more than 65° tends to deposit formulation within the anterior oral cavity. Therefore, using a plume angle, the average of all three formulations (i.e., liposomes, ethanol-based proliposomes, and proniosomes) demonstrated a plume angle of less than 60° and hence suggested that all spray devices will have higher chances of drug deposition in the posterior parts of the oral cavity (Table 2).

For spray pattern images on the TLC sheets, only a liposome formulation was used, as no significant difference was found between formulation types on spray pattern generation. Upon examination of the spray pattern, spray devices A and C were found to exhibit round and oval shapes with a dense spray pattern when compared to the crescent/hemispherical shape by device B and irregular shape by device D (Fig. 3). These images of the spray pattern at a distance of 3 cm were also confirmed by the ovality ratio. In the ovality ratio, value 1 represents a complete round shape of the spray pattern produced by a device. Hence, spray device A exhibited a much closer value of ovality ratio to 1, followed by devices C, D, and B (1.05, 1.07, 1.15, and 1.19), respectively (Table 3). The same trend of the ovality ratio closer to 1 can be seen in Fig. 4 for spray devices (i.e., A, C, D, and B), which further suggests that device A would have better coverage and drug deposition in the oral cavity as compared to other spray devices.

Furthermore, upon comparing the spray pattern at a distance of 12.5 cm (Figs. 3 and 4), again spray device A demonstrated a high degree of round spray pattern, followed by D, C, and B. The pattern of spray was observed to be round, crescent, star, and irregular to oval in shape for spray devices A, B, C, and D. However, upon examination of the spray pattern at a distance of 30 cm, where a pattern is blurry due to the longer distance covered by the formulation, device A still kept its round shape, followed by devices C, D, and B, respectively (Figs. 3 and 4). Thus, it was found that throat spray device A is superior to other spray devices (regardless of formulation type) as it can cover a greater area of the throat with a larger angle, greater plume width, and longer plume length for better therapeutic effect.

Moreover, each actuation generated from throat spray devices was observed and analysed for these three phases, i.e., formation, evolution, and dissipation (CDER, 2002), using a liposome formulation (Fig. 5). Throat spray devices A and C exhibited longer formation, evolution, and dissipation phases when compared to spray devices B and D. However, a comparatively longer ($p < 0.05$) evolution phase was determined by device A (150 ms) when compared to counterpart spray devices (Fig. 5). It is suggested that the longer evolution phases (by devices A and C) and shorter evolution phases (by devices B and D) may be related to the total plume length (to complete the evolution phase) (Table 2). Thus, it was proposed that spray devices A and C may be more efficient in terms of

