




This item was submitted to Loughborough's Institutional Repository (<https://dspace.lboro.ac.uk/>) by the author and is made available under the following Creative Commons Licence conditions.


 **creative commons**
C O M M O N S D E E D


Attribution-NonCommercial-NoDerivs 2.5


You are free:

- to copy, distribute, display, and perform the work

Under the following conditions:

 **Attribution.** You must attribute the work in the manner specified by the author or licensor.


 **Noncommercial.** You may not use this work for commercial purposes.

 **No Derivative Works.** You may not alter, transform, or build upon this work.

- For any reuse or distribution, you must make clear to others the license terms of this work.
- Any of these conditions can be waived if you get permission from the copyright holder.

Your fair use and other rights are in no way affected by the above.

This is a human-readable summary of the [Legal Code \(the full license\)](#).

[Disclaimer](#) 

For the full text of this licence, please go to:
<http://creativecommons.org/licenses/by-nc-nd/2.5/>

Lidocaine carboxymethylcellulose with gelatine co-polymer hydrogel delivery by combined microneedle and ultrasound

Atul Nayak, Hiten Babla, Tao Han, Diganta Bhusan Das*

Department of Chemical Engineering, Loughborough University, Loughborough, LE11 3TU, UK

(*Corresponding Author; Email: D.B.Das@lboro.ac.uk; Tel: 0044 1509 22509;

Fax: 0044 1509 223923)

ABSTRACT

A study that combines microneedles and sonophoresis pre-treatment was explored to determine their combined effects on percutaneous delivery of lidocaine from a polymeric hydrogel formulation. Varying ratios of carboxymethylcellulose and gelatine (NaCMC:gel range are 1:1.60-1:2.66) loaded with lidocaine were prepared and characterised for zeta potential and particle size. Additionally, variations in the formulation drying techniques were explored during the formulation stage. Ex-vivo permeation studies using Franz diffusion cells measured lidocaine permeation through porcine skin after pre-treatment with stainless steel microneedles and 20 kHz sonophoresis for 5 and 10 minute durations. A stable formulation was related to a lower gelatine mass ratio because of smaller mean particle sizes and high zeta potential. Lidocaine permeability in skin revealed some increases in permeability from combined microneedle and ultrasound pre-treatment studies. Furthermore, up to 4.8 fold increase in the combined application was observed compared with separate pre-treatments after 30 minutes. Sonophoresis pre-treatment alone showed insignificant enhancement in lidocaine permeation during the initial 2 hours period. Microneedle application increased permeability at a time of 0.5 h for up to ~17 fold with an average up to 4 fold. The time required to reach therapeutic levels of lidocaine was decreased to less than 7 minutes. Overall, the attempted approach promises to be a viable alternative to conventional lidocaine delivery methods involving painful injections by hypodermic needles. The mass transfer effects were fairly enhanced and the lowest amount of lidocaine in skin was 99.7% of the delivered amount at a time of 3 hours for lidocaine NaCMC/GEL 1:2.66 after low frequency sonophoresis (LFS) and microneedle treatment.

Keywords Carboxymethylcellulose, gelatine, microneedles, sonophoresis, lidocaine, percutaneous

Abbreviations: Degree of substitution (D.S.), sodium carboxymethylcellulose (NaCMC), gelatine (gel)

1. Introduction

This paper is concerned with the delivery of lidocaine, a common anaesthetic, from a lidocaine carboxymethylcellulose with gel co-polymer hydrogel formulation such as discussed recently by Nayak et al. (2013). An ideal anaesthetic can be described as one that provides rapid, prolonged and effective localised anaesthesia via a mechanism of blocking sensory nerve fibres in the

37 periphery that induces no pain and causes no adverse local tissue reaction (Rudin, 2013; Richards
38 and McMahon, 2013; Milewski and Stinchcomb, 2011). Lidocaine hydrochloride is a water soluble
39 weak acid, fully ionised at pH 5.0 and administered into the plasma rich layer under the skin surface
40 (González-Rodríguez et al., 2013; Igaki et al., 2013). However, this administration is conventionally
41 performed via hypodermic needles as a low cost and fast acting method (Kim et al., 2012; Hedge et
42 al., 2011). This is known to cause significant pains (Scarfone et al., 1998). Alternatives, such as
43 eutectic mixture of local anaesthetics (EMLA), a topical form to administer lidocaine, require at least
44 an hour of application to achieve effective analgesia, thus limiting its use especially in emergency
45 situations (Nayak and Das, 2013). Therefore, there are important rationales for the pursuits of
46 alternative lidocaine administration (Nayak et al., 2013; Nayak and Das, 2013). This can be
47 evidenced in the European paediatric drug legislation which backs innovative approaches to
48 develop 'easy to administer' and 'minimally invasive' drug delivery methods (Shah et al., 2011). The
49 alternative rationales for lidocaine delivery include the need for increased safety amongst the
50 patients and healthcare providers, increased compliance with those who possess a fear of needles,
51 reduced discomfort and pain especially in the case of applying anaesthetics as well as improved
52 ease of delivery (Gill and Prausnitz, 2007; Giudice and Campbell, 2006; Li et al., 2010). Oral
53 administration can overcome many of the disadvantages associated with direct injection of drugs
54 (Bal et al., 2010). However, one constraint is low bioavailability of some drugs which limits the
55 effectiveness as therapeutic targets (Shipton, 2012; Benet et al., 1996; De Boer et al., 1979; Huet
56 and Lelorier, 1980). Lidocaine's oral bioavailability is approximately reduced by 65 - 96%, mainly by
57 hepatic enzymes (Fasinu et al., 2011; Fen-Lin et al., 1993). In principle, innovative percutaneous
58 delivery method could be used to overcome the barriers associated with direct injection and oral
59 administration of drugs (Polat et al., 2011) such as lidocaine. The rate of passive diffusion (PD) of
60 drugs by percutaneous delivery depends on the molecular structure, size and hydrophobicity in
61 conjunction with the drug concentration gradients. However, many studies have used combinations
62 of PD and non-invasive techniques with varying success, e.g., microneedles and ultrasound (Han
63 and Das, 2013; Chen et al., 2010). This is the topic of this paper and it is discussed in more detail
64 below.

65
66 Microneedles are needle-like structures of the size order of microns commonly arranged in a matrix
67 (Gill and Prausnitz, 2007; Zhang et al., 2014; Olatunji et al., 2014). The geometry of microneedle
68 influences its ability to pierce the skin but importantly, it can be designed to control/optimize the rate
69 of drug delivery. The lidocaine NaCMC/GEL hydrogels pseudoplasticity permits the viscous
70 formulation in allowing seepage into microneedle cavities to bypass the stratum corneum skin layer
71 compared with passive diffusion (Nayak et al., 2013). Research has shown that a significant
72 increase in skin permeability can be achieved when optimised microneedle arrays are used where
73 the important factors include microneedle length, number of microneedles, the length and width
74 aspect ratio and surface area of the microneedle patch (Al-Qallaf and Das, 2008; Al-Qallaf and Das,
75 2009; Olatunji et al., 2012; Olatunji et al., 2013; Guo et al., 2013). It has been suggested that

76 microneedles can be adapted to aid lidocaine delivery yielding many fold increase in delivery rate
77 (Kwon, 2004; Li et al., 2008; Wilson et al., 2008; Kochhar et al., 2013; Ito et al., 2013; Zhang et al.,
78 2012; Zhang et al., 2012; Nayak et al., 2013).

79
80 In a recent review paper, Nayak and Das (2013) have discussed the possibility of delivering
81 lidocaine using biodegradable micro-needles. In exploring alternative applications of microneedles,
82 a number of other studies have successfully delivered numerous active molecules using
83 microneedles, e.g., hepatitis B vaccine (Guo et al., 2013), Solaraze[®] gel in extending pore opening
84 (Ghosh et al., 2013b) and naltrexone co-drug with diclofenac drug (Banks et al., 2013). In another
85 recent study, it has been shown that microneedles can be combined with ultrasound for increasing
86 the delivery rate of a large macromolecular drug (Han and Das, 2013). These studies have further
87 raised the hypothesis that microneedles and ultrasound combination could be used for greater
88 epidermal lidocaine delivery in order to determine the significance of optimum sonophoretic power
89 related the effects on lidocaine permeation.

90
91 In this context, it is important to state the classification of sonophoresis which is generally based on
92 the frequency of ultrasound. The low frequency sonophoresis (LFS) is defined to be within the range
93 of 20-100kHz and the high frequency sonophoresis (HFS) is usually for above 0.7MHz (Polat et al.,
94 2011). The mechanism by which enhanced permeability is achieved via ultrasound can be linked to
95 a number of physical phenomena including thermal effects, formation of cavitation, mechanical
96 effects and convective localised fluid velocities in skin (Lavon and Kost, 2004). However, in the
97 ultrasound pretreatment experiment, it is generally accepted that inertial cavitation is the largest
98 contributor to the enhancement in skin permeability. It is more so with LFS as shown by Merino et al
99 (2003) due to larger bubble size at low frequency range. Inertial cavitation occurs due to pressure
100 variations induced by ultrasound, resulting in rapid growth and collapse of bubbles formed in the
101 coupling medium. The collapsing of the aforementioned bubbles near skin surface will cause micro-
102 jets due to asymmetrically release of energy. These micro-jets have been confirmed as the main
103 contributors to the permeability increment (Wolloch and Kost, 2010). The effects of ultrasound have
104 been studied for the enhancement of transdermal lidocaine administration with significant
105 enhancement demonstrated with both pulsed and continuous output mode of LFS (Ebrahimi et al.,
106 2012). However, as far as we are aware of, these techniques are yet to be combined and studied for
107 permeability enhancement levels, particularly for lidocaine.

108
109 The potential for adapting microneedles for lidocaine delivery via hydrogel microparticles has been
110 discussed previously with the conclusion that there is significant commercial potential for lidocaine
111 microneedle products (Zhang et al., 2012; Nayak et al., 2013). Polymeric hydrogel microparticles
112 are good for the purpose of controlling spreading (i.e., controllable spreading radius, droplet height
113 and contact angle) of the drug formulation over skin (Nayak et al., 2013). A hydrogel drug vehicle
114 comprises the electrostatic polyionic interaction of a branched structural polymer with a relatively

115 linear polymer in the formation of a cross-linked matrix to encapsulate Lidocaine molecules (Zhao et
116 al., 2006; Lastumäki et al., 2003).

