Developing Transcutaneous Nano-enabled Anaesthetics for Eyelid Surgery

Aikaterini Lalatsa[1*], Krisztina Emeriewen[2], Vasiliki Protopsalti, Gabrielle Skelton, George M Saleh[2,3]

[1]Department of Pharmaceutics, School of Pharmacy and Biomedical Sciences, University of Portsmouth, St Michael's Building, White Swan Road, Portsmouth PO1 2DT, UK

[2]Moorfields Eye Hospital, South Wing, Kempston Road, Bedford, MK42 9DJ, UK

[3]The National Institute for Health Research Biomedical Research Centre at Moorfields Eye Hospital and the UCL Institute of Ophthalmology, Kempston Road, Bedford, MK42 9DJ, UK

[*]Corresponding author: Dr Aikaterini Lalatsa, Senior Lecturer in Pharmaceutics, School of Pharmacy and Biomedical Sciences, St Michael's Building, University of Portsmouth, White Swan Road, Portsmouth, PO1 2DT, Tel.: +44 (0)23 9284 3929; E-mail address: katerina.lalatsa@port.ac.uk

SUBTITLE:

Nano-enabled anaesthetics for eyelid surgery; an assessment of the transcutaneous permeability of lipidic and polymeric engineered anaesthetic nanomedicines as needless alternatives to subcutaneous anaesthetics for eyelid surgery.

KEYWORDS: cellulose membranes, Franz diffusion cells, eyelid surgery, lidocaine, nanoparticles

WORD COUNT: 2855

This is a peer-reviewed, accepted author manuscript of the following article: Lalatsa, A., Emeriewen, K., Protopsalti, V., Skelton, G., & Saleh, G. M. (2016). Developing transcutaneous nanoenabled anaesthetics for eyelid surgery. British Journal of Ophthalmology , 100(6), 871-876. https://doi.org/10.1136/bjophthalmol-2015-308250

ABSTRACT

Purpose: Local anaesthesia in eyelid surgery carries inherent risks, which has spurned ongoing investigation to identify needleless alternatives. Nanomedicines (particles ranging between 10nm and 1000nm in size) have shown promise in the transcutaneous delivery of certain drugs. In this study, we explore the feasibility of nano-enabled lidocaine delivery across an artificial skin analogue.

Materials and Methods: Three different lidocaine-loaded nanocarriers were characterised. Diffusion studies were performed through cellulose membranes using customised Franz cells. The nanocarriers included polymeric micelles (PM) (Soluplus), solid lipid nanoparticles (SLN) [Tripalmitin: Lecithin: Labrasol: polysorbate-20: water; 3.33:1:40:1:4.67 w/w] and Self-nanoemulsifying drug delivery systems (SNEDDS) [Capryol-90: Transcutol: Labrasol; 1:3:6 w/w]. Particles were characterised in terms of size, zeta-potential and morphology. One-way analysis of variance (ANOVA) with post hoc Tukey tests were used to assess differences in permeation at a significance of $p<0.05$.

Results: Lidocaine loading was highest in SNEDDs (50 ± 2.1 mg g^{-1}) compared with PMs (13.4 ± 0.6 mg ml^{-1}) and SLNs (2.8±0.5 mg ml^{-1}). All particles possessed a size below 150nm, illustrated good colloidal stability with a negative zeta-potential and a spherical morphology as demonstrated by transmission electron miscroscopy images. Cumulative lidocaine concentration after 6 hours was significant for both PMs (345.7+/-23.8 μg/cm^2/h) and SNEDDS (224.8+/-118.2 μg/cm^2/h) compared to SLNs (127.3+/-25.4 μg/cm^2/h). However, SLNs provided controlled release of lidocaine with a linear gradient that continued to increase up to 6 hours.

Discussion: These results highlight the potential capability of nanoparticle lidocaine delivery in eyelid surgery. The achieved flux for all nanomedicines was higher than that reported for currently approved topical lidocaine formulations (including EMLA cream).

INTRODUCTION

Local anaesthesia in eyelid surgery carries inherent risks such as haemorrhage, swelling, tissue distortion, obscuration of the surgical landmarks and needle phobia. This has spurned ongoing investigation to identify less invasive needleless alternatives for anaesthetic delivery [1].

Local anaesthetics (LAs) work through the reversible inhibition of action potential generation and propagation with the pharmacological properties determined by their molecular structure (consisting of an aromatic ring (conferring lipophilicity and potency), an intermediate chain (determining stability) and an amine group [2 3]. Due to these properties, most LAs require a needle to bypass the skin barrier and provide effective dermal anesthesia.