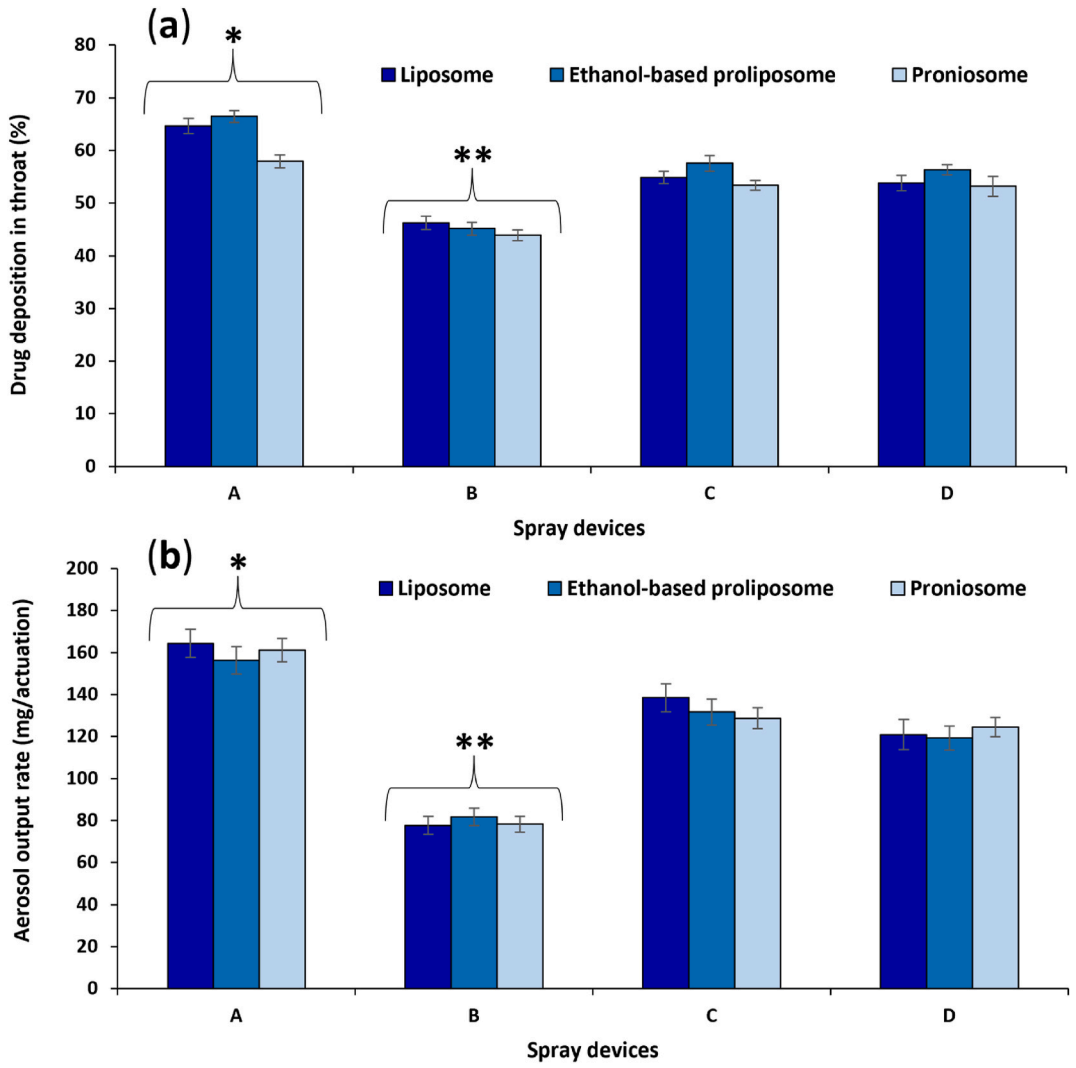

Fig. 7. Liposome, ethanol-based proliposome, and proniosome formulations were aerosolized using four throat spray devices (i.e., A, B, C, and D) for the determination of (a) drug deposition in the throat part (representing the upper stage of TSI) and (b) aerosol output rate. Data are mean ± SD, n = 3. (a) *$p < 0.05$ for spray device A drug deposition in throat compared to other spray devices; **$p < 0.05$ for spray device B drug deposition in throat compared to other spray devices. (b) *$p < 0.05$ for spray device A aerosol output rate compared to spray devices B, C, and D; **$p < 0.05$ for spray device B aerosol output rate compared to spray devices A, C, and D.

covering a large area of the oral cavity due to a longer evolution phase with a higher cone angle and length covered by the spray plume.

3.5. Mass output, drug output and aerosol output rate analysis

Aerosolization to "dryness" *via* total actuation did not result in complete atomization of the formulation from the throat spray devices, and hence 100 % mass output was not achieved (Fig. 6a). Some of the formulation remained as the "dead or residual" volume at the end of aerosolization/total actuations. Mass output was significantly higher ($p < 0.05$) than the drug output for all formulations using all four spray devices (Fig. 6a and 7b). It may be attributed to the design of the spraying device, the presence of free/unentrapped drug, or the vesicles with larger sizes containing entrapped drugs that did not aerosolize upon actuation. Moreover, using spray device A, aerosol mass output was significantly higher ($p < 0.05$), followed by throat spray devices C, D, and lastly B. Higher mass output of spray device A may be associated with the higher numbers of full actuations (Fig. 2a) and their close ovality ratio to one (exhibiting full and complete spray pattern per actuation) (Table 3 and Fig. 3). A similar trend was also observed for the drug output (Fig. 6b), where a higher amount of drug was aerosolized *via* actuation using spray device A than the counterpart devices.