117
118 In this particular study, the drug vehicle for lidocaine encapsulation is polyanionic, carbohydrate
119 based NaCMC crosslinked with polycationic, protein based gel in forming a hydrogel (Nayak et al.,
120 2013). Previously lidocaine formulation bypassing the stratum corneum (SC) epidermal layer was
121 outlined, the viscoelastic properties in adapting a NaCMC/gel network hydrogel prevents slippage of
122 the drug formulation when applied to the skin and the possibility of non-convective flow through the
123 opened cavities of the skin from microneedle treatment (Milewski and Stinchcomb, 2011; Ghosh et
124 al., 2013a). To try and exploit this potential the main aim of the study is to combine the techniques in
125 microneedle array and ultrasound technology as a pre-treatment to meet the definition of an ideal
126 anaesthetic delivery method. Furthermore, this study will focus on using solid microneedles utilising
127 the 'poke and patch' technique. The main advantage of this approach is the technical simplicity
128 required for reproduction of the required micro-needles leading to reduction of cost. The other major
129 advantage is that an extended release is possible using this approach. A carbohydrate based
130 visceral hydrogel formulation was prepared as a model anaesthetic as this provides flexible
131 properties and ability to encapsulate considerable amounts of liquid drug, lidocaine in this instance
132 (Milewski and Stinchcomb, 2011), as discussed in the following section. Furthermore, the spreading
133 behaviour of the prepared formulation was studied and compared with the spreading behaviour
134 lidocaine solution as a Newtonian liquid. Unlike numerous studies performed using synthetic
135 substrates, this study implements porcine skin as a lipophilic substrate as was attempted by Chow
136 et al. (2008).

137

138 **2. Materials and methods**

139 NaCMC and gel emulsion was crosslinked to form hydrogels with encapsulated lidocaine in batch
140 scale production. This formulation setup is highly beneficial because of fairly efficient preparation
141 times in achieving a finished drug formulation and low heat treatment in adaptation of green
142 chemistry.

143

144 *2.1 Materials and equipments*

145 Sodium carboxymethylcellulose (D.S. 0.9; M.W. 250kD), sorbitan mono-oleate (SPAN 80),
146 glutaraldehyde 50% w/w, paraffin liquid (density range: 0.827-0.89 g/ml), lidocaine hydrochloride
147 (M.W. 288.81 g/mol), methylene blue (50 % v/v) and porcine gelatine (type A) were purchased from
148 Sigma Aldrich Ltd (Dorset, UK). Analytical grade acetic acid, high performance liquid
149 chromatography (HPLC) grade acetonitrile and n-hexane (95% w/w) were purchased from Fisher
150 Scientific UK (Loughborough, UK). A Gemini-NX column (C18) of particle size 3 µm was purchased
151 from Phenomenex (Cheshire, UK) for HPLC instrumentation. Amputated porcine ears (age of pig: 5-
152 6 months) were purchased from a local butcher and dissected into 20 mm x 20 mm squares before

153 storage at $-20 \pm 1^\circ\text{C}$. Also 10 mm x 10 mm squares of same porcine skin were dissected as a
154 substrate for droplet spreading. Microneedle patch (stainless steel, flat arrow head geometry and
155 1100 μm length) was purchased from nanoBioSciences (Sunnyvale, CA, USA). Branson Digital
156 Sonifier 450 (Danbury, USA) was chosen as the ultrasound output system. This ultrasound system
157 includes an auto-calibrated transducer and a digital output controller. The frequency of the
158 ultrasound is fixed at 20 kHz but the output powers are adjustable between 4 and 400 W. The
159 equipment for droplet spreading studies were AVT Pike F-032 high performance camera (Allied
160 Vision Technologies UK), Camera i-speed LT high speed video (Olympus, UK),

161 2.2 Formulation of lidocaine NaCMC:gel hydrogel

162 Paraffin oil (100 ml) was sheared continuously for up to 400 rpm in a stirred vessel bought from IKA
163 (Staufen, Germany). Span 80 (0.5% w/w) was dispensed in ambient conditions. To this NaCMC
164 (1.24 % w/w) in ultrapure water was added dropwise, and depending on the polymeric ratio (c %
165 w/w), gel in ultrapure water was also added dropwise at 35-40 $^\circ\text{C}$ (Table 1). A subsequent pH
166 reduction of the solution to pH 4.0 was performed by the addition of acetic acid (~3% w/w). While
167 shearing at 400 rpm, lidocaine HCl (2.44 % w/w) was added dropwise in ultrapure water at 20 $^\circ\text{C}$ into
168 the polymer mixture. The polymeric mixture was then cooled to 5-10 $^\circ\text{C}$ for 30 minutes to initially
169 harden the microparticles. Glutaraldehyde (0.11 % w/w) was added to the emulsion as a cross linker.
170 Upon returning to 20 $^\circ\text{C}$ temperature the hydrogel mixture was sheared for 2 hours at approximately
171 1000 rpm to ensure thorough mixing. The lidocaine NaCMC/gel formulation was then left to stand
172 until a distinct w/o boundary was observed after which this formulation was left overnight at 1-5 $^\circ\text{C}$.
173 Excess paraffin liquid was removed via n-hexane separation shaking (50 % v/v); top organic layer
174 was discarded before placing the hydrogel formulation in a vacuum oven (Technico, Fistreem
175 International Ltd, Loughborough, UK) under full vacuum and a temperature of 20 $^\circ\text{C}$ for 8 hours.
176 Following this, the formulation was washed with deionised (DI) water and filtered using commercial
177 filter papers with pore size 6 μm (Whatman, Ltd, Oxon, UK) for removal of unbound lidocaine before
178 further characterisation. In the case of F5 residual paraffin and n-hexane were removed by rotary
179 evaporation (Heidolph Instruments, Essex, UK). Similarly, the formulation was washed with DI water
180 and filtered as previously outlined.

181

| |
|---------|
| Table 1 |
|---------|

182 2.3 Zeta potential of lidocaine NaCMC:gel hydrogel

183 The zeta potential was measured using a Zetasizer (3000 HSA, Malvern Instruments,
184 Worcestershire, UK). Lidocaine NaCMC/gel (2.0 \pm 0.5 g/ml) in DI water was injected into the sample
185 port, temperature maintained at 25.0 $^\circ\text{C}$ and the results were obtained in triplicate. The zeta-potential
186 (ζ) was measured in terms of electrophoretic mobility (μ) via an optical technique, and ζ (mV) (Park
187 et al., 2005) of the diluted hydrogel was computed from the Smoluchowski equation (2) where μ is
188 referenced with latex ($\text{m}^2 \text{v}^{-1} \text{s}^{-1}$), η is the DI volume viscosity ($\text{m}^2 \text{s}^{-1}$), ϵ_0 and ϵ_r are the permittivity in
189 a vacuum and relative permittivity of DI water as medium respectively (Sze et al, 2003).

$$\zeta = \frac{4\pi\mu\eta}{(\varepsilon_r\varepsilon_0)} \quad (2)$$

190

191

192 2.4 Viscometric analysis of lidocaine NaCMC:gel hydrogel

193 A well-mixed sample volume (25 ml) of lidocaine NaCMC/gel hydrogel sample was determined for
194 variations to viscoelastic properties at standard temperatures of 20°C. An inducing shear rotating
195 viscometer (Viscotester VT550, Haake, Germany) with rotor and cup (NV1) assemblies and a
196 constant ravine of 0.35 mm, in between the assembly was adapted in viscometric analysis. More
197 details on this aspect of our work are presented by Nayak et al. (2013).

198

199 2.5 Optical micrographs of lidocaine NaCMC:gel hydrogel

200 Micrographs were obtained using an optical microscope (BX 43, Olympus, Southend-on-sea, UK)
201 and a camera attachment captured coloured still images (Retiga-2000R, QImaging, British
202 Columbia, Canada). Micrographs were pictured in triplicate for each formulation. An image
203 processing software (*ImageJ*) was adapted in pixel measurement via graticule calibration to
204 interpret particle size diameters from a random selection of 50 microparticles per image. ImageJ is a
205 Java-based open source image processing and analysis program developed at the National Institute
206 of Health (NIH), USA.

207

208 2.6 Controlled release of lidocaine from NaCMC/gel hydrogel

209 Lidocaine NaCMC/gel hydrogel (0.1 ± 0.05 g) was placed in an amber vial and 25.0 ml of DI water
210 was dispensed before the sample was placed in a pre-heated thermo-stat bath at 37.0 ± 0.5°C
211 (Grant Instruments, Cambridge, UK). Subsequently 1 ± 0.0005 ml of heated sample removed by
212 autopipette (Eppendorf, Stevenage, UK), filtered using Nylon membranes (Posidyne, 0.1 µm) and
213 analysed for lidocaine content using HPLC instrumentation. The results were measured in triplicate
214 and the standard deviation from sample mean was taken.

215

216 2.7 Ex vivo skin permeation study of lidocaine NaCMC/gel hydrogel

217 Jacketed Franz diffusion cells (FDC) (Logan Instruments, New Jersey, USA) were used as
218 previously annotated for determining the *ex vivo* drug permeation rate through porcine skin (Nayak
219 et al., 2013). Porcine ear skin was used in this analysis because of the histological similarity with
220 human skin. Dissected square skin sections (20 x 20 mm) were defrosted at 25°C for a maximum
221 time of 1 hour before the commencement of this study. The FDC receptor chamber (5.0 ml) was
222 filled with deionised water and constantly stirred using a magnetic flea. The FDC receptor volume
223 was constantly maintained at 37 ± 1°C through a water jacket. A square section of full thickness skin
224 (subcutaneous fat and connective tissue removed) was placed on the top of the aperture surface of
225 diffusion cell with a diffusion area of 1.33cm². The average skin thickness was recorded in the range
226 of 760-787µm (± 25 µm). The continuous viscoelastic properties of skin are unlikely to allow for

227 microneedles to penetrate beyond 200 μm when considering 1500 μm needle length rollers
228 penetrating a depth of 150 μm (Roxhed et al., 2007; Badran et al., 2009). The lidocaine NaCMC:gel
229 hydrogel ($0.10 \pm 0.03\text{g}$) was placed on to the skin's donor compartment, the split second timer
230 initiated and then the skin was securely clamped with a donor lid. A fixed 1.5ml receptor volume was
231 syringe removed periodically from the receptor chamber and replaced with 1.5ml of deionised water.
232 Following this the samples were analysed for free lidocaine using HPLC instrument (Agilent 1100
233 series, Hewlett Packard, U.S.). Similar FDC method was used for all drug release experiments
234 concerning passive diffusion, microneedles only pre-treatment, LFS only pre-treatment,
235 microneedles and LFS pre-treatment. Microneedles were carefully applied to the skin ensuring
236 penetration and held in place using a constant pressure device comprising of a pneumatic piston
237 (0.05MPa) for 3 or 5 minutes. LFS was supplied using a probe set to 20 kHz frequency for 5-10
238 minutes. Continuous application of ultrasound was implemented due to no significant difference
239 being observed during pre-treatment applications (Herwadkar et al., 2012). The inter-coupling
240 distance between the skin and probe was set to 2mm with coupling medium of deionised water. A
241 minimum lidocaine concentration of 1.5 $\mu\text{g/ml}$ was deduced from literature as the permissible
242 effective drug therapeutic value in plasma (Schulz et al., 2012; Grossman et al., 1969).