Transcutaneous delivery offers an alternative to injecting the LAs. The drug, however, has to be in a solubilized form, to allow passive diffusion through the skin, which is driven by a concentration gradient. Of the most commonly used topical products for dermal anesthesia, EMLA cream (an 1:1 oil/water emulsion with the oily phase being an eutectic mixture of lidocaine 2.5% w/w and prilocaine 2.5%w/w cream) and Pliaglis [lidocaine (7% w/w) and tetracaine (7%) in a creamy vehicle that upon drying on exposure to air turns into a pliable, occlusive membrane] rely on eutectic mixtures of the aneasthetics to lower their melting point, hence solubilising the originally crystalline anesthetic drugs to allow for their incorporation within the formulation in high concentration and thus, enhancing their permeation across the skin [2 3]. Ametop gel, another popular topical product, uses xantham gum gel to suspend its anesthetic component, tetracaine [2 3]. The limitation of these products is, that lengthy application and dermal occlusion are needed to achieve maximal effect [2 3].

Nanocarriers work in a fundamentally different way. By encapsulating the therapeutic drug, the resulting particles are of nano size range (between 10 to 1000 nm), which is smaller than the original crystalline drug size while being are able to increase the aqueous solubility of the anaesthetics several fold [4]. Their increased surface area-to-volume ratio results in enhanced permeation via all routes across the skin including intracellular, intercellular and transappendageal [4]. There are several different categories of nanocarriers depending on the material used in the preparation (Figure 1). Polymeric micelles (PMs) are amphiphilic block co-polymers, which contain both hydrophilic and hydrophobic monomers that are able to self-assemble into nanoparticles which are stable in aqueous media. They can solubilise substantial amounts of hydrophobic compounds in their inner core retaining high levels of structural stability [5 6]. Soluplus (polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft co-polymer) is a generally regarded as safe (GRAS) polymer used to formulate PMs. Unlike the polymeric micelles, the self-nanoemulsifying drug delivery systems (SNEDDS) are isotropic mixtures of

natural or synthetic oils, solid or liquid surfactants, or alternatively, one or more hydrophilic solvents and cosolvents/surfactants such as Labrasol (Caprylocaproyl macrogol-8 glyceride) and Transcutol (diethylene glycol monoethyl ether) that have the ability of forming fine oil-in-water (o/w) micro emulsions spontaneously or upon mild agitation when mixed with biological fluids [7]. Alternatively, lipidic nanomedicines prepared by solid lipids at room temperature, known as solid lipid nanoparticles (SLNs) have shown promise in delivery across the stratum corneum. SLNs can be composed by different type of solid lipids such as glycerides, waxes, and fatty acids, and stabilised by a wide range of surfactants in an aqueous media [8]. However, for all the above systems, critical properties for nanoparticulate drug delivery across the stratum corneum involve the size, surface charge, morphology of the particles as well as their drug loading efficiency.

Lidocaine is the most commonly used LA in eyelid surgery due to its potency, rapid onset and moderate duration of action. In this work, we aim to explore the potential of these three different nanocarriers (PMs, SLNs and SNEDDs), as transcutaneous drug delivery systems for lidocaine, across an artificial skin barrier.

MATERIALS AND METHODS:

Source of Materials Used

All chemicals and solvents were purchased from Sigma Aldrich (Dorset, UK). Excipients for SNEDDs [Labrasol (Caprylocaproyl macrogol-8 glycerides EP), Transcutol (diethylene glycol monoethyl ether EP) and Capryol 90 (Propylene glycol monocaprylate (type II) NF)] were donated by Gatefosse (Alpha Chemicals, Berkshire, UK), while phosphatidylcholine (soya lecithin) was donated by Lipoid AG (Lipoid S100, Lipoid AG, Steinhausen, Switzerland) and Soluplus (polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft co-polymer, 90-140 kDa) from BASF (Nottingham, UK). The artificial skin (cellulose acetate membranes, Visking dialysis tubing, cut-off: 12-14KDa, thickness: 2 mm) was purchased from Medicell Membranes Ltd (London, UK). The Franz cells were custom made by Soham Scientific Ltd (Fordham, UK).