The deposition of drug, regardless of formulation type, exhibited the same trend as mass output and drug output using spray device A when compared to the counterpart spray devices. Upon using the TSI model for all formulations, an average of 63 % of the drug was deposited ($p < 0.05$) from the total drug output in the throat part (i.e., the upper stage of the TSI) compared to the lowest deposition of lidocaine by spray device B (i.e., 45 %) (Fig. 7a). Higher or lower deposition of drug by spray devices A and B in the throat may be directly related to the mass and drug output efficiency as well as the speed of spray upon actuation. A typical targeted dose for throat spray is approximately between 10 and 20 mg per actuation. On average of all three formulations (i.e., liposomes, ethanol-based proliposomes, and proniosomes), spray device A demonstrated a deposition of 8.88 mg of lidocaine per actuation in the throat (when using TSI), whereas spray devices B, C, and D showed a deposition of 4.82, 7.45, and 6.03 mg per actuation, respectively. Which means that spray devices A and C showed better drug deposition when compared to spray devices B and D. In addition, the plume geometry, and ovality ratio (Section 3.4) further confirmed that spray device A (produced a uniform, round-shaped plume) is superior for drug deposition in the upper stage of TSI as compared to the remaining

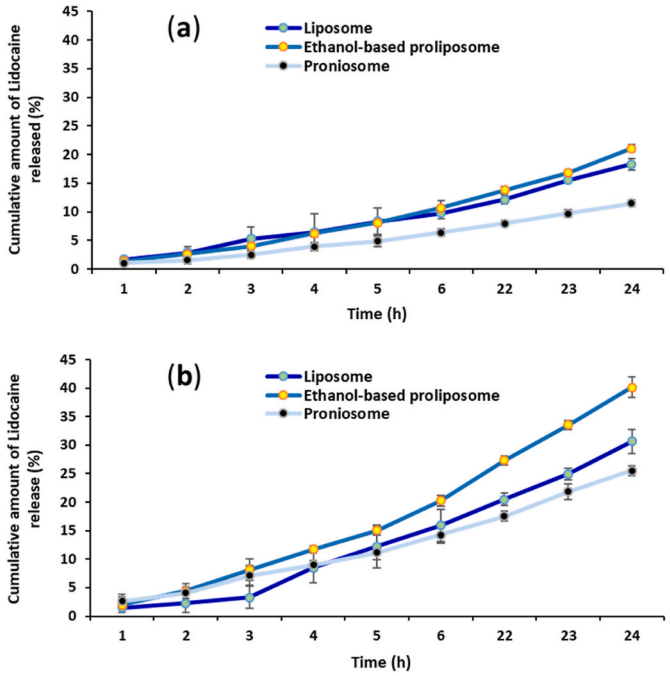

Fig. 8. *In-vitro* study profile of lidocaine entrapped in liposome, ethanol-based proliposome, and proniosome formulations prepared with (a) cholesterol, and (b) without cholesterol were investigated for 24 h. Data are mean ± STD, n = 3.

counterpart spray devices.

Overall, throat spray device A generated aerosols with higher mass output and drug output. Additionally, spray device A completed an average of 22 total actuations (including priming, full actuation, and tailing-off) or when it reached "dryness" for all formulations when compared to an average of total actuations by spray devices B, C, and D (i.e., 29, 23, and 28), respectively (Fig. 2a). Consequently, a much higher mass output rate was attained using spray device A compared to the remaining spray devices (Fig. 7b). Moreover, an average aerosol output rate for all three formulations was 160 mg/actuation for spray device A, while only 79 mg/actuation was recorded for spray device B (Fig. 7b). Hence, the lower actuation numbers, higher mass output, and drug output, as well as the higher output rate using throat spray device A, may offer advantages over the other spray devices in terms of dose consistency and reliability.

3.6. *In-vitro sustained release study of formulation*

Upon using lidocaine alone as a control, 100 % drug release was achieved after 2 h (data not shown). However, the three nano-formulations evaluated in this work (thin-film method, ethanol-based proliposomes, and proniosomes) were not to prevent drug release but specifically designed and formulated to achieve sustained release, with the aim of prolonging the analgesic effect of lidocaine. The rationale behind using nanoformulations was not only to sustain the release but also to improve the drug's local retention and tissue penetration [50]. Although some fraction of the formulation may be washed away due to the dynamic environment of the throat, lipid bilayers and surfactants used in these drug delivery systems may adhere to the throat mucosa, thereby reducing clearance and allowing a more prolonged drug residence time [51]. Moreover, nano-sized vesicles can penetrate mucosal barriers more effectively than free drug, allowing deeper and more sustained delivery to the target tissue [52]. Even partial retention of these nanocarriers can result in local deposition and controlled drug release, which may be more effective than rapid clearance of free drug. Therefore, this concept is based on established principles of mucosal drug delivery and controlled release technology [53]. The proof-of-concept study presented here demonstrates that nanocarrier systems can indeed modulate lidocaine release kinetics and potentially extend the duration of analgesia, which is highly desirable for throat pain management.