243

244 *2.8 Spreading of lidocaine NaCMC:gel 1:2.33 across porcine skin*

245 The setup for measurement of spreading radius, droplet height and apparent contact angle of
246 droplet was similar to Chao et al (2014). A square section of porcine skin (10 mm x 10 mm) was
247 placed flat in a closed sample box. A sample droplet ($3.0 \pm 0.5\mu\text{l}$) was dispensed on the porcine
248 skin, camera frame rate capture of 1.85 frames per second (fps) was maintained and the results
249 recorded. Results were obtained in duplicate for the optimum particle size controlled formulation and
250 compared with a duplicate set of lidocaine solution of the same lidocaine loading weight (2.44 % wt).

251

252 *2.9 Histological study*

253 The determination of microneedle insertion depth into skin by post microneedle treatment of skin
254 was adapted from Cheung et al (2014). First, the skin sample is pretreated using 1100 μm
255 microneedle patch for 5 min. Then, the porcine skin sample is stained using methylene blue (50%
256 v/v) and merged into embedding compound (Bright Cryo-m-Bed, Huntingdon, UK) which is filled in a
257 cuboid mould. The whole sample is then put inside the microtome (Bright Cryostat 5030,
258 Huntingdon, UK) to solidify. The frozen sample is cut into 15 μm slices and analysed under the
259 microscope for the histology.

260

261 **3. Results and discussions**

262 *3.1. Lidocaine NaCMC/gel hydrogel microparticle size diameters and morphology*

263 Lidocaine encapsulated hydrogel microspheres based on NaCMC and gelatine were prepared using
264 glutaraldehyde in transforming emulsion droplets to defined microparticles. As the mechanisms for
265 ionic interactions in forming spherical microparticles are known (Gupta et al., 2000; Berger et al.,

266 2004), it is not discussed in detail in this paper. The morphological observations of lidocaine
267 NaCMC:GEL microparticles are spherical, well-formed and slightly agglomerated for a significant
268 number of them (Fig. 1a, 1b). Mean particle size diameters (Table 1) in the formulation ranged from
269 5.89-14.60 μm depending on the formulation with an increase in mean particle size observed with an
270 increased gelatine ratio. This is the likelihood of increased gelatine component of the hydrogel,
271 producing larger droplets during the w/o emulsification and subsequent hardening after the addition
272 of glutaraldehyde. The rotary evaporation method yielded significantly larger particle sizes in
273 comparison to vacuum drying. Interestingly, a positively skewed particle size distribution was
274 observed for all lidocaine hydrogel formulations (Fig. 2).

275  Fig. 1

276  Fig. 2

277

278 3.2. Dispersion of lidocaine NaCMC/gel hydrogel microparticles

279 Zeta potential studies in lidocaine NaCMC/gel hydrogels demonstrated a stable and fairly dispersed
280 microparticulate system. The results (Fig. 3) expressed a trend of decreasing stability with an
281 increase in the gelatine ratio, which in theory should impact a greater level of microparticle
282 agglomeration thus likely affecting the permeability through skin. The pH of all formulations was kept
283 constant and therefore it should not have affected the zeta potential although the slight decline of ζ -
284 potential in the positive direction is linked to the increase in gelatine ratio caused by gelatine in
285 conjunction to lidocaine possessing a positively charged tertiary amide group at pH 4.0 and thus
286 contributing to the increasing negative surface charge. The anionic polymer, sodium carboxymethyl
287 cellulose has a ζ -potential value of -30mV (Ducel et al., 2004) and electric charge neutralisation did
288 not occur or was not significantly induced by gelatine or lidocaine, so the overall lidocaine
289 NaCMC:gel hydrogel charge was greater than -30mV. Nevertheless, reduced agglomeration is the
290 result of a medium pKa, higher dielectric constants in comparison to a polymeric hydrogel
291 components converging to significantly low overall ζ -potential range of -35 to -40mV and effect of
292 electrostatic particle repulsion (Xu et al., 2007).

293  Fig. 3

294

295

296 3.3. Viscoelasticity of lidocaine NaCMC/gel hydrogel

297 Viscosity determination (Fig. 4) revealed a lenient pseudoplastic nature for the formulation with
298 lidocaine NaCMC/gel hydrogel with good correlative best fit curves observed for individual set of
299 data points ($R^2 > 0.93$). The dynamic viscosity plots showed similar mild pseudoplastic behaviour
300 between the formulations with lidocaine NaCMC/gel 1:2.66 hydrogel being marginally higher when
301 considering the upper viscosity range of 0.5 to 0.6 Pa.s at a starting shear of 25 s^{-1} and then more
302 defined shear thinning behaviour observed above 100 s^{-1} . Lidocaine with sodium
303 carboxymethylcellulose as a polyanionic vehicle alone will not be sufficient in enhancing

304 pseudoplastic properties and a recent study has shown that the profile of a dynamic viscosity plot is
 305 Newtonian (Alaie, 2013).

306 Fig. 4

307
 308 *3.4 Control of lidocaine NaCMC/gel 1:2.33 spreading on porcine skin.*

309 The spreading radius and height of lidocaine NaCMC/gel 1:2.33 outline significant control on its
 310 spreading behaviour compared with lidocaine solution of the same mass loading (Fig. 5a and 5b).
 311 The beginning of the plateau effect is observed after 10 seconds and therefore, there is expected to
 312 be a localisation effect on the skin surface (Fig. 5a and 5b). The apparent contact angles of
 313 lidocaine NaCMC/gel 1:2.33 droplets are considerably higher than the lidocaine solution contact
 314 angle droplets, near to the skin impact time of 0 seconds (Fig 5b). Apparent contact angle stability is
 315 noticed after 40 seconds (Fig. 5c). Our results also show that the lidocaine solution is a Newtonian
 316 liquid that can spread a much faster than lidocaine NaCMC/gel microparticles.

317 Fig. 5

318
 319 *3.5. The percentage release of lidocaine from controlled release of lidocaine.*

320 All four lidocaine NaCMC/gel hydrogels outline rapid release of lidocaine directly in DI water during
 321 the first 1 hour with steady state conditions observed in the next three hours (Fig. 6a). A 0.3 fold
 322 decrease in cumulative release is observed in the first hour when comparing lidocaine NaCMC/gel
 323 1:1.6 with lidocaine NaCMC/gel 1:2.66 as the highest releasing outline. Also, a 0.1 fold decrease in
 324 cumulative release was observed in the next three hours when comparing lidocaine NaCMC/gel
 325 1:1.6 with lidocaine NaCMC/gel 1:2.66. This shows that the variation between hydrogel ratios is not
 326 significantly large as permeation release profiles explained in the following sections. The percentage
 327 release of lidocaine from NaCMC/gel hydrogels were determined by the following equation 1:

$$328 \text{ Percentage drug release} = \frac{M_s - M_t}{M_s} \times 100 \quad 1)$$

329 Where M_s is the maximum mean cumulative steady state concentration of drug and M_t is the mean
 330 cumulative concentration of lidocaine taken specifically at release time. The highest amount of
 331 Lidocaine released was from NaCMC/gel 1:1.6 hydrogel in which 32.3% was detected in the DI
 332 water media in one hour (Fig. 6b). This is because the smaller particles sizes of Lidocaine
 333 NaCMC/gel 1:1.6 ratio allow for a greater surface area and encapsulated lidocaine thus rapidly
 334 dissolves in DI water. The lidocaine NaCMC/gel 1:2.66 ratio comprises larger microparticles and
 335 therefore a smaller surface area is exposed for DI water dissolution so the percentage of lidocaine
 336 released was 17.4% in one hour. Significantly less amounts of lidocaine is released for all
 337 NaCMC/gel hydrogel formulations after 1 hour reflecting the steady state conditions of the hydrogel
 338 as the DI water media becomes a saturated solution.

Fig. 6

339
340

341 3.6 *Histological analysis on the microneedles*

342 The microneedles that are employed in the histological experiment are 1100 μm in length. The
343 purpose of the histological experiment is to determine the insertion depth of this microneedle patch
344 under thumb pressure for which post-microneedle treated skin is micrograph imaged (Fig. 7).
345 According to Fig.7, the insertion depth is between 300 μm and 400 μm which are much lower than
346 the real length of microneedles. This is caused by several reasons, such as the viscoelastic
347 properties of the skin, the geometry of the microneedles and the insertion force. This reduced
348 insertion depth can further affect the permeation results.

349
350

Fig. 7

351 3.7 *Passive diffusion of lidocaine NaCMC/gel hydrogel*

352 Skin passive diffusion experiments were carried out in order to provide a control from which any pre-
353 treatment enhancement results can be compared and contrasted. The lowest polymeric
354 microparticle ratio 1:1.6 of lidocaine (Fig. 8a) outlines the most desirable cumulative permeation for
355 lidocaine in crossing the minimum threshold therapeutic level after 0.57 hours. This is the shortest
356 lag time for reaching the pain receptors for lidocaine in the deep dermis region rich in watery plasma
357 and nerves. The hydrogel microparticle chemistry is a combination of significantly high negative zeta
358 potential and smaller mean particle size contributing to an increased permeation. All lidocaine
359 NaCMC/gel ratio hydrogels have demonstrated a very low initial permeation at a maximum of
360 0.3 $\mu\text{g}/\text{ml}$ reached in 0.5 hours. This is the normal lag time because of a longer path length for
361 microparticle permeation when considering the topmost SC layer surface area bigger than the
362 accessible VE layer microcavities. However, lidocaine NaCMC/gel 1:2.0 and lidocaine NaCMC/gel
363 1:2.66 hydrogels are the next two favourables after the most desirable formulation containing a
364 polymeric mass ratio 1:1.6 for bypassing the minimum therapeutic threshold at a shorter time
365 interval. Initially, lidocaine is diffusing through the fresh skin because of microparticulate disruption
366 to the hydrogel formula caused by natural skin moisture hence the low initial concentration rates
367 proceeding upto 0.5 hours. Due to the requirements of lidocaine as an fast acting anaesthetic the
368 current results confirm enhancement of permeation is required if minimum therapeutic threshold of
369 lidocaine (1.5 $\mu\text{g}/\text{ml}$) are to be reached within a suitable time frame for this technique to be of
370 practical use. The lag time to cross a minimum therapeutic level is slightly greater than 1 hour in
371 lidocaine NaCMC/gel 1:2.33 hydrogel and just over 2 hours for lidocaine NaCMC/gel 1:2.66
372 hydrogel, rotary evaporation method with respect to passive diffusion alone which is considerably a
373 long, unreasonable waiting time for a promising polymeric hydrogel ointment drug. The cumulative
374 lidocaine thresholds tend to stabilise post 4 hours, where equilibrium is reached and no more drug is

375 released into the concentrated dermal region. This means that the lidocaine hydrogel ointment can
376 be washed off the skin. Lidocaine NaCMC/gel hydrogel was compared with lidocaine solution
377 permeation from literature (Sekkat et al., 2004). Prior to this passive diffusion comparison with
378 lidocaine solution passive diffusion, the permeation units of $\mu\text{g}/\text{ml}$ were converted into $\mu\text{g}/\text{cm}^2$ by the
379 product of the known receptor volume followed by the quotient of the adjustment factor value of 2.36
380 ($3.14 \text{ cm}^2/ 1.33 \text{ cm}^2$) due to the increase in FDC diffusion area when comparing a similar study
381 using a smaller aperture diameter (Sekkat et al., 2004). The current lidocaine NaCMC/gel 1:1.6
382 hydrogel crosses the minimum therapeutic threshold by 1.8 fold than lidocaine solution on similar
383 full thickness skin despite lidocaine solution permeating initially at 1.4 fold faster before a half an
384 hour time frame and not anywhere near the minimum therapeutic threshold (Sekkat, 2004).
385 Lidocaine NaCMC/gel 1:2.66 and lidocaine NaCMC/gel 1:2.66 hydrogel formulated by rotary
386 evaporation were chosen to be studied for further enhancement via pre-treatment. The factor of
387 permeation enhancement can be deduced when making this comparison.