Preparation of polymeric micelles (PMs)

Lidocaine (20mg) and Soluplus (10mg) were dispersed in a phosphate buffer (10mM, 1mL) and then probe sonicated (15 minutes, 30% amplitude, Ultra Sonicator, UP200S, 200 Watt, Chesire, UK). The resulting suspension was centrifuged (8,000 rpm for 10 minutes, Juan B3i centrifuge, Fisher Scientific, Loughborough, UK) and the supernatant was isolated.

Preparation of solid lipid nanoparticles (SLNs)

SLNs were prepared using a thin film hydration method followed by probe sonication [8]. Tripalmitin, phosphatidylcholine, and lidocaine (3 mg) were dissolved in a dichloromethane and methanol mixture (2:1 v/v) and rota-evaporated until a thin film was produced (Rotavapor-R, Buchi, Flawil, Switzerland) at 50°C. The film was hydrated with 1% Tween 20 (polyethylene-20-sorbitan monolaurate) solution and a 40% Labrasol aqueous suspension to a final composition lidocaine: tripalmitin: phosphatidylcholine: Tween 20: Labrasol: water of 0.8/3.33/1/40/1/53.87 w/w. The resulting suspension was centrifuged (8,000 rpm for 10 minutes, Juan B3i centrifuge, Fisher Scientific, Loughborough, UK) and the supernatant was isolated for further testing.

Preparation of Self Nanoemulsifying Drug Delivery Systems (SNEDDS)

Solubility studies were carried out in water and other excipients to determine the maximum experimental solubility of lidocaine as it has not been previously reported. Excess lidocaine was dispersed in water, Capryol 90, Transcutol and Labrasol. The samples were left in a waterbath without shaking at 37°C for 24 hours prior to centrifugation (12,000 rpm for 15 minutes, Juan B3i centrifuge, Fisher Scientific, Loughborough, UK) and the supernatant was isolated for further quantification using HPLC (as explained below).

Ternary phase diagrams were constructed with surfactant (Labrasol), co-surfactant (Transcutol (diethylene glycol monoethyl ether)), oil (Capryol 90) and water, using a water titration method [9]. A ratio of oil: co-surfactant of 1:6:3 w/w/w was deemed optimal in terms of particle size and optical clarity of the resultant microemulsion type II.

Particle size measurements

All formulations were diluted with de-ionised water (1 to 100 v/v or 1 to 300 v/v) prior to particle size and zeta-potential measurements, which were performed using photon correlation spectroscopy (Malvern Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK). The accuracy of the instrument was accessed periodically using a drop of latex beads (polystyrene, mean size: 0.1µm, Sigma) in 50 mM sodium chloride. All measurements (n=15) were performed in triplicate.

Transmission electron microscopy (TEM) Imaging

A drop of the solution was placed on Formvar/Carbon Coated Grid (F196/100 3.05 mm, Mesh 300, TAAB labs Ltd, England). Excess sample was filtered off and negatively stained with 2% freshly prepared aqueous uranyl acetate. Imaging was carried out under a Jeol JEM 1400 Transmission Electron Microscope (Hertfordshire, UK). Digital images were taken using an AMTV600 (digital) camera.

In vitro Franz cells diffusion studies

In vitro static diffusion cells are a validated and widely used method to evaluate skin permeability of newly developed formulations (Figure 2) [10 11]. Artificial membranes in conjunction with Franz-type diffusion studies are used to model human skin [10 11]. Modified Franz diffusion cells were specially made for this study. The cells displayed an approximate diffusional area of 0.07 cm^2. After the compartments were rinsed with an acetate buffer (20mM, pH 5) and a 5x2mm stirrer bar was added to the receiver compartment, the compartment was filled up with 2mL of acetate buffer. The washed cellulose acetate membranes (MWCO: 12-14 kDa) were cut into square pieces (0.2 cm^2) and mounted to adequately cover the receptor chambers. The donor compartment was placed on top of the receptor compartment covered with the cellulose membrane, tightly clamped and sealed with Parafilm to avoid leakage. The formulation (1mL or 1 g) was placed in the donor compartment, which was sealed with Parafilm and the Franz cells were submerged in a pre-heated waterbath (37^oC, SBB Aqua 26 Plus, Grant, Shepreth, UK) with the stirrer (350 rpm, MIX 15, 2MAG stirrer, Munchen, Germany). Samples were taken at 0, 1, 2, 4, 6 hours from the receiver compartment. Syringes were activated 5 times prior to sampling to ensure homogeneity (200 µL) and an equal volume of pre-heated acetate buffer was replaced.