Upon comparison between formulations (with or without cholesterol), a significant difference ($p < 0.05$) was found for the lidocaine release between liposome, ethanol-based proliposome, and proniosome formulations. A maximum release of lidocaine was found in vesicles generated from ethanol-based proliposomes after 24 h, which was 21 % and 40 % with and without cholesterol (Fig. 8). This may be related to the presence of ethanol, which acts as a penetration enhancer. This means that ethanol fluidizes and disrupts the phospholipid bilayers, reducing the order of phospholipid hydrocarbon chains, thereby making them less rigid and increasing the membrane permeability and fluidity. Moreover, it was also identified that formulations without cholesterol have higher release when compared to formulations with cholesterol inclusion. It is suggested that drug release may be associated with the interaction of cholesterol with the bilayers. It is important to know that cholesterol incorporation may increase vesicle rigidity, reduce drug leakage, and enlarge vesicle size by filling extra spaces in the bilayers, which can decrease or retard the accommodation of lidocaine compared to formulations prepared without cholesterol (Section 3.1; Table 1). Upon the addition of a cholesterol molecule, it may fill or occupy the spaces between phospholipid or surfactant molecules during annealing time (where cholesterol interacts with the central core of the vesicle). The presence of cholesterol may improve the stability of vesicular drug delivery systems. Liposomal doxorubicin (i.e., Doxil®) contains cholesterol in order to stabilize the circulation duration of vesicles [54]. In oral delivery, liposomes containing cholesterol coated with chitosan demonstrated higher stability in simulated gastric fluid, which showed that cholesterol may also prevent the premature leakage/breakdown of liposomes by gastric fluids and bile salts [55]. Liposomes also showed stability when cholesterol was used with diclofenac for topical administration [56]. Without cholesterol presence, the vesicles are more flexible (having more gaps between phospholipid or surfactant molecules), and hence the drug release is much faster when compared to the formulations with cholesterol incorporation. Therefore, formulations without cholesterol released almost twice the drug when compared to formulations with cholesterol (Fig. 8). Similarly, more than twice the drug release (i.e., Celecoxib) was demonstrated by Deniz et al. [48] when liposomes were prepared without cholesterol when compared to cholesterol inclusion in the formulations. It was also found by Briuglia et al. [49] that a higher amount of cholesterol incorporation in phospholipid vesicles enables slower release of the drug (i.e., Quinine). Furthermore, it was also confirmed by Ali et al. [57] and Yasmin et al. [58] that lipid-based vesicles without cholesterol release a higher percentage of drug over the same period of time when compared to formulations with cholesterol incorporation. Thus, it was identified that cholesterol inclusion in lipid bilayer formulations may decrease the rotational freedom of the hydrocarbon chains and therefore significantly decrease the release of the drug from vesicles and hence take a longer time for the drug to release.

4. Conclusion

In this study, three vesicular formulations (liposomes, liposomes generated from ethanol-based proliposomes, and niosomes hydrated from proniosomes), each with and without cholesterol, were prepared and characterized using four throat spray devices (referred to as devices A, B, C, and D). This study demonstrated that all three vesicular formulations without cholesterol showed better results in terms of particle size and entrapment efficiency. These formulations also provided a sustained release of lidocaine from these vesicular formulations in order to improve patient compliance compared to conventional formulations. Spray device A was found to be better in terms of characterization as compared to other spray devices without being affected by formulation

type. Therefore, it was concluded that throat spray device A demonstrated significantly better performance using nanoformulation compared to the counterpart spray devices and may be a more appropriate device to be used for the management of throat pain. These findings highlight the potential of novel vesicular delivery systems in enhancing better anaesthetic therapy. However, further *in-vivo* studies as well as the incorporation of bioadhesive and mucoadhesive agents (to prevent rapid clearance by swallowing and allow the sustained-release properties of liposomes) are needed to confirm localized drug delivery, clinical effectiveness, and long-term safety.