Fig. 8

391 3.8 Ultrasound only pre-treatment of lidocaine NaCMC/gel ratio 1:2.66 hydrogel

392 To observe the effect of power and application time of LFS has on permeation, LFS was applied
393 continuously with varying power and exposure time as shown in Fig. 8b. Theoretically, the exposure
394 of LFS should form inertial cavities in the coupling medium and develop micro-jets toward the skin
395 surface to aid permeation. However, lidocaine transport through the skin saw no significant
396 enhancement up to 2 hours after which a significant enhancement, especially power induction, 18W
397 at 10 mins for lidocaine NaCMC/gel 1:2.66 (T-test $P < 0.026$) outlined a greater permeation profile.
398 The results conclude that an increase in power has a greater enhancement effect compared to an
399 increase in LFS exposure time; however, no significant increase in lidocaine transport through the
400 skin was observed during the initial stages after varying respective power induction and time
401 durations while maintaining constant NaCMC/gel ratios of lidocaine hydrogel drug application. It is
402 predicted that a higher LFS power level would further increase diffusion; however, the risk of
403 thermal effects would be too high for this to be of practical use.

404 3.9 Microneedle pre-treatment of lidocaine NaCMC/gel ratio 1:2.66 hydrogel

405 PD permeation (Fig. 8d) and microneedle assisted (MN) permeation (Fig. 8c) with a post application
406 time limit of 3 and 5 minutes concurrently were compared altogether. Microneedle only pre-
407 treatment of lidocaine NaCMC/gel 1:2.66 hydrogel generated a substantial increase in lidocaine
408 permeation for both the 3 and 5 minute post MN duration (Fig. 8c). A statistically significant
409 difference ($P < 0.04$) was observed for MN application duration. Initial ($t = 0.5\text{h}$) permeation for the 3
410 and 5 minute patch duration resulted in increases of 9 and 17 fold respectively. An average 3 fold
411 increase in permeation was observed for the 3 minute microneedle application and comparatively
412 an increase by 4 fold for a 5 minute microneedle application. The results indicate that therapeutic

413 levels of lidocaine could be reached within 0.15 hours or 9 minutes post application MN, in
414 comparison to no pre-treatment requiring 40 minutes (Fig. 8c, 8d). The reason for this short lag time
415 is due to lidocaine microparticles traveling at a shorter path length to the deep dermis layer. The
416 stratum corneum layer has been bypassed by artificial microneedle cavities. Microneedle assisted
417 cumulative release study with respect to lidocaine formulations has not been performed *ex vivo* to
418 date. However, *in vivo* release studies have been performed using non degrading polymeric
419 microneedle array coating of lidocaine alone, sustained approximately 15 minutes of delivery thus
420 proven successful for rapid emergency anaesthesia (Zhang et al., 2012). *In vivo* release studies
421 with *ex vivo* cumulative release studies are completely incomparable due to obvious differences in
422 experimental procedures and removal of active drug for characterisation. The lidocaine NaCMC/gel
423 1:1.6 ratio (Fig. 8a) hydrogel crosses the therapeutic level at significantly slower time duration,
424 greater than 30 minutes in lidocaine NaCMC/gel 1:2.66 ratio in comparison of microneedle and LFS
425 treatment. This is due to the fact that microneedles and ultrasound are involved in either cavity
426 engulfing of larger sized hydrogel microparticles.

427 428 *3.10 Microneedle and ultrasound (dual) pre-treatment of lidocaine NaCMC/gel ratio 1:2.66 hydrogel*

429 Both pre-treatments (dual) were combined and studied for further permeation enhancement in
430 comparison to microneedle or LFS pre-treatment only. Lidocaine NaCMC/gel 1:2.66 hydrogel in
431 which combining a 10 minute application of 18W LFS after a 5 minute application of microneedles
432 demonstrated an initial faster permeation by 23 fold with an average 4.8 fold increase over 30
433 minutes of application when compared with separate device treatments and passive diffusion (Fig.
434 8d). Therapeutic levels of lidocaine could theoretically be reached after 7 minutes post application in
435 terms of reaching the deep dermis layer of skin as the target. A general increase in permeation
436 throughout the period of experimentation can be noticed rather than post 2 hours as seen with LFS
437 pre-treatment only, this could be due to efficiency of LFS pre-treatment is further enhanced on
438 porous skin sample formed via the microneedle patch.

439 440 *3.11 Dual pre-treatment of lidocaine NaCMC/gel 1:2.66 hydrogel via rotary evaporation method*

441 Lidocaine NaCMC/gel 1:2.66 hydrogel with the rotary evaporation method as described earlier,
442 favoured an additional time of nearly 0.9 hour or fifty minutes after the application of 18W LFS at 10
443 minutes ($P < 0.04$) to reach minimum therapeutic level in conjunction to a two fold average increase
444 in permeation after 1 hour, compared with the same formulation without rotary evaporation method
445 (Fig. 8d, 8e). This was the likelihood of higher heating temperatures compromising the
446 glutaraldehyde fixation and thus resulting in larger microparticle as previously reported. Higher
447 heating temperatures were required in the large volume removal of n-hexane and paraffin oil
448 mixture by solvent evaporation. A 5 minute application of the microneedle array led to an initial
449 increase by 2.8 fold and subsequently an average 3.4 fold increase was observed with respect to
450 the deep dermis layer skin target. Combining the two pre-treatments resulted in an initial permeation

451 increase by 3.8 fold followed by an average increase by 4.1 fold in comparison to passive diffusion
452 only (Fig. 8d, 8e). Therapeutic levels of lidocaine were reduced from just over 2 hours to less than 1
453 hour on average.

454 3.12 Mass transfer of lidocaine from NaCMC/gel 1:2.66 hydrogel

455 The percentage of lidocaine remaining inside *ex vivo* skin was determined by the subtraction of the
456 mass of lidocaine initially encapsulated during formulated preparation (125000 μg) by the
457 cumulative amount detected in DI water from controlled release studies. The purpose of using
458 controlled release studies is to determine the amount of lidocaine contained in the vehicle as mass
459 balance before the subtraction of the mass of lidocaine in the receptor in which the DI water in the
460 receptor is the deep dermis. All mass balances were carried out in μg and converted from
461 cumulative concentration units of $\mu\text{g}/\text{ml}$ before the percentage of lidocaine remaining inside the skin
462 was determined (Fig. 9). Overall the mass transfer of lidocaine with respect to all treatment
463 applications appeared to outline a gradual, slow process of diffusing through the full thickness
464 appendage. However there is a fairly substantial decline in the percentage of lidocaine remaining in
465 the skin when microneedle and ultrasound treatment (LFS) method was applied. This can be
466 interpreted as diffusion of lidocaine molecules through skin cells and layers before clearance into
467 the blood stream. The lowest percentage of lidocaine remaining in the skin is 99.7 % after a time of
468 3 hours (Fig. 9).

469  Fig. 9

470 4 Conclusions

471 This study aimed to use low frequency sonophoresis and microneedles as a pre-treatment to skin in
472 order to enhance permeation of lidocaine encapsulated in a formulation. A significantly more
473 microparticle stability was found with lower gelatine ratios (1:1.60); however all formulations were
474 sufficiently stable (zeta potential: $\geq -30\text{mV}$). Our diffusion experiments revealed a small increase in
475 diffusional permeation when low frequency sonophoresis was used in combination with a
476 microneedle array pre-treated skin. However, rotary evaporation during the final polymeric drug
477 formulation stage caused significant reductions in lidocaine permeation levels. Nota bene that the
478 main purpose for utilising rotary evaporation was for reduced time in removal of a large volume of
479 residual paraffin and n-hexane as the final operative method compared to vacuum oven drying (data
480 not shown). Lidocaine NaCMC/gel 1:2.66 and lidocaine NaCMC/gel hydrogel 1:2.66 formulated by
481 rotary evaporation showed a decreased time required to reach minimum therapeutic levels of
482 lidocaine by 5.7 and 2 fold, respectively. Generally, lidocaine permeation was significantly increased
483 with higher sonophoresis power and increasing exposure duration demonstrated a minor increase of
484 the permeation rate for lidocaine NaCMC/gel hydrogel formulations. Also the microneedle
485 application time duration of 5 minutes resulted in a highly favourable increase in lidocaine
486 permeation. Furthermore, combining microneedle and low frequency sonophoresis pre-treatments
487 allowed for the time to reach minimum therapeutic lidocaine levels to be significantly reduced, For

488 example, in the case of lidocaine NaCMC/gel, 1:2.66 hydrogel therapeutic thresholds of lidocaine
489 were reached within 7 minutes of application. The mass transfer effects in which the percentage of
490 lidocaine remained in the full skin depicted the gradual movement of drug in targeting pain receptors
491 below the SC layer. The lidocaine NaCMC/gel 1:2.66 hydrogel treated by microneedles and LFS
492 shows a greater mass transfer profile. The US and MN treated lidocaine NaCMC/gel 1:2.66 has a
493 0.18 % mass transfer of lidocaine through skin within 2 hours compared with 0.01 % mass transfer
494 of lidocaine through skin. Therefore, this method is promising and could be of medical use as a
495 painless, easy to administer technique for drug delivery overcoming the time constraints associated
496 with delivery of lidocaine. Lidocaine NaCMC/gel 1:2.66 hydrogel is likely to be the most desirable
497 drug formulation candidate for further developmental studies reaching potentially important pre-
498 clinical and final post clinical stage developments. In order to develop a less polydisperse but low
499 micron scale lidocaine hydrogel formulation requires a longer time frame and added investment.
500 The resources and materials in developing a lidocaine NaCMC/gel 1:2.66 hydrogel without rotary
501 evaporation is economical on a batch scale at present. Lidocaine. NaCMC/gel 1:2.33 formulation
502 with defined morphological appearance is able to remain on the surface of the skin for longer
503 durations compared with a lidocaine solution of the same mass loading.

504 5. Acknowledgements

505 The authors acknowledge the help of Craig Chao for his assistance towards the characterisation of
506 droplet spreading on skin (Figure 5).

