Physicochemical characteristics and in vitro permeation of loratadine solid lipid nanoparticles for transdermal delivery

Abstract

Aim: To prepare loratadine-loaded solid lipid nanoparticles (SLNs) using a modified two-step ultrasound- assisted phase inversion temperature (PIT) process. **Results/methodology:** Loratadine was dissolved in beeswax and Tween 80 dissolved in water. The two phases were mixed together to prepare a water in oil emulsion preconcentrate (w/o) at a PIT of 85 °C, followed by gradual water addition at 25 ° C to trigger nanoparticles formation (o/w). Kinetic stability was investigated. No change in the size was observed within six months. Fourier-transform infrared spectroscopy (FTIR) demonstrated stability of the emulsions via molecular structure of water at the interface of the o/w nanoemulsions. SLNs enhanced the in vitro skin permeation of loratadine. **Conclusion:** Stable SLNs was successfully prepared by ultrasound-assisted PIT.

Keywords:

Solid lipid nanoparticles; Phase inversion temperature; Transdermal; Franz diffusion cell; Permeation; Pig skin; Synthetic membrane.

22 **Introduction**

23 Solid lipid nanoparticles are considered as a modern aqueous dispersion alternative to conventional
24 emulsions and liposomes [1] and are used as a passive approach to enable the transdermal delivery
25 of lipophilic and hydrophilic drugs. The SLN core is composed of solid lipids and is dispersed in
26 water and stabilized by surfactant and can range from 10 to 1000 nm in size [2].

27 The small size of nanoparticles gives the system critical functional characteristics that affect their
28 performance during application. It decreases the agglomeration of particles and enhances the
29 physical stability of the system and offers large overall surface area to achieve sustained release.
30 SLNs are made of materials with high safety profiles, including waxes, fatty acids, fatty alcohols
31 and triglycerides. This makes them biocompatible and biodegradable and can be readily excreted
32 through urine or bile [3]. The solid lipid content of SLNs varies between 0.1 and 30% and
33 surfactant varies between 0.5 and 5% (w/w) [4]. The size of particles is related to lipid content
34 with some studies reporting that the smallest size was obtained with 5% lipid content [3]. The
35 proper selection of components and lipid-to-surfactant ratios can affect the particle size, drug
36 loading and release characteristics and stability over long periods of time.

37 Different high-energy methods have been employed to prepare SLNs and each one presents unique
38 limitations. In the formulation of SLNs, the application of external source of energy is required to
39 break the kinetic energy barrier between oil and water. The use of ultrasound is justified by its
40 capacity to produce intensive disruptive forces needed to overcome the forces holding the oil and
41 water droplets in a spherical shape [5].

42 Ultrasound, if used alone to prepare SLNs, requires a relatively high amount of surfactant which
43 is inappropriate in terms of safety. Another limitation, is that very low amounts of mechanical
44 energy (< 0.1%) is actually transferred to the sample during the emulsification process [6].

45 Alternatively, a method employing the intrinsic physicochemical properties of surfactant to form
46 emulsion referred to as PIT was first reported in 1969 [7]. A notable feature in employing PIT
47 alone for the preparation of SLNs is the use of high amounts of surfactant relative to the oil content
48 to prepare an emulsion preconcentrate of micrometric droplet size in the PIT region at low water
49 content. This is defined as one-phase microemulsion as described by Wadle's group [8]. The
50 preconcentrate is subsequently used to obtain the SLNs via a cooling-dilution process at ambient
51 conditions with high water content. One potential problem with the formulation of SLNs by the
52 PIT approach is the polymorphic transformation of crystallized solid lipid into a more stable form
53 after cooling [9].
54 Loratadine is a second-generation antihistamine with long duration of pharmacological action.
55 According to the biopharmaceutics classification system (BCS), it possesses low solubility and
56 high permeability which makes it BCS Class II drug [10]. It is available as 10-mg dose, however,
57 this dose has side effects such as headaches, fatigue, nausea, sleepiness, dizziness and dry mouth
58 due to the transient high blood concentration of the drug. Loratadine is a highly lipophilic
59 compound and associated with significant biotransformation and tissue distribution.
60 The transdermal route is an attractive alternative delivery system for loratadine that would provide
61 sufficient plasma levels with no or reduced side effects. Supersaturated vehicles with high
62 thermodynamic activity are reported to be able to increase drug absorption by the skin [11]. In this
63 regard, emulsion preconcentrate offer two features for transdermal drug delivery. Firstly, they act
64 as system with low aqueous solubility for lipophilic compounds in the presence of high quantities
65 of water and forms the basis for nanoemulsions formation using the dilution method. Secondly,
66 when the drug is loaded into the preconcentrate, it exhibits in vitro crystallization at a slower rate