High Performance Liquid Chromatography (HPLC) analysis

Lidocaine concentration in the receiver fluid was determined from a standard calibration curve prepared from a lidocaine stock solution (10mg mL^{-1} in dimethylsulfoxide) that was diluted with an acetate buffer (pH 5.0) to elicit a linear calibration curve (1 -100 µg mL^{-1}). Two Phenomenex Onyx Monolithic C18 columns attached to a guard column (20+100+100 mm x 4.6 mm, 5µm) were maintained at 25^oC for analysis using an Agilent 1100 HPLC. Samples (40µL) were eluted at 1.2mL min^{-1} using a gradient method. The mobile phase consisted of an acetate buffer (20mM, pH 5 ± 0.1) and acetonitrile and detection was performed at 220 and 254 nm. The gradient method expressed as time (min): acetonitrile % was as follows: 0:5, 5:10, 10:20, and 20:30, with a retention time of 22.1 minutes. For each of the combinations a minimum of three cellulose membranes and three iterations of HPLC were done.

Data analysis and statistical analysis

Microsoft Office Excel 2007 (Microsoft corporation, Seattle, USA) was used to analyse all data, calculate standard deviations, and fit linear equations. One-way analysis of variance (ANOVA) with post hoc Tukey test was used to assess differences in permeation across cellulose membranes, where $p<0.05$ was considered statistically significant using Minitab 17 (Minitab Ltd, Coventry, UK).

RESULTS:

The characteristics of all three nanomedicines are summarised in Table 1. Lidocaine SNEDDS illustrated the highest loading (50 ± 2.1 mg g^{-1}) from all nanomedicines followed by polymeric micelles (13.4 ± 0.6 mg mL^{-1}) and SLNs (2.8 ± 0.5 mg mL^{-1}). This is likely to be attributed to the high solubility of lidocaine within the selected excipients (Figure 3). All nanoparticulate formulations possessed a particle size below 150 nm and an anionic charge in excess of -10mV illustrating acceptable colloidal stability and spherical or quasispherical morphology (Tables 1, Figure 4B-4D). The cumulative release of the lidocaine-loaded nanomedicines over a 6-hour period is reported in Table 2. SNEDDS and polymeric micelles illustrated superior flux (277 and 193 µg/cm^2/h) across cellulose membrane compared to SLNs (86 µg/cm^2/h).

Table 1: Characteristics of Lidocaine-loaded nanocarriers (Values are presented with their standard deviation)

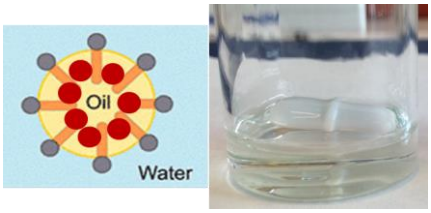
Nanomedicine	Polymeric Micelles (PM)	Solid Lipid Nanoparticles (SLNs)	Self-nanoemulsifying drug delivery systems (SNEDDS)
Appearance			
Maximum Lidocaine Loading (mg mL^{-1})	13.4 ± 0.6	2.8 ± 0.5	50 ± 2.1
Particle size (nm)	91 ±1	104 ± 1	103 ± 23
Colloidal Stability (zeta-potential, mV)	Weakly stable -9.8 ± 2.5	Stable - 23.8 ± 0.4	Very Stable - 77.6 ± 0.6

Table 2: Concentration of Lidocaine-loaded nanocarriers over time across cellulose acetate membrane in Franz cell diffusion studies

Time (h)	Polymeric Micelles (10mg mL^{-1})		SLNs (2mg mL^{-1})		SNEDDs (50mg g^{-1})	
	Concentration (µg ml^{-1})	SD	Concentration (µg ml^{-1})	SD	Concentration (µg ml^{-1})	SD
0	0	0	0	0	0	0
1	88.3	21.7	6.9	8.7	46.4	41.2
2	221.9	28.6	32.6	12.0	200.0	72.8
4	351.9	27.2	80.5	14.0	268.9	145.8
6	345.7	23.8	127.3	25.4	224.8	118.2

DISCUSSION:

In this study, three different types of nanomedicines (lipidic nanoparticles, polymeric nanoparticles and microemulsions), all prepared using safe (GRAS) excipients, were compared for permeation across artificial skin under sink conditions. We show that nano-enabled formulations with high loading of lidocaine can be successfully formulated showing potential in carrying the anaesthetic across an artificial skin barrier. Lidocaine SNEDDS illustrated apart from high colloidal stability, the highest loading (50 ± 2.1 mg g^{-1}) compared to other nanomedicines which is almost 18 fold higher than lidocaine aqueous solubility followed by polymeric micelles and SLNs (Table 1). All prepared particles indicated a nanoparticulate size below 150 nm and spherical or quasi-spherical morphology (Table 1).