507

508 6. References

- 509 Alaie, J., Vasheghani-Farahani, E., Rahmatpour, A., Semsarzadeh, MA. (2013). Gelation rheology
510 and water absorption behavior of semi-interpenetrating polymer networks of polyacrylamide
511 and carboxymethyl cellulose. *Journal of Macromolecular Science, Part B*, 52, 604-613.
- 512 Al-Qallaf, B., Das, D.B. (2009). Optimizing microneedle arrays to increase skin permeability for
513 transdermal drug delivery. *Annals of the New York Academy of Sciences*, 1161, 83-94.
- 514 Al Qallaf, B and Das, DB (2008) Optimization of square microneedle arrays for increasing drug
515 permeability in skin, *Chemical Engineering Science*, 63, 2523-2535, DOI:
516 10.1016/j.ces.2008.02.007.
- 517 Badran M.M, Kuntsche J., Fahr A. (2009) Skin penetration enhancement by microneedle device
518 (Dermaroller®) in vitro: Dependency on needle size and applied formulation. *European Journal*
519 *of pharmaceutical Sciences*, 36, 511-523.
- 520 Bal, S.M., Ding, Z., van Riet, E., Jiskoot, W., and Bouwstra, J.A. (2010). Advances in
521 transcutaneous vaccine delivery: Do all ways lead to Rome? *Journal of Controlled Release*,
522 148, 266-282.
- 523 Banks, S.L., Paudel, K.S., Brogden, N.K., Loftin, C.D., and Stinchcomb, A.L. (2013). Diclofenac
524 enables prolonged delivery of naltrexone through microneedle-treated skin. *Pharmaceutical*
525 *Research*, 28, 1211-1219.

- 526 Benet, L.Z., Oie, S., and Schwartz, J.B. (1996). Design and optimisation of dosage regimens:
527 pharmacokinetic data. In J.G. Hardman, L.E. Limbard, P.B. Molinoff, R.W. Rudon, and A.G.
528 Gilman (Eds.), *The pharmacological basis of therapeutics* (pp. 1707-1792). McGraw Hill, New
529 York.
- 530 Berger, J., Reist, M., Mayer, J.M., Felt, O., Peppas, N.A., and Gurny, R. (2004). Structure and
531 interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical
532 applications. *European Journal Pharmaceutics and Biopharmaceutics*, 57, 19-34.
- 533 Chao TZ, Trybala A., Starov V., Das D.B. (2014). Influence of haematocrit level on the kinetics of
534 blood spreading on thin porous medium during blood spot sampling. *Colloids and Surfaces A:
535 Physicochemical and Engineering Aspects*, 451, 38-47.
- 536 Chen, B., Wei, J., and Iliescu, C. (2010). Sonophoretic enhanced microneedles array (SEMA)—
537 Improving the efficiency of transdermal drug delivery. *Sensors and Actuators B: Chemical*, 145,
538 54-60.
- 539 Cheung K., Han T., Das D.B. (2014) Effect of force of microneedle insertion on the permeability of
540 insulin in skin. *Journal of Diabetes Science and Technology*. DOI: 10.1177/1932296813519720
541 (in press).
- 542 Chow KT, Chan LW, Heng PWS (2008) Characterization of spreadability of nonaqueous
543 ethylcellulose gel matrices using dynamic contact angle. *Journal of Pharmaceutical Sciences*
544 97: 3467-3481.
- 545 Davidson, A., Al-Qallaf, B., and Das, D.B. (2008). Transdermal drug delivery by coated
546 microneedles: Geometry effects on effective skin thickness and drug permeability. *Chemical
547 Engineering Research and Design*, 86, 1196-1206.
- 548 De Boer, A.G., Breimer D.D., Mattie, H., Pronk, J., and Gubbens-Stibbe, J.M. (1979). Rectal
549 bioavailability of lidocaine in man: partial avoidance of "first-pass" metabolism. *Clinical
550 pharmacology and therapeutics*, 26, 701-709.
- 551 Ducel, V., Richard, J., Saulnier, P., Popineau, Y., and Boury, F. (2004). Evidence and
552 characterization of complex coacervates containing plant proteins: application to the
553 microencapsulation of oil droplets. *Colloids Surface A*. 232, 239–247
- 554 Ebrahimi, S., Abbasnia, K., Motealleh, A., Kooroshfard, N., Kamali, F., and Ghaffarinezhad, F.
555 (2012). Effect of lidocaine phonophoresis on sensory blockade: pulsed or continuous mode of
556 therapeutic ultrasound? *Physiotherapy*, 98, 57-63.
- 557 Escobar-Chávez, J.J., Rodríguez-Cruz, I.M., and Domínguez-Delgado, C.L. (2012). Chemical and
558 physical enhancers for transdermal drug delivery. In Dr Luca Gallelli (Ed), *Pharmacology*,,
559 ISBN: 978-953-51-0222-9
- 560 Fasinu, P., Viness Pillay, V., Ndesendo, V.M.K., Du Toit, L.C., and Choonara Y.E. (2011). Diverse
561 approaches for the enhancement of oral drug bioavailability. *Biopharmaceutics and drug
562 disposition*, 32, 185-209
- 563 Fen-Lin, W., Razzaghi A., and Souney, P.F. (1993). Seizure after lidocaine for bronchoscopy: case
564 report and review of the use of lidocaine in airway anesthesia. *Pharmacotherapy*, 13, 72-78

- 565 Ferrari, M., Desai, T., and Bhatia, S. (2007). *BioMEMS and Biomedical Nanotechnology*. Vol. 3.
566 New York: Springer.
- 567 Gill, H.S., and Prausnitz, M.R. (2007). Coated microneedles for transdermal delivery. *Journal of*
568 *Controlled Release*, 117, 227-237.
- 569 Giudice E.L., and Campbell, J.D. (2006). Needle-free vaccine delivery. *Advanced Drug Delivery*
570 *Reviews*, 58, 68-89.
- 571 Ghosh, P., Brogden, N.K., and Stinchcomb, A.L. (2013a). Effect of formulation pH on transport of
572 naltrexone species and pore closure in microneedle-enhanced transdermal drug delivery.
573 *Molecular Pharmaceutics*, 10, 2331-2339.
- 574 Ghosh, P., Pinninti, R.R., Hammell, D.C., Paudel, K.S., and Stinchcomb, A.L. (2013b). Development
575 of a codrug approach for sustained drug delivery across microneedle-treated skin. *Journal of*
576 *Pharmaceutical Sciences*, 102, 1458-1467.
- 577 González-Rodríguez, M.L., Barros, L.B., Palma, J., González-Rodríguez, P.L., and Rabasco A.M.
578 (2007). Application of statistical experimental design to study the formulation variables
579 influencing the coating process of lidocaine liposomes. *International Journal of Pharmaceutics*,
580 337, 336-345.
- 581 Grossman J.I., Cooper J.A., Frieden J. (1969) Cardiovascular effects of infusion of lidocaine on
582 patients with heart disease. *The American Journal of Cardiology*, 24, 191-197.
- 583 Guo, L., Qiu, Y., Chen, J., Zhang, S., Xu, B., and Gao, Y. (2013). Effective transcutaneous
584 immunization against hepatitis B virus by a combined approach of hydrogel patch formulation
585 and microneedle arrays. *Biomedical Microdevices*. DOI 10.1007/s10544-013-9799-z.
- 586 Gupta, K.C., and Ravi Kumar, M.N.V. (2000). Semi-interpenetrating polymer network beads of
587 crosslinked chitosan–glycine for controlled release of chlorpheniramine maleate. *Journal of*
588 *Applied Polymer Science*, 76, 672–683.
- 589 Han, T., and Das, D.B. (2013). Permeability enhancement for transdermal delivery of large molecule
590 using low frequency sonophoresis combined with microneedles, *Journal of Pharmaceutical*
591 *Sciences*, 102 (10), 3614-3622
- 592 Hedge, N.R., Kaveri, S.V., and Bayry, J. (2011). Recent advances in the administration of vaccines
593 for infectious diseases: microneedles as painless delivery devices for mass vaccination. *Drug*
594 *discovery today*, 16, 1061-1068.
- 595 Herwadkar, A., Sachdeva, V., Taylor, L.F., Silver, H., and Banga, A.K. (2012). Low frequency
596 sonophoresis mediated transdermal and intradermal delivery of ketoprofen. *International*
597 *Journal of Pharmaceutics*, 423, 289-296.
- 598 Huet, P.M., and Lelorier, J. (1980). Effects of smoking and chronic hepatitis B on lidocaine and
599 indocyanine green kinetics. *Clinical pharmacology and therapeutics*, 28, 208-215.
- 600 Hynynen, K. (2010). MRI-guided focused ultrasound treatments, *Ultrasonics*, 50, 221–229
- 601 Igaki, M., Higashi, T., Hamamoto, S., Kodama, S., Naito, S., and Tokukara, S. (2013). A study of the
602 behavior and mechanism of thermal conduction in the skin under moist and dry heat
603 conditions. *Skin Research and Technology*, 0, 1-7.

- 604 Ito, Y., Ohta, J., Imada, K., Akamatsu, S., Tsuchida, N., Inoue, G., Inoue, N., Takada, K.
605 Dissolving microneedles to obtain rapid local anesthetic effect of lidocaine at skin tissue
606 (2013) *Journal of Drug Targeting*, 21, 770-775.
- 607 Kalluri, H, and Banga, A. (2011). Transdermal delivery of proteins. *AAPS PharmSciTech*, 12, 431-
608 441.
- 609 Kim, Y., Park, J., and Prausnitz, M.R. (2012). Microneedles for drug and vaccine delivery. *Advanced*
610 *Drug Delivery Reviews*, 64, 1547-1568.
- 611 Kochhar, J.S., Lim, W.X.S., Zou, S., Foo, W.Y., Pan, J., Kang, L.
612 Microneedle integrated transdermal patch for fast onset and sustained delivery of lidocaine
613 (2013) *Molecular Pharmaceutics*, 10, 4272-4280.
- 614 Kwon, S.Y. (2004). In vitro evaluation of transdermal drug delivery by a micro-needle patch.
615 *Controlled Release Society 31st Annual Meeting Transactions. TheraJect Inc.* no. 115.
- 616 Lastumäki, T.M., Lassila, L.V.J., and Vallittu, P.K. (2003). The semi-interpenetrating polymer
617 network matrix of fibre-reinforced composite and its effect on the surface adhesive properties.
618 *Journal of Materials Science-Materials in Medicine*, 14, 803-809.
- 619 Li, X., Zhao, R., Qin, Z., Zhang, J., Zhai, S., Qiu, Y., Gao, Y., Xu, B., and Thomas, S.H. (2010).
620 Microneedle pretreatment improves efficacy of cutaneous topical anesthesia. *American*
621 *Journal of Emergency Medicine*, 28, 130-134.
- 622 Li, X.-G., Zhao, R.-S., Qin, Z.-L., Gao, Y.-H., Zhang, J., Zhai, S.-D., Xu, B.
623 Painless microneedle transdermal patch enhances permeability of topically applied lidocaine
624 (2008) *Chinese Journal of New Drugs*, 17 (7), pp. 597-601. Merino, G., Kalia, Y.N., Delgado-
625 Charro, M.B., Potts, R.O., and Guy, R.H. (2003). Frequency and thermal effects on the
626 enhancement of transdermal transport by sonophoresis. *Journal of Controlled Release*, 88,
627 85-94.
- 628 Milewski, M., and Stinchcomb, A. (2011). Vehicle composition influence on the microneedle-
629 enhanced transdermal flux of naltrexone hydrochloride. *Pharmaceutical Research*, 28, 124-
630 134.
- 631 Mitragotri, S. (2013). Devices for overcoming biological barriers: The use of physical forces to
632 disrupt the barriers. *Advanced Drug Delivery Reviews*, 65, 100-103.
- 633 Mitragotri, S., and Kost, J. (2004). Low-frequency sonophoresis: A review. *Advanced Drug Delivery*
634 *Reviews*, 56, 589-601.
- 635 Naik, A., Kalia, Y.N., and Guy, R.H. (2000). Transdermal drug delivery: overcoming the skin's barrier
636 function. *Pharmaceutical Science and Technology Today*, 3, 318-326.
- 637 Nayak, A., Das, D.B., and Vladisavljević, G.T. (2013). Microneedle assisted permeation of lidocaine
638 carboxymethylcellulose with gelatine co-polymer hydrogel. *Pharmaceutical Research*. DOI:
639 10.1007/s11095-013-1240-z (in press)
- 640 Nayak, A., and Das D. (2013). Potential of biodegradable microneedles as a transdermal delivery
641 vehicle for lidocaine. *Biotechnology Letters*, 35, 1351–1363