67 and this may last for up to 10-14 days without any crystal formation in the first 2-3 days after
68 achieving supersaturation [11].
69 SLNs have a great potential as transdermal drug delivery systems and are composed of safe
70 biocompatible lipids which do not cause skin irritation and were found to increase skin hydration
71 to 31% [12]. They also contain surfactants which are chemical permeation enhancers. The lipids
72 and surfactants used are also found to modulate the skin penetration of drugs, while the small
73 particle size further enhances the permeation, thus facilitates drug transport through the layers of
74 skin.
75 Polydimethylsiloxane (PDMS) membranes are frequently used in topical and transdermal studies
76 as a cheap model to provide reproducible data for predicting skin permeation [13]. The stability of
77 nanoparticles in relation to the particle size is crucial in the formulation development.
78 Understanding the molecular mechanisms that are underlie the interactions at the interface are
79 addressed by employing vibrational spectroscopic methods such FTIR spectroscopy [14].
80 The aim of this work was to investigate the feasibility of using the ultrasound-assisted PIT
81 approach to prepare stable SLNs using emulsion preconcentrate template of micrometric droplet
82 size loaded near the saturation concentration of loratadine in beeswax (C_{sat}) and low surfactant
83 content for transdermal drug delivery [15]. Beeswax was selected as solid lipid to prepare the
84 SLNs. The suitability of PDMS membrane to evaluate the effect of SLNs formulation parameters
85 including the oil-to-surfactant ratios on the permeation compared to pig skin, was also evaluated.
86 The second objective was to assess the effect of ultrasound-assisted PIT process on the physical
87 stability of SLNs by FTIR spectroscopy.
88
89

Materials and methods

Materials

Loratadine was obtained as a gift from Gulf Pharmaceutical Industries (Ras Al-Khaimah, UAE). Beeswax was purchased from Acros Organics (Geel, Belgium). Pure grade Tween 80 was acquired from Sigma Aldrich Co. (Missouri, USA). Cellulose acetate dialysis membrane (MWCO 12-14 kDa) was purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Silicone membrane - polydimethylsiloxane (PDMS)- was obtained from Samco Silicone Products (Warwickshire, UK). All other chemicals were of analytical grade.

Determination of loratadine solubility in beeswax

Loratadine solubility in beeswax was determined following the method reported in [14, 15]. 50 mg of loratadine was placed in a mortar maintained at 10 °C above the melting point of beeswax, and the lipid was added gradually in increments of 50 mg and stirred, till loratadine was completely dissolved. The amount of lipid required to solubilize loratadine was noted by visualizing the disappearance of drug crystals and formation of a transparent homogeneous system and this was recorded [17].

Preparation of loratadine-loaded SLNs

PIT and ultrasonication approaches were combined to prepare the SLNs. In this investigation, three different lipid-to-surfactant ratios (1:5, 1:7 and 1:10) were studied based on preliminary studies (data not shown). SLNs which are listed in Table 1, were prepared based on the PIT method described in [15]. 75 mg of loratadine was dissolved in 375 mg of beeswax to achieve a total drug loading of 20% w/w and melted at 85 °C with the quantity of beeswax used, dependent on the