CRediT authorship contribution statement

Iftikhar Khan: Writing – review & editing, Supervision, Project administration, Conceptualization. **Kai Chang:** Writing – original draft, Methodology, Formal analysis. **Ismail Alsaadi:** Resources, Data curation. **Nozad Rashid Hussein:** Validation, Data curation. **Anila Mathew Thevarkattil:** Visualization, Methodology. **Saeed Ahmed Khan:** Validation, Software. **Sajid Khan Sadozai:** Visualization, Investigation. **Adeeb Shehzad:** Visualization, Software. **Ruba Bnyan:** Writing – review & editing, Resources.

Declaration of competing 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.

Data availability

Data will be made available on request.

References

[1] P. Szmuk, P. Olomuk, R.B. Pop, A.C. Farrow-Gilespie, General anesthetics and neurotoxicity in the developing brain: a review of current literature, Jurnalul Roman de Anestezie Terapie Intensiva/Rom. J. Anaesth. Intensive Care 17 (2010) 117–122.

[2] E.A. Shipton, New formulations of local anaesthetics-part I, Anesthesiol. Res. Pract. 2012 (2012) 546409, https://doi.org/10.1155/2012/546409.

[3] B.-S. Kim, G.-T. Park, M.-H. Park, Y.G. Shin, C.-W. Cho, Preparation and evaluation of oral dissolving film containing local anesthetic agent, lidocaine, J. Pharm. Invest. 47 (2017) 575–581, https://doi.org/10.1007/s40005-016-0298-0.

[4] G.W. Hanks, I. White, Local anaesthetic creams, Br. Med. J. 297 (1988) 1215–1216, https://doi.org/10.1136/bmj.297.6658.1215.

[5] M. Kumar, R. Chawla, M. Goyal, Topical anesthesia, J. Anaesthesiol. Clin. Pharmacol. 31 (2015) 450–456, https://doi.org/10.4103/0970-9185.169049.

[6] A. Kanai, A. Suzuki, H. Okamoto, Comparison of cutaneous anesthetic effect of 8% lidocaine spray with lidocaine patch using current perception threshold test, Pain Med. 11 (2010) 472–475, https://doi.org/10.1111/j.1526-4637.2009.00790.x.

[7] R. Grillo, N.F.S. de Melo, D.R. de Araújo, E. de Paula, A.H. Rosa, L.F. Fraceto, Polymeric alginate nanoparticles containing the local anesthetic bupivacaine, J. Drug Target. 18 (2010) 688–699, https://doi.org/10.3109/10611861003649738.

[8] M. Khodadoostan, S. Sadeghian, A. Safaei, A.R. Shavakhi, A. Shavakhi, Viscous lidocaine solution versus lidocaine spray for pharyngeal local anesthesia in upper gastroesophageal endoscopy, J. Res. Med. Sci. : Off. J. Isfahan Univ. Med. Sci. 23 (2018) 102, https://doi.org/10.4103/jrms.JRMS_579_17.

[9] L. Zorzetto, P. Brambilla, E. Marcello, N. Bloise, M. De Gregori, L. Cobianchi, A. Peloso, M. Allegri, L. Visai, P. Petrini, From micro- to nanostructured implantable device for local anesthetic delivery, Int. J. Nanomed. 11 (2016) 2695–2709, https://doi.org/10.2147/ijn.S99028.

[10] A.R. Strazar, P.G. Leynes, D.H. Lalonde, Minimizing the pain of local anesthesia injection, Plast. Reconstr. Surg. 132 (2013) 675–684, https://doi.org/10.1097/PRS.0b013e31829ad1e2.

[11] R. Bnyan, I. Khan, T. Ehtezazi, I. Saleem, S. Gordon, F. O'Neill, M. Roberts, Formulation and optimisation of novel transfersomes for sustained release of local anaesthetic, J. Pharm. Pharmacol. 71 (2019) 1508–1519, https://doi.org/10.1111/jphp.13149.

[12] A.L. Balocco, P.G.E. Van Zundert, S.S. Gan, T.J. Gan, A. Hadzic, Extended release bupivacaine formulations for postoperative analgesia: an update, Curr. Opin. Anaesthesiol. 31 (2018) 636–642, https://doi.org/10.1097/aco.0000000000000648.

[13] J.V. Natarajan, C. Nugraha, X.W. Ng, S. Venkatraman, Sustained-release from nanocarriers: a review, J. Contr. Release : Off. J. Control. Release Soc. 193 (2014) 122–138, https://doi.org/10.1016/j.jconrel.2014.05.029.