- 642 Olatunji, O, Das, DB, Nassehi, V (2012) Modelling transdermal drug delivery using microneedles:
643 Effect of geometry on drug transport behaviour, *Journal of Pharmaceutical Sciences*, 101(1),
644 pp.164-175, ISSN: 0022-3549. DOI: 10.1002/jps.22736.
- 645 Olatunji, O., Das, D.B., Garland, M.J., Belaid, L., and Donnelly, R.F. (2013). Influence of array
646 interspacing on the force required for successful microneedle skin penetration: theoretical and
647 practical approaches. *Journal of Pharmaceutical Sciences*, 102, 1209-1221.
- 648 Olatunji, O, Igwe, CC, Ahmed, AS, Alhassan, OA, Asieba, GO, **Das, DB** (2014) Microneedles from
649 fish scale biopolymer, *Journal of Applied Polymer Science*, DOI: 10.1002/app.40377 (in
650 press).
- 651 Park, N., Kwon, B., Kim, I.S., and Cho, J. (2005). Biofouling potential of various NF membranes with
652 respect to bacteria and their soluble microbial products (SMP): Characterizations, flux decline,
653 and transport parameters. *Journal of Membrane Science*, 258, 43-54.
- 654 Polat, B., Hart, D., Langer, R., and Blankschtein, D. (2011). Ultrasound-mediated transdermal drug
655 delivery: mechanisms, scope, and emerging trends. *Journal of Controlled Release*, 152, 330-
656 348.
- 657 Richards, N., and McMahon, S.B. (2013). Targeting novel peripheral mediators for the treatment of
658 chronic pain. *British Journal of Anaesthesia*, 111 (1), 46-51.
- 659 Roxhed, N., Gasser T.C., Griss, P. (2007) Penetration-enhanced ultrasharp microneedles and
660 prediction on skin interaction for efficient transdermal drug delivery. *Journal of*
661 *microelectromechanical systems*, 16, 1429-1440.
- 662 Rudin, N.J. (2013). Topical analgesics for chronic pain. *Current Physical Medicine and*
663 *Rehabilitation Reports*. DOI 10.1007/s40141-013-0028-8.
- 664 Scarfone, R.J., Jasani, M., and Gracely, E.J. (1998). Pain of Local Anesthetics: Rate of
665 Administration and Buffering. *Annals of Emergency Medicine*, 31 (1), 36-40
- 666 Schulz, M., Iwersen-Bergmann, S., Andresen, H., and Schmoldt, A. (2012). Therapeutic and toxic
667 blood concentrations of nearly 1,000 drugs and other xenobiotics. *Critical Care*, 16, R136
- 668 Sekkat, N., Kalia, Y.N., Guy, R.H. (2004). Porcine ear skin as a model for the assessment of
669 transdermal drug delivery to premature neonates. *Pharmaceutical Research*, 21, 1390-1397.
- 670 Shah, U.U., Roberts. M., Orlu Gul, M., Tuleu, C., and Beresford, M.W. (2011). Needle-free and
671 microneedle drug delivery in children: A case for disease-modifying antirheumatic drugs
672 (DMARDs). *International Journal of Pharmaceutics*, 416,1-11.
- 673 Shipton, E.A. (2012). Advances in delivery systems and routes for local anaesthetics. *Trends in*
674 *Anaesthesia and Critical Care*, 2, 228-233.
- 675 Sze, A., Erickson, D., Ren, L., Li, D. (2003). Zeta-potential measurement using the Smoluchowski
676 equation and the slope of the current-time relationship in electroosmotic flow. *Journal of*
677 *Colloid and Interface Science*, 261, 402-410.
- 678 Wilson, J.R., Kehl, L.J., Beiraghi, S.
679 (2008). Enhanced topical anesthesia of 4% lidocaine with microneedle pretreatment and
680 iontophoresis. *Northwest dentistry*, 87, 40-41.

- 681 Wolloch L, Kost J (2010) The importance of microjet vs shock wave formation in sonophoresis.
682 *Journal of Controlled Release*, 148, 204-211.
- 683 Xu, R., Wu, C., and Xu, H. (2007). Particle size and zeta potential of carbon black in liquid media.
684 *Carbon*. 45 :2806–2809
- 685 Zhang, Y., Brown, K., Siebenaler, K., Determan, A., Dohmeier, D., and Hansen, K. (2012).
686 Development of lidocaine-coated microneedle product for rapid, safe, and prolonged local
687 analgesic action. *Pharmaceutical Research*, 29, 170-177.
- 688 Zhang, Y., Siebenaler, K., Brown, K., Dohmeier, D., and Hansen, K. (2012). Adjuvants to prolong
689 the local anesthetic effects of coated microneedle products. *International Journal of*
690 *Pharmaceutics*, 439, 187-192.
- 691 Zhang, D, Das, DB, Rielly, CD (2014) Potential of microneedle-assisted micro-particle delivery by
692 gene guns: a review, *Drug Delivery*, DOI: 10.3109/10717544.2013.864345.
- 693 Zhao, Y., Kang, J., and Tan, T. (2006). Salt-, pH- and temperature-responsive semi-interpenetrating
694 polymer network hydrogel based on poly(aspartic acid) and poly(acrylic acid). *Polymer*, 47,
695 7702-7710.

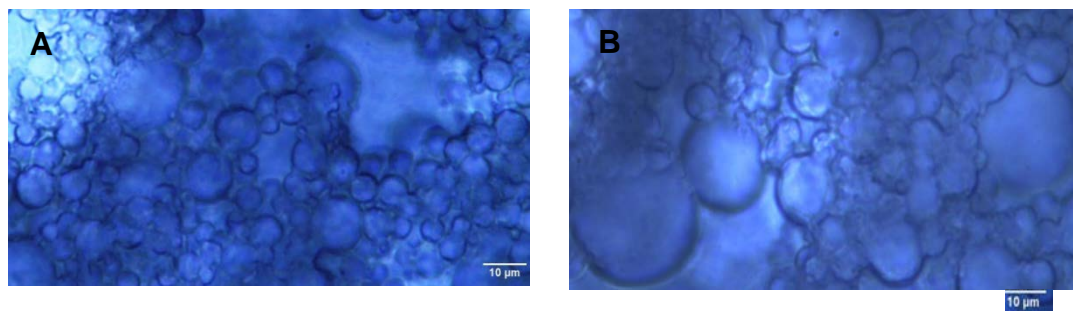
Table 1

Lidocaine NaCMC/gel hydrogel mass ratio with particle size values

| Sample ID | NaCMC (% w/v) | Gelatine (c % w/w) | Lidocaine (% w/w) | NaCMC:Gelatine ratio | Drier Type | Mean Particle Diameter \pm S.D. (μm) | Particle Diameter range (μm) |
|-----------|---------------|--------------------|-------------------|----------------------|------------|---|---|
| F1 | 1.2 | 2.0 | 2.4 | 1:1.6 | Vacuum | 5.89 \pm 0.0026 | 1 - 13 |
| F2 | 1.2 | 2.4 | 2.4 | 1:2.00 | Vacuum | 6.04 \pm 0.0027 | 1 - 14 |
| F3 | 1.2 | 2.8 | 2.4 | 1:2.33 | Vacuum | 6.81 \pm 0.0029 | 2 - 17 |
| F4 | 1.2 | 3.2 | 2.4 | 1:2.67 | Vacuum | 7.42 \pm 0.0029 | 3 - 17 |
| F5 | 1.2 | 3.2 | 2.4 | 1:2.67 | Rotary | 14.60 \pm 0.0067 | 4 - 31 |

696

697



698

699

700

701

Fig. 1. Micrograph of **a.** lidocaine NaCMC:gel 1:2.33 hydrogel showing distinctly formed microparticles.
b. lidocaine NaCMC:gel 1:2.66 hydrogel showing larger and slightly more agglomerated microparticles.