113 solubility of loratadine in the lipid which was determined earlier (as described in the previous
114 section). The final concertation of loratadine in the SLNs preparation was 0.4 mg/mL upon dilution
115 with water. A predetermined quantity of Tween 80 was dissolved in water and heated at 85 °C as
116 well, then added slowly in a dropwise fashion to the loratadine beeswax mixture and homogenized
117 (14000 rpm, 5 min) using the Ultra-Turrax® IKA T25, (Germany). The temperature was increased
118 above the PIT by 10 °C during the homogenization. The resulting emulsion was probe sonicated
119 for 5 min also at 10 °C above the PIT using a 300 V/T ultrasonic homogenizer (BioLogics Inc,
120 USA). The operating frequency was 20 kHz and the applied power was 150 W. Using paddle
121 stirrer, rapid dilution of the emulsion with water was carried out at room temperature under
122 moderate stirring, which resulted in very quick generation of the nanoemulsions. The final
123 concentration of water was always within the range of 96 to 98%.

124 **►Table 1◄**

125 *Drug loading and encapsulation efficiency*

126 Loratadine loading into SLNs was determined by ultracentrifugation at 25000 rpm for 30 min
127 [18] and the resulting supernatant was analyzed using HPLC. The drug loading and
128 encapsulation efficiency was calculated based on the amount of free loratadine measured using
129 the following equations:

130
$$Drug\ loading\ (\%) = \frac{Wa - Ws}{(Wa - Ws) + Wl}$$

131 *Wa* Amount of drug added to the formulation

132 *Ws* Amount of unencapsulated drug measured in the supernatant
133
134 *Wl* Weight of the lipid
135

136
$$Encapsulation\ efficiency\ (\%) = \frac{Wa - Ws}{Wa}$$

137

138

139 *Dynamic light scattering (DLS) and microscopic analysis*

140 The sizes and zeta potential of the SLNs was determined using DLS on a Zetasizer Nano-ZS90

141 (Malvern Instruments, UK). Samples were diluted with distilled water and the DLS measurements

142 (n = 3, ± SD) performed at a scattering angle of 173°, laser wavelength of 633 nm and temperature

143 set at 25 °C. Cryogenic-transmission electron microscopy (TEM) measurements were performed

144 [15].

145

146

147 *Skin preparation*

148 In the study, frozen pig ears were used to obtain full thickness skin. The outer side of the ear was

149 cut with a scalpel and visually examined for any possible defects, washed and frozen at -18 °C and

150 used within a month. Whenever required, a piece of skin was thawed at 25 °C and cut into smaller

151 pieces that fit the Franz cell diffusion area. The pieces of skin were mounted between the donor

152 and receptor compartments and allowed to hydrate for an hour prior to the commencement of the

153 permeation analysis.

154

155 *In vitro drug release*

156 For in vitro drug release studies, the two compartments were separated by a cellulose acetate

157 membrane using Franz diffusion cells (V3A-02 PermeGear, USA.). Each Franz cell had a diffusion

158 surface area of 0.64 cm^2 and a receptor compartment volume of 5.3 mL filled with hydroethanolic

159 solution (water: ethanol 1:1). The hydroethanolic solution was used to ensure that all the drug

160 dissolved in the media and to also maintain sink conditions. The receptor media was stirred at 600

161 rpm and at 37 ± 0.5 °C using a thermostatic water pump (Haake SC 100, Thermo Fisher Scientific,

162 USA). The cellulose acetate membrane was cut and placed on the donor compartment. Before use,

163 the membrane was soaked overnight in distilled water, and did not exerts any resistance on
164 particles that move across.
165 The Franz cell donor compartment was filled with 1 mL of SLNs formulation suspension and was
166 sealed with Parafilm M® to minimize solvent loss. At predetermined time intervals, 1 mL was
167 withdrawn from the receptor compartment and replenished with the same volume of fresh
168 dissolution media at the same temperature over a 24-hour release period. Loratadine concentration
169 was determined by HPLC and all analyses were repeated for a minimum of six experiments ($n \geq$
170 6).
171 *PDMS and pig skin permeation studies*
172 The same procedure was followed as in the in vitro drug release section above. The receptor
173 compartment of Franz diffusion cells was filled with 5.3 mL of hydroethanolic solution comprising
174 of 1:1 ratio of water and ethanol to ensure pseudo-sink conditions by increasing loratadine
175 solubility in the receiving phase [19,20]. 1 mL of SLN sample was placed in the donor
176 compartment and medium was magnetically stirred (600 rpm) to ensure homogeneity at a
177 temperature of 37 ± 0.5 °C. The membrane (PDMS or full-thickness pig skin) was placed between
178 the donor and receiver compartments. The permeation studies were carried out over a 24 h period
179 and loratadine concentration was determined by HPLC. All analyses were repeated for a minimum
180 of six experiments ($n \geq 6$). The permeation fluxes were calculated as the slope divided by the skin
181 surface area:

182 $$J_{ss} = \left(\frac{dQ}{dt}\right)_{ss} \cdot \frac{1}{A}$$

183 where J_{ss} is the steady-state permeation flux ($g/cm^2/h$), A is the area of skin tissue (cm^2) through
184 which drug permeation takes place, and $(dQ/dt)_{ss}$ is the amount of drug passing through the skin
185 per unit time at a steady state (g/h).
186

187 *Fourier transform infrared spectroscopy (FTIR)*
188 Samples were analyzed on a Cary 630 FTIR instrument (Agilent Technologies, California, USA).
189 IR spectra were obtained for all materials including loratadine, Tween 80 and lipids, and for
190 formulations prepared with different lipids using ZnSe crystal surface at wavenumber range of
191 650–4000 cm^{-1}.
192
193 *Stability studies*
194 The effect of different lipid-to-surfactant ratios on the SLNs stability was studied at room (25 °C)
195 and cold (8 °C) temperatures over a period of six months. The SLNs dispersions were regularly
196 examined for particle size as well as changes in physical appearance such as gelation, precipitation
197 and loratadine crystallization.
198
199 *HPLC analysis*
200 Loratadine was quantified by HPLC using analytical column Restek®, Allure (USA) C18 (150 ×
201 4.6 mm I.D, 5μ) as a stationary phase, mobile phase comprising phosphate buffer (0.05 M) pH 3.0,
202 acetonitrile and methanol (38:45:17 v/v) in isocratic mode at a flow rate of 1.0 mL min^{-1}. Injection
203 volume was 20 μL for each run and detected at 247 nm [21]. A calibration curve was plotted from
204 loratadine standards with concentrations ranging from 2 μg/mL to 100 μg/mL (R^2 = 0.998). Limit
205 of quantification (LOQ) was found to be 0.09 μg/mL.
206
207 *Statistical analysis*
208 All statistical analyses were performed using Minitab version 14 for one- way ANOVA and was
209 performed with a significance level of 0.05.
210
211

212 **Results**

213 *The formation of loratadine- SLNs*

214 In this study, loratadine- SLNs were prepared by two steps of ultrasound-assisted phase inversion.
215 The first step was to prepare w/o preconcentrate formed by keeping the PIT at 85 °C. Subsequently,
216 stepwise dilution was done with water at room temperature to induce the formation of SLNs o/w
217 nanoemulsions template. Subsequently, low frequency ultrasound was applied to further reduce
218 the droplet size to form SLNs with long-term stability.

219 *SLNs characterization*

220 Maximum loratadine loading in beeswax was determined by adding various amounts of beeswax
221 to 50 mg of the drug. The formulations were observed visually for precipitation and no observed
222 precipitation detected, which was similar to the observations reported by Chattopadhyay
223 coworkers [22]. Loratadine showed a high encapsulation efficiency of approximately 92% and a
224 satisfactory drug loading of 18 % for BW-10 (Table 2). For BW-5 and BW-10, the encapsulation
225 efficiency decreased to approximately 75% and 15% drug loading.

226 Cryogenic TEM image of BW-7 (Fig. 1 A) showed many visible aggregates which can be
227 attributed to the ultrasound over-processing effect, which causes droplet coalescence at high shear
228 rate [23]. The cavitational forces of ultrasound drive SLNs to the nodes and antinodes of the
229 acoustic field, which results in increased droplet coalescence, and over-processing [22, 23]. These
230 visible structures derived from small intact droplets with size ranging from 10 to 20 nm with
231 monodispersed distribution [25], are shown in Fig. 1 B.