[14] I. Khan, M. Sabu, N. Hussein, H. Omer, C. Houacine, W. Khan, A. Elhissi, S. Yousaf, Trans-resveratrol-loaded nanostructured lipid carrier formulations for pulmonary drug delivery using medical nebulizers, J. Pharmaceut. Sci. (2025) 103713, https://doi.org/10.1016/j.xphs.2025.103713.

[15] I. Khan, K. Edes, I. Alsaadi, M.Q. Al-Khaial, R. Bnyan, S.A. Khan, S.K. Sadozai, W. Khan, S. Yousaf, Investigation of spray drying parameters to formulate novel spray-dried proliposome powder formulations followed by their aerosolization performance, Pharmaceutics 16 (2024) 1541.

[16] A. Mathew Thevarkattil, S. Yousaf, C. Houacine, W. Khan, R. Bnyan, A. Elhissi, I. Khan, Anticancer drug delivery: investigating the impacts of viscosity on lipid-based formulations for pulmonary targeting, Int. J. Pharm. 664 (2024) 124591, https://doi.org/10.1016/j.ijpharm.2024.124591.

[17] M. Apostolou, A.A. Fatokun, S. Assi, I. Khan, Targeted lipid-based drug delivery systems for lung cancer therapy, Appl. Sci. 14 (2024) 6759.

[18] A. Elhissi, D. Elkhalifa, I. Khan, W. Ahmed, Manufacturing strategies for liposome and proliposome-based drug delivery systems, in: A. Elhissi, D. Elkhalifa, I. Khan, W. Ahmed (Eds.), Proliposomes: a Manufacturing Technology of Liposomes for Pulmonary Drug Delivery, Springer Nature Switzerland, Cham, 2025, pp. 35–61.

[19] A.F. Rogobete, M. Dragomirescu, O.H. Bedreag, D. Sandesc, C.A. Cradigati, M. Sarandan, M. Papurica, S.E. Popovici, C. Vernic, G. Preda, New aspects of controlled release systems for local anaesthetics: a review, Trends Anaesth. Crit. Care 9 (2016) 27–34, https://doi.org/10.1016/j.tacc.2016.06.004.

[20] M. Azim, S.A. Khan, N. Osman, S.K. Sadozai, I. Khan, Ameliorated delivery of amphotericin B to macrophages using chondroitin sulfate surface-modified liposome nanoparticles, Drug Dev. Ind. Pharm. 51 (2025) 38–49, https://doi.org/10.1080/03639045.2024.2443007.

[21] I. Khan, S. Sunita, N.R. Hussein, H.K. Omer, A. Elhissi, C. Houacine, W. Khan, S. Yousaf, H.A. Rathore, Development and characterization of novel combinations and compositions of nanostructured lipid carrier formulations loaded with trans-resveratrol for pulmonary drug delivery, Pharmaceutics 16 (2024), https://doi.org/10.3390/pharmaceutics16121589.

[22] A. Ascenso, S. Raposo, C. Batista, P. Cardoso, T. Mendes, F.G. Praca, M.V. Bentley, S. Simoes, Development, characterization, and skin delivery studies of related ultradeformable vesicles: transfersomes, ethosomes, and transethosomes, Int. J. Nanomed. 10 (2015) 5837–5851, https://doi.org/10.2147/ijn.S86186.

[23] H. Shrestha, R. Bala, S. Arora, Lipid-based drug delivery systems, J. Pharm. 2014 (2014) 801820, https://doi.org/10.1155/2014/801820.

[24] C. Desai, F.M. Wood, S.A. Schug, R.W. Parsons, C. Fridlender, V.B. Sunderland, Effectiveness of a topical local anaesthetic spray as analgesia for dressing changes: a double-blinded randomised pilot trial comparing an emulsion with an aqueous lidocaine formulation, Burns : J. Int. Soc. Burn Inj. 40 (2014) 106–112, https://doi.org/10.1016/j.burns.2013.05.013.

[25] P. Armstrong, C. Young, D. McKeown, Ethyl chloride and venepuncture pain: a comparison with intradermal lidocaine, Can. J. Anaesth. = J. Canad. Anesth. 37 (1990) 656–658, https://doi.org/10.1007/bf03006485.