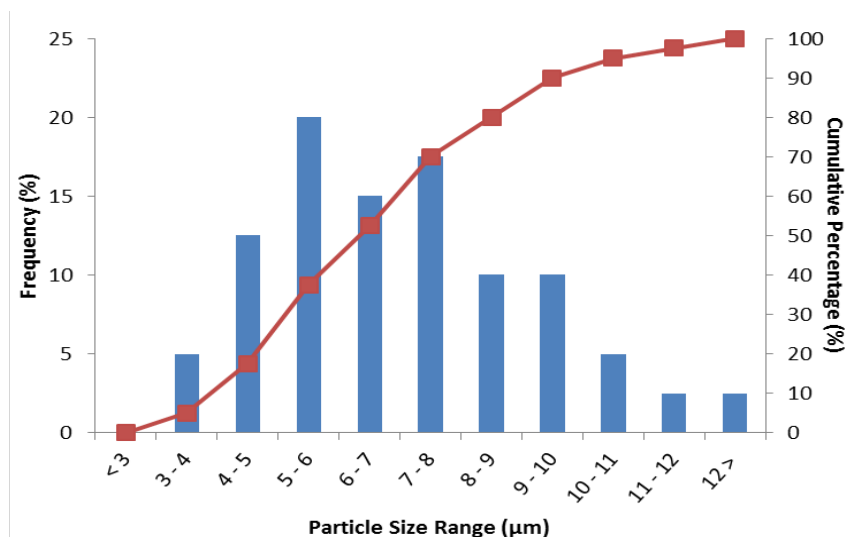


Fig. 2. Particle size distribution of Lidocaine NaCMC/gel hydrogels

702

703

704

705

706

707

708

709

710

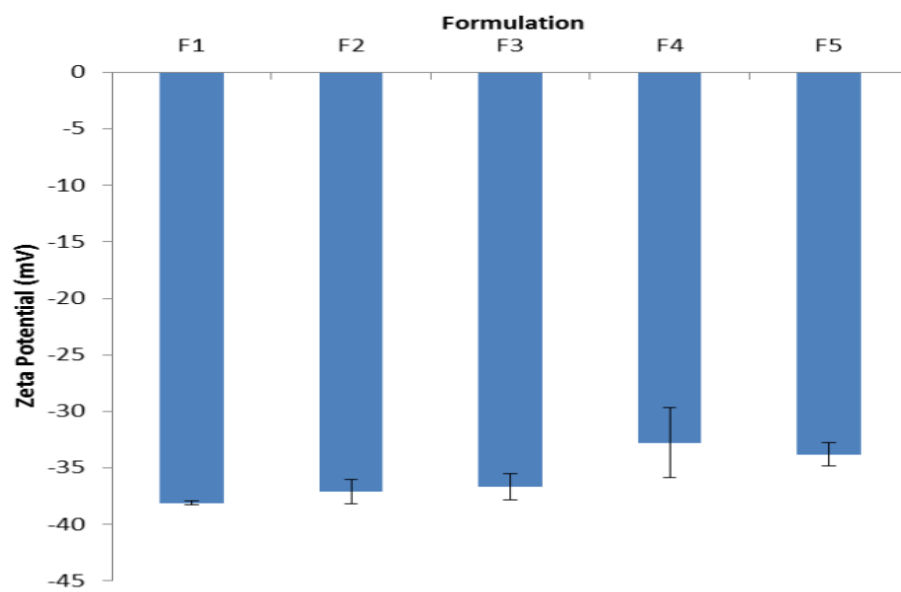


Fig. 3. Lidocaine NaCMC/GEL 1:1.6 to 1:2.66 (F1 to F4) and lidocaine NaCMC/GEL1:2.66 by rotary evap prep. (F5) for zeta potential

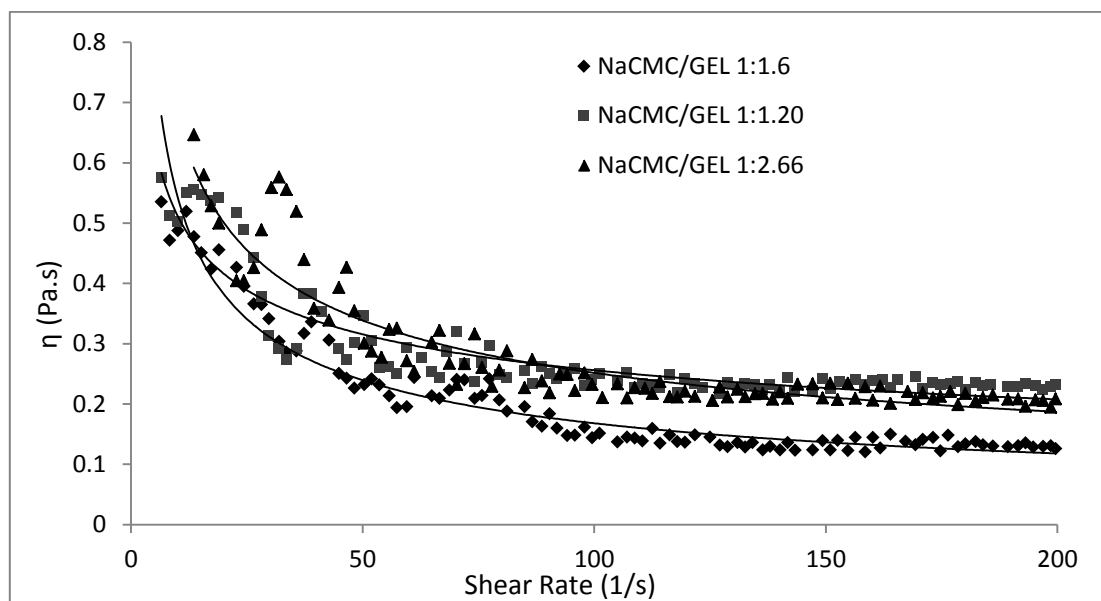


Fig. 4. Lidocaine 2.44 % w/w NaCMC/GEL ratio pseudoplasticity

712

713

714

715

716

717

718

719

720

721

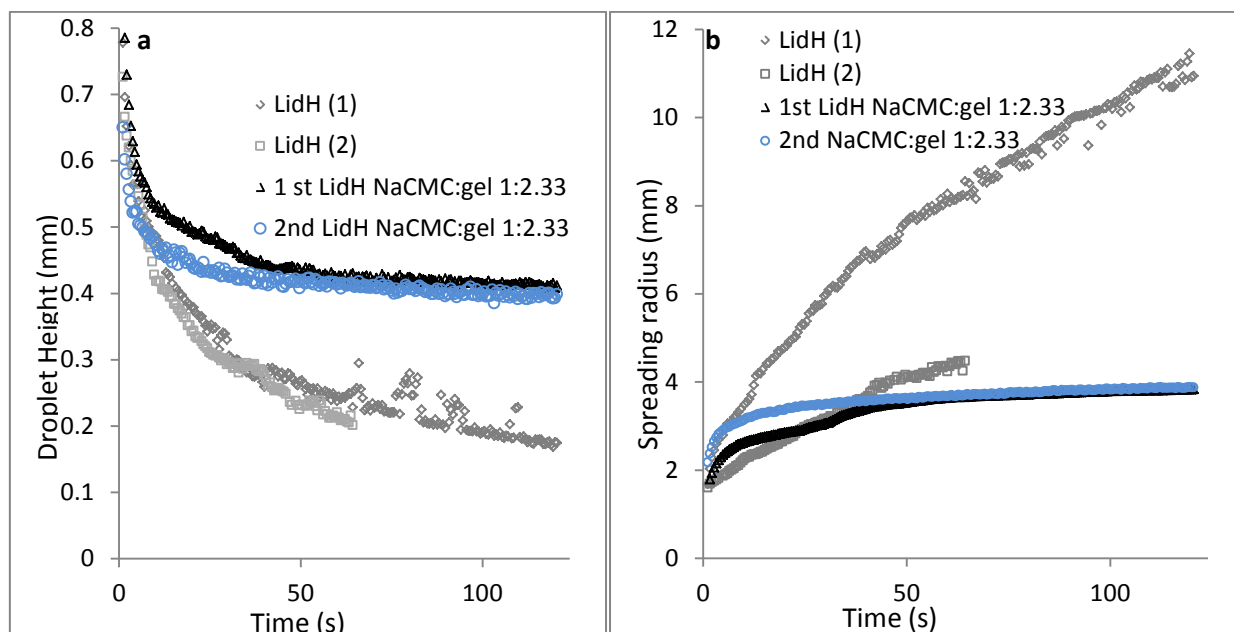
722

723

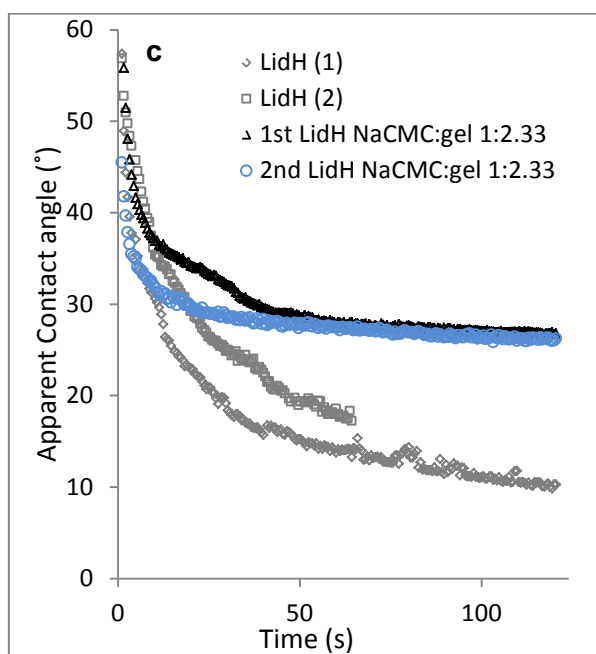
724

725

726



727



728

729 **Fig. 5** Lidocaine NaCMC/gel 1:2.33 comparison with Newtonian lidocaine solution according to **a.** droplet heights **b.**
 730 spreading radii **c.** apparent contact angles. The results suggest that the spreading of lidocaine NaCMC/gel 1:2.33 on
 731 the skin surface is much more predictable/controllable as compared to lidocaine solution.

732

733

734

735

736

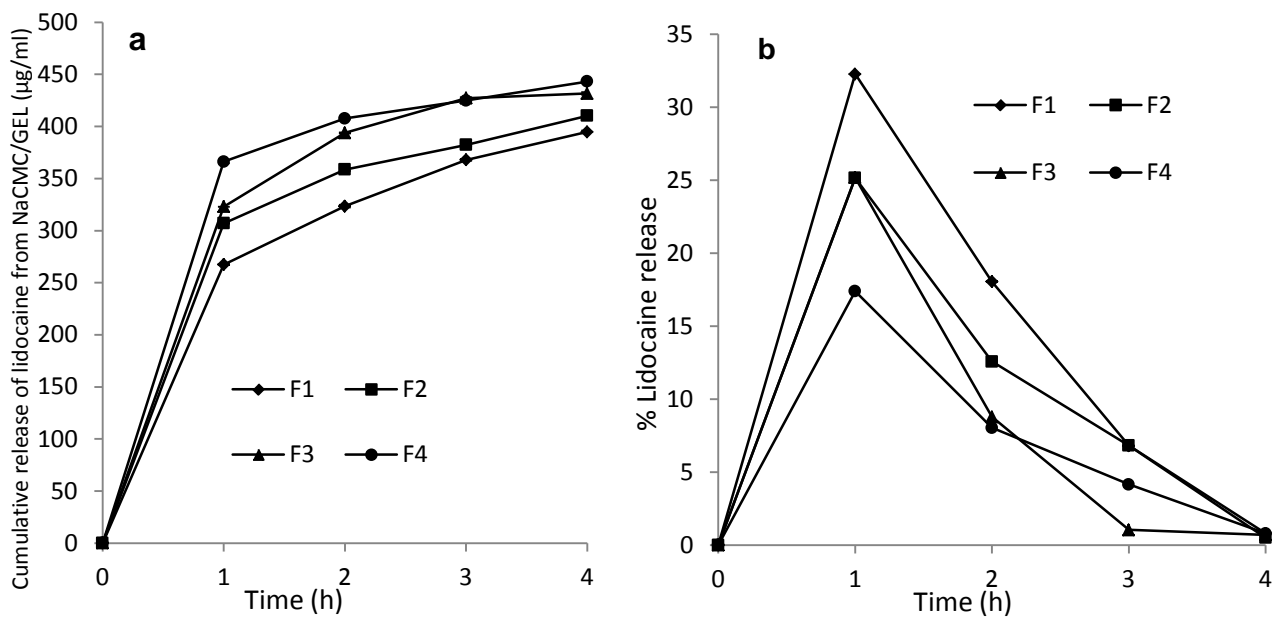


Fig. 6. The controlled release of Lidocaine 2.44% w/w encapsulated **a.** NaCMC/GEL 1:1.6 (F1), NaCMC/GEL 1:2.0 (F2), NaCMC/GEL 1:2.33 (F3) and NaCMC/GEL 1:2.66 (F4) **b.** as a percentage into DI water medium from NaCMC/GEL 1:1.6 (F1), NaCMC/GEL 1:2.0 (F2), NaCMC/GEL 1:2.33 (F3) and NaCMC/GEL 1:2.66 (F4). The error bars in a) the standard deviation of mean represents the error. b) No error bars indicated