232 As described by Rao and McClements [26], there are three possible regimes for oil solubilization
233 in surfactant solution based on the C_{sat} as defined by McClements [27] “it is the number of grams
234 of oil solubilized per gram of surfactant under specified environmental conditions”. Regime I is
235 when the oil concentration is lower than C_{sat}; when the oil concentration is approximately equal to

236 C_{sat}, it is considered as Regime II and when the oil concentration exceeds C_{sat}, Regime III prevails.
237 One important characteristic of Regime II is the presence of large and small populations of
238 particles. TEM findings indicate the existence of Regime II as a result of the presence of nano-
239 sized droplets as well as swollen micelles as reported in [27].
240
241 **►Fig. 1 A and B ◄**
242
243 The impact of the concentration of surfactant on the characteristics of SLNs was studied by
244 preparing SLNs with beeswax and Tween 80 at three lipid-to-surfactant ratios of 1:5, 1:7 and 1:10
245 (w/w) [28] and showed transparent to translucent SLN solutions at the three ratios.
246 The data also showed that the particle size can be significantly modulated with the concentration
247 of the surfactant used. The smallest droplet sizes were obtained in the SLN dispersion prepared at
248 lipid-to-surfactant ratio of 1:5 (w/w) (Table 2) with PDI of 0.36 nm. The particle size and the PDI
249 increased at higher surfactant concentrations, compared to 1:5 ratio with no significant difference
250 ($p > 0.05$) between 1:7 and 1:10 ratios (Table 2). This is consistent with data reported by different
251 groups [6,28–30]. Our formulation showed a particle size distribution that falls within the preferred
252 distribution range similar to that reported by Chattopadhyay and co-workers [22].
253
254 **►Table 2◄**
255 For the freshly prepared SLNs samples, the method was able to produce a unimodal droplet size
256 distribution at all surfactant-to-oil ratios (Fig. 2) which indicates a good potential for long-term
257 stability.
258 Zeta potential is an important property for evaluating particle surface charge of SLNs. BW-5, BW-
259 7 and BW-10 showed -12.4 ± 5.6, -11.6 ± 4.8 and -6.4 ± 4.3 mV, respectively. The zeta potential

260 decreased as the Tween 80 concentration increased. Ideally, a stable dispersed system should have
261 a zeta potential higher than+30 mV or lower than −30 mV to be considered physically stable [31].
262 Nevertheless, good electrostatic stability could be achieved by Tween 80 with absolute minimum
263 values of zeta potential between 8 and 9 mV [32, 33].
264 **►Fig. 2◄**
265
266
267 *Solid lipid nanoparticles storage stability*
268 The stability of colloidal dispersions is an important property that determines the shelf life of a
269 formulation. The stability of the SLNs dispersions at 25 ° C and 8 ° C, was assessed over a 6-
270 month period (Table 2). For the formulations containing beeswax and Tween 80 at 1:5 (w/w), there
271 was no statistically significant ($p > 0.05$) change in particle size of loratadine SLNs, indicating
272 SLNs stability towards droplet aggregation at different storage temperatures. This also indicates
273 that the droplet surface coverage with Tween 80 is optimal at lipid-to-surfactant ratio of 1:5 (w/w)
274 and in association with the hydrophobic loratadine, is able to hinder (or lessen) destabilization
275 processes such as Ostwald ripening [34]. No phase separation was observed in any of the
276 formulations throughout the storage period.
277 Interestingly, there were significant decreases ($p < 0.5$) in the particle size for SLNs at lipid-to-
278 surfactant ratios of 1:7 and 1:10 (w/w) after storage for six months at 25°C and 8 °C (Table 2).
279 The same effect was reported by Rao and McClements [26] and it was proposed that the oil phase
280 would take a longer time for full solubilization within Tween 80 micelles and thus results in
281 reduction of particle size.
282 Because the method of preparation produced diluted SLNs, it is important to understand this
283 behavior in relation to droplet surface coverage [34]. Further, SLNs stored at 8 °C showed