[26] K.F. Payne, P. Kalirai, V. Thirunavukarasu, The use of topical local anaesthetic spray for suturing paediatric intra-oral lacerations, J. Maxillofac. Oral Surg. 15 (2016) 565, https://doi.org/10.1007/s12663-016-0894-6.

[27] NHS, Advice Following Gastroscopy with Local Anaesthetic Throat Spray, 2015, pp. 1–3.

[28] Y. Tanaka, T. Nakayama, M. Nishimori, Y. Tsujimura, M. Kawaguchi, Y. Sato, Lidocaine for preventing postoperative sore throat, Cochrane Database Syst. Rev. (2015) Cd004081, https://doi.org/10.1002/14651858.CD004081.pub3.

[29] K. Hara, K. Maruyama, Effect of additives in lidocaine spray on postoperative sore throat, hoarseness and dysphagia after total intravenous anaesthesia, Acta Anaesthesiol. Scand. 49 (2005) 463–467, https://doi.org/10.1111/j.1399-6576.2005.00632.x.

[30] D.E. Becker, K.L. Reed, Essentials of local anesthetic pharmacology, Anesth. Prog. 53 (2006) 98–108, https://doi.org/10.2344/0003-3006(2006)53[98:Eolap]2.0.Co;2, quiz 109-110.

[31] CDER, Guidance for industry: Nasal spray and inhalation solution, suspension, and spray drug products, Chem. Manufact. Controls Doc. (2002) 1–43.

[32] EMEA, Guideline on the Pharmaceutical Quality of Inhalation and Nasal Products, EMEA/CHMP/QWP/49313/2005 Corr, 2006, pp. 1–27.

[33] D. Marx, G. Nadler, Throat Spray System – Key Considerations when Developing a Throat Spray Solution, 2018, pp. 1–4.

[34] CDER, Guidance for Industry: Bio-Availability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action, 2003, pp. 1–25.

[35] B. pharmacopoeia, British pharmacopoeia, Appendix XII C. Consistency Formulated Preparation, 2025.

[36] K. Taylor, M.E. Aulton, in: Kevin M.G. Taylor, Michael Aulton (Eds.), Aulton's Pharmaceutics the Design and Manufacture of Medicines, sixth ed., Elsevier, Amsterdam, 2021.

[37] S. Shaker, A.R. Gardouh, M.M. Ghorab, Factors affecting liposomes particle size prepared by ethanol injection method, Res. Pharm. Sci. 12 (2017) 346–352, https://doi.org/10.4103/1735-5362.213979.

[38] I. Khan, R. Needham, S. Yousaf, C. Houacine, Y. Islam, R. Bnyan, S.K. Sadozai, M. A. Elrayess, A. Elhissi, Impact of phospholipids, surfactants and cholesterol selection on the performance of transfersomes vesicles using medical nebulizers for pulmonary drug delivery, J. Drug Deliv. Sci. Technol. 66 (2021) 102822, https://doi.org/10.1016/j.jddst.2021.102822.

[39] A. Elhissi, D. Elkhalifa, I. Khan, W. Ahmed, Formulation approaches for proliposomes in pulmonary drug delivery, in: A. Elhissi, D. Elkhalifa, I. Khan, W. Ahmed (Eds.), Proliposomes: a Manufacturing Technology of Liposomes for Pulmonary Drug Delivery, Springer Nature Switzerland, Cham, 2025, pp. 63–112.