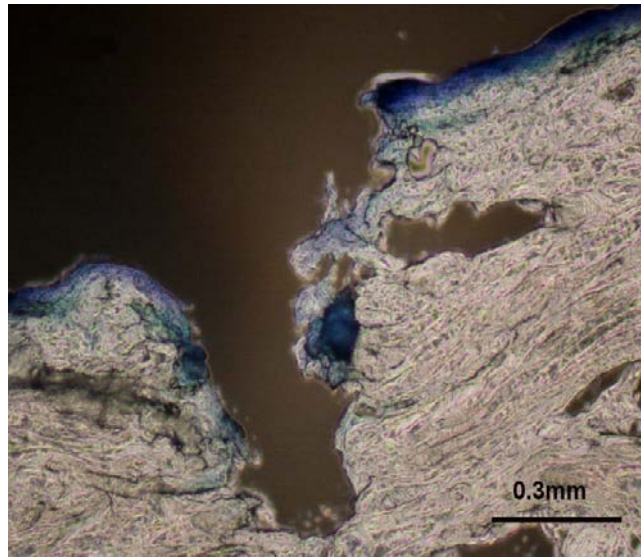
737

738

739

740

741



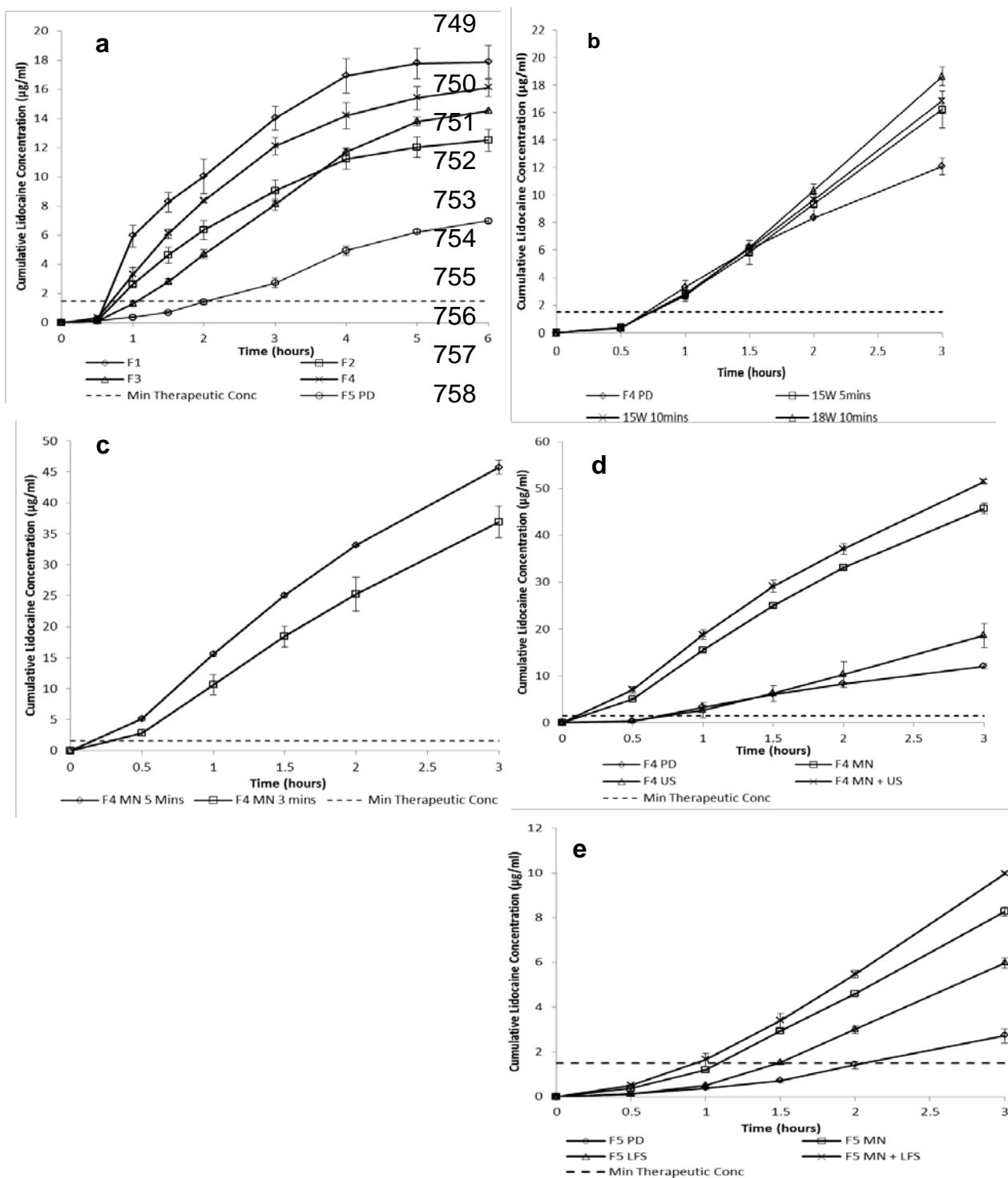
742

743 **Fig. 7** The microneedle insertion depth of skin sample using 1100 μm microneedles under thumb
744 pressure. The histological studies shows that although the microneedles are 1100 μm , for the
745 microneedle density in the array and force applied, they creates holes of approximately 400 μm .

746

747

748



759

760

761

762

763

764

765

766

767

768

769

770

771

772

773

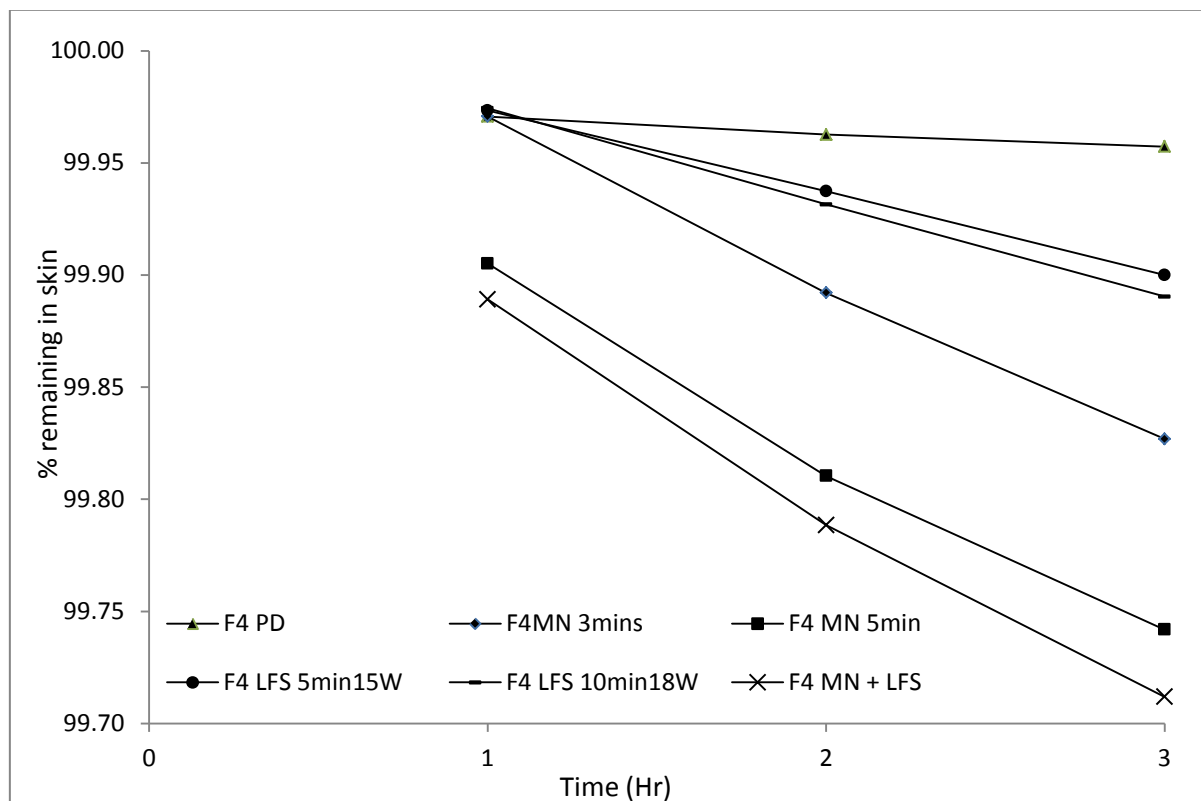
774

775

Fig. 8. Cumulative lidocaine permeation from Lidocaine. **a** NaCMC/GEL 1:1.6 (F1), NaCMC/GEL 1:2.0 (F2), NaCMC/GEL 1:2.33 (F3), NaCMC/GEL 1:2.66 (F4) and passive diffusion (PD) NaCMC/GEL 1:2.66 by rotor evaporation prep stage (F5). **b** F4 PD and comparative pre-treatment with ultrasound at 15W and 18W for 5 and 10 minutes respectively. **c** F4 adapting a microneedle (MN) patch for a 3 minute and 5 minute pre-treatment duration for Lidocaine NaCMC/GEL 1:2.66. **d** F4 adapting NaCMC/GEL 1:2.66 (F4 PD), NaCMC/GEL 1:2.66 (F4 US, 18W 10min.), NaCMC/GEL 1:2.66 (F4 MN, 5 min.) and NaCMC/GEL 1:2.66 (F4 MN 5 min and US 18W 10min). **e** NaCMC/GEL 1:2.66 (F5 PD), NaCMC/GEL 1:2.66 (F5 LFS, 18W 10min.), NaCMC/GEL 1:2.66 (F5 MN, 5 min.) and NaCMC/GEL 1:2.66 (F5 MN 5 min, LFS, 18W 10min).

776

777



778

779 **Fig. 9.** Percentage of lidocaine contained in (F4) NaCMC/GEL 1:2.66 (▲ Passive Diffusion), (◆ microneedles, 3 min),
 780 (■ microneedles, 5 min.), (● LFS 5 min 15W), (■ LFS 10 min 18W), (✕ MN + LFS). (Error bars outline a
 781 random error range of 0.005 %)