The diffusion studies results (Figure 4A) indicate that SNEDDS and polymeric micelles result in permeation with a short lag time and achieving significant concentrations in the receptor compartment within the first hour. SLNs, on the other hand, provided controlled release of lidocaine but with a linear gradient that continued to increase up to 6 hours. The achieved flux for all nanomedicines is higher than that experimentally identified across eyelid skin in our setting (65.68 µg/cm^2/h, data not shown) and that reported for EMLA cream tested in skin (73.81 µg/cm^2/h for lidocaine hydrochloride and 53.93 µg/cm^2/h for prilocaine hydrochloride)[12]. Polymeric micelles permeation across cellulose acetate membranes [that lack a formidable lipidic barrier posed by the anucleate corneocytes of the stratum corneum] may be favoured. However, this would not necessarily hinder the release of the nanoemulsion or solid lipid

nanoparticles as they have been shown to be able to permeate the stratum corneum due to their lipidic nature [13 14].

Active pharmaceutical ingredients (APIs) must be in the solubilised state to penetrate the stratum corneum via a passive transport mechanism driven by the concentration gradient between the formulation and the skin. The selection of the oil phase and surfactant in this study was based on the high solubility of lidocaine within these excipients (Figure 3) which is higher than other commonly used penetration enhancers such as isopropyl myristate and isopropyl palmitate (131 and 110 mg mL^{-1} respectively) which are used in commercially available organogels for local delivery of lidocaine [15]. Transcutol was added to the formulation as it enables high drug loading and the generation of a steep concentration gradient. Transcutol also disorganises the intercellular space between corneocytes which would facilitate diffusion in actual skin. Additionally, the ease of manufacture and scale-up is one of the most important advantages that make SNEDDS unique compared to other novel drug delivery systems, such as liposomes and nanoparticles.

To our knowledge, no studies have been conducted to explore the potential of nanomedicines for eyelid surgery. The results presented here demonstrate promising characteristics (particle size, stability and loading efficiency) for three categories of nanoparticles in the delivery of lidocaine across an artificial skin analogue. Further experiments are under way to ascertain the behaviour of these lidocaine-loaded nanomedicines across human skin tissue and their long-term safety before broader application can be considered.

FUNDING:

This work was funded by the Royal Society (RG130542) and the National Institute for Health Research (NIHR) Biomedical Research Centre based at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology and NIHR Eastern CRN. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

COMPETING INTERESTS:

Dr. Lalatsa reports grants from Royal Society and Dr Saleh from National Institute for Health Research (NIHR) Biomedical Research Centre during the conduct of the study.

CONTRIBUTORS:

AL planned, designed, implemented experiments, monitored data collection experiments, analysed data including statistical analysis and drafted and revised the paper. KE has revised the draft paper. VP and GS have performed experiments, analysed data and revised the paper. GS initiated the

collaborative project, monitored data collection experiments, and revised the draft paper. DL and SC have aided in TEM imaging of nanoparticulate aqueous suspensions.

ACKNOWLEDGEMENTS:

The authors would like to acknowledge the Royal Society (RG130542) for funding and support from the National Institute for Health Research (NIHR) Biomedical Research Centre based at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology and NIHR Eastern CRN. Finally, the authors would like to acknowledge Miss Diana Leite and Prof Simon Cragg for help with TEM imaging.

REFERENCES:

1. Trapasso M, Veneroso A. Local anesthesia for surgical procedures of the upper eyelid using filling cannula: our technique. Plastic and reconstructive surgery. Global open 2014;**2**(5):e143 doi: 10.1097/GOX.0000000000000071[published Online First: Epub Date]|.
2. Alster T. Review of lidocaine/tetracaine cream as a topical anesthetic for dermatologic laser procedures. Pain and therapy 2013;**2**(1):11-9 doi: 10.1007/s40122-013-0010-2[published Online First: Epub Date]|.
3. Sobanko JF, Miller CJ, Alster TS. Topical anesthetics for dermatologic procedures: a review. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al.] 2012;**38**(5):709-21 doi: 10.1111/j.1524-4725.2011.02271.x[published Online First: Epub Date]|.
4. Lee RW, Shenoy DB, Sheel R. Micellar Nanoparticles: Applications for Topical and Passive Transdermal Drug Delivery. In: Kullkarni VS, ed. Handbook of Non-Invasive Drug Delivery Systems. San Antonio, Texas: Elsevier, , 2010:37-58.
5. Liu H, Farrell S, Uhrich K. Drug release characteristics of unimolecular polymeric micelles. Journal of controlled release : official journal of the Controlled Release Society 2000;**68**(2):167-74
6. Lalatsa A, Schatzlein AG, Mazza M, et al. Amphiphilic poly(L-amino acids) - new materials for drug delivery. Journal of controlled release : official journal of the Controlled Release Society 2012;**161**(2):523-36 doi: 10.1016/j.jconrel.2012.04.046[published Online First: Epub Date]|.
7. Souto EB, Fnagueiro JF, Muller RH. Solid Lipid Nanoparticles (SLN^{TM}). In: Uchegbu IF, Schatzlein AG, Cheng WP, et al., eds. Fundamental of Pharmaceutical Nanoscience. New York: Springer 2013:91-116.
8. Mukherjee S, Ray S, Thakur RS. Solid lipid nanoparticles: a modern formulation approach in drug delivery system. . Indian J Pharm Sci 2009;**71**(4):349-58
9. Venkatesh G, Majid MI, Mansor SM, et al. In vitro and in vivo evaluation of self-microemulsifying drug delivery system of buparvaquone. Drug development and industrial pharmacy 2010;**36**(6):735-45 doi: 10.3109/03639040903460446[published Online First: Epub Date]|.
10. FDA-SUPAC-SS. In vitro Release Testing and In Vivo Bioequivalence Documentation. Guidance for Industry. SUPAC-SS Non-sterile

Semisolid Dosage Forms. In: Scale-up and Postapproval Changes: Chemistry MaC, ed. Bathesda, US: Food and Drug Administration, 1997:19-24.
11. Ng SF, Rouse JJ, Sanderson FD, et al. Validation of a static Franz diffusion cell system for in vitro permeation studies. . AAPS PharmSciTech 2010;**11**(3):1432-41
12. Kruger L. Pheroid technology for the transdermal delivery of lidocaine and prilocaine. North West University, 2009.
13. Matsui R, Hasegawa M, Ishida M, et al. Skin permeation of lidocaine from crystal suspended oily formulations. Drug development and industrial pharmacy 2005;**31**(8):729-38 doi: 10.1080/03639040500216147[published Online First: Epub Date]|.
14. Pathak P, Nagarsenker M. Formulation and evaluation of lidocaine lipid nanosystems for dermal delivery. . AAPS PharmSciTech 2009;**10**(3):985-92
15. Almeida H, Amaral MH, Lobao P, et al. Pluronic(R) F-127 and Pluronic Lecithin Organogel (PLO): main features and their applications in topical and transdermal administration of drugs. Journal of pharmacy & pharmaceutical sciences : a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques 2012;**15**(4):592-605

FIGURE LEGENDS:

Figure 1. Permeation pathways for local anaesthetics and local anaesthetic loaded nanomedicines across the skin; nanomedicines utilise apart from the intercellular and trancellular route also the shunt routes (transfollicular, transglandular).

Figure 2. Preparation of Franz Cells for diffusion studies; A: Schematic diagram of Franz cells compartments, B: Specially prepared Franz cells for use with small surface narrow samples, C: Sampling at various time points from Franz cells, D-F: Step by step preparation of Franz cells for diffusion studies.

Figure 3. Solubility studies of Lidocaine at various generally regarded as safe (GRAS) excipients.

Figure 4. A: Cumulative release of the three Lidocaine-loaded nanocarriers across cellulose membrane in Franz cell diffusion studies; Lidocaine-loaded PMs (triangles), Lidocaine-loaded SNEDDs (squares) and Lidocaine-loaded SLNs (circles). Transmission electron microscopy (TEM) images of aqueous dispersions of (B) Lidocaine-loaded PMs (10 mg mL^{-1}), (C) Lidocaine-loaded SNEDDS (50mg g^{-1}) and (D) Lidocaine-loaded SLNs (2.8mg mL^{-1}). Staining: 1% Uranyl Acetate, Bar: 100nm.