[40] S.K. Pathak, R. Mishra, S. Kumar, G. Prakash, R. Parthasarthy, Effect of cholesterol concentration on size of liposome, J. Pharm. Biol. Sci. 1 (2012) 50–53.
[41] S.-C. Lee, K.-E. Lee, J.-J. Kim, S.-H. Lim, The effect of cholesterol in the liposome bilayer on the stabilization of incorporated retinol, J. Liposome Res. 15 (2005) 157–166, https://doi.org/10.1080/08982100500364131.
[42] L.P. Tseng, H.J. Liang, T.W. Chung, Y.Y. Huang, D.Z. Liu, Liposomes incorporated with cholesterol for drug release triggered by magnetic field, J. Med. Biol. Eng. 27 (2007) 29–34.
[43] H. Lecuyer, D.G. Dervichian, Structure of aqueous mixtures of lecithin and cholesterol, J. Mol. Biol. 45 (1969) 39–57, https://doi.org/10.1016/0022-2836(69)90208-3.
[44] D. Needham, R.S. Sarpal, Binding of paclitaxel to lipid interfaces: correlations with interface compliance, J. Liposome Res. 8 (1998) 147–163, https://doi.org/10.3109/08982109809035523.
[45] A.R. Mohammed, N. Weston, A.G.A. Coombes, M. Fitzgerald, Y. Perrie, Liposome formulation of poorly water soluble drugs: optimisation of drug loading and ESEM analysis of stability, Int. J. Pharm. 285 (2004) 23–34, https://doi.org/10.1016/j.ijpharm.2004.07.010.
[46] K. Ruckmani, V. Sankar, Formulation and optimization of zidovudine niosomes, AAPS PharmSciTech 11 (2010) 1119–1127, https://doi.org/10.1208/s12249-010-9480-2.
[47] A. Sharma, U.S. Sharma, Liposomes in drug delivery: progress and limitations, Int. J. Pharm. 154 (1997) 123–140, https://doi.org/10.1016/s0378-5173(97)00135-x.
[48] A. Deniz, A. Sade, F. Severcan, D. Keskin, A. Tezcaner, S. Banerjee, Celecoxib-loaded liposomes: effect of cholesterol on encapsulation and in vitro release characteristics, Biosci. Rep. 30 (2010) 365–373, https://doi.org/10.1042/bsr20090104.
[49] M.-L. Briuglia, C. Rotella, A. McFarlane, D.A. Lamprou, Influence of cholesterol on liposome stability and on in vitro drug release, Drug Deliv. Transl. Res. 5 (2015) 231–242, https://doi.org/10.1007/s13346-015-0220-8.
[50] S.H. Abd El-Alim, A.A. Kassem, M. Basha, Proniosomes as a novel drug carrier system for buccal delivery of benzocaine, J. Drug Deliv. Sci. Technol. 24 (2014) 452–458, https://doi.org/10.1016/S1773-2247(14)50087-1.
[51] M. Pandey, H. Choudhury, J.N.S. Ying, J.F.S. Ling, J. Ting, J.S.S. Ting, I.K. Zhia Hwen, H.W. Suen, H.S. Samsul Kamar, B. Gorain, et al., Mucoadhesive nanocarriers as a promising strategy to enhance intracellular delivery against oral cavity carcinoma, Pharmaceutics 14 (2022), https://doi.org/10.3390/pharmaceutics14040795.
[52] M. Üner, Therapeutic potential of solid lipid nanoparticles and nanostructured lipid carriers for buccal delivery of drugs, Int. J. Adv. Multidiscip. Res. Stud. 4 (2024) 630–641.
[53] H. Sato, K. Yamada, M. Miyake, S. Onoue, Recent advancements in the development of nanocarriers for mucosal drug delivery systems to control oral absorption, Pharmaceutics 15 (2023) 2708.
[54] Y. Barenholz, Doxil®–the first FDA-Approved nano-drug: lessons learned, J. Control. Release 160 (2012) 117–134, https://doi.org/10.1016/J.JCONREL.2012.03.020.
[55] M.S. El-Samaligy, N.N. Afifi, E.A. Mahmoud, Increasing bioavailability of silymarin using a buccal liposomal delivery system: preparation and experimental design investigation, Int. J. Pharm. 308 (2006) 140–148, https://doi.org/10.1016/j.ijpharm.2005.11.006.
[56] E. Touitou, H.E. Junginger, N.D. Weiner, T. Nagai, M. Mezei, Liposomes as carriers for topical and transdermal delivery, J. Pharmacol. Sci. 83 (1994) 1189–1203, https://doi.org/10.1002/jps.2600830902.
[57] M.H. Ali, D.J. Kirby, A.R. Mohammed, Y. Perrie, Solubilisation of drugs within liposomal bilayers: alternatives to cholesterol as a membrane stabilising agent, J. Pharm. Pharmacol. 62 (2010) 1646–1655, https://doi.org/10.1111/j.2042-7158.2010.01090.x.
[58] M. Yasmin Begum, K. Abbulu, M. Sudhakar, Aneesa, Celecoxib – encapsulated liposomes of long alkyl chain lipids: formulation, characterization and in vitro performance, Der Pharm. Sin. 3 (2012), 177-125.