284 excellent stability compared to room temperature conditions, because cooling well below the cloud
285 point ensures that the droplets are stable to coalescence [35]. SLNs stored at 25°C showed larger
286 particle size, which could have been due to the storage temperature being well below the PIT.
287 Langmuir isotherm model was used to calculate the surface coverage of oil droplets by the
288 surfactant (Γ) (Table 1) using the equation below:

289 $$\Gamma = \Gamma_{sat} C/(k_d + C)$$

290 where Γ_{sat} is the saturation adsorption, C is the surfactant concentration, and k_d is the adsorption
291 coefficient. Tween 80 (polyoxyethylene (20) sorbitan monooleate (POE 20)) has Γ_{sat} and k_d values
292 of 1.4×10^{-10} mol cm^{-2} and 2.7 µM respectively [36].
293 Considering the bimodal size distributions demonstrated (Figs. 3 and 4) for these SLNs upon
294 storage, it could not be determined if the cube of the mean diameter of droplet undergoing Ostwald
295 ripening/coalescence is linearly dependent on the storage time. The same was observed by
296 Thompson et al. [37]. Moreover, using the linear increase in the cube of the average drop radius
297 as the only indication of Ostwald ripening is not definitive, since coalescence often results in a
298 linear r^3-time plot in nanoparticles [34] and cannot be decoupled from each other.
299
300 **►Fig. 3 A and B◄**
301 **►Fig. 4 A and B◄**
302
303 *FTIR spectroscopy of SLNs*
304 We proposed the use of FTIR to investigate the physical stability of SLNs, however, based on the
305 preliminary data we could not acquire good spectrum for BW-5 and BW-7. The IR spectrum of
306 BW-10 SLNs is shown in Fig. 5. The spectrum of SLNs is similar to that of pure water with
307 prominent OH bending band at 1636 cm^{-1} and broad OH stretching band between 2800 to 3700
308 cm^{-1}. Kiefer and co-workers [14] reported that hydrogen bonding environment could be used as

309 indicator for emulsion stability through the OH stretching to understand the molecular interactions
310 between different emulsion components. Symmetrically and asymmetrically hydrogen-bonded
311 waters can be assigned at 3268 and 3337 cm^{-1} bands, respectively, as reported by other groups
312 [14,38–40]. The observed peaks showed low intensities which indicates that hydrogen-bonding
313 network in the probed water was weakened by the interaction with Tween 80 molecules and thus
314 prevents droplet coalescence. The presence of the band at 1636 cm^{-1} was attributed to OH bending
315 due to the hydrophilic nature of Tween 80 resulting in more water being entrapped at higher
316 temperature of 85 °C as reported by Whittinghill and co-workers [41], while the 1088 cm^{-1} band
317 is due to C-O stretching of Tween 80.
318
319 **►Fig. 5 ◄**
320 *In vitro release and permeation studies*
321 Fig. 6 shows cumulative permeation amounts of loratadine released from SLNs, where panel A
322 shows the amount permeating through the cellulose membrane plotted against time and, panel B
323 shows the amount permeating through the PDMS membrane plotted against time. Fig. 7 shows the
324 amount permeating through full-thickness pig skin plotted against time.
325 Loratadine aqueous saturated solution (0.005 mg/mL) permeation through PDMS resulted in a flux
326 of 1.17 ± 0.04 μg/cm^2/h, which is low due to the drug's high lipophilicity. On the other hand, the
327 SLNs demonstrated an increased permeation given the fact that all SLNs were loaded with near
328 saturation concentration of loratadine based on [15]. This method is employed to form drug-
329 enriched core SLNs [42] since drug supersaturation occurs in the process of cooling the
330 nanoemulsion template, which induces precipitation of the drug before that of the lipid. This allows
331 the precipitating lipid to encapsulate the precipitated drug, resulting in the formation of a
332 membrane surrounding the drug [42].

333 BW-5 (F1) showed enhanced permeation which was 11 times more than the aqueous saturated
334 solution with a drug flux of 12.67 ± 0.7 µg/cm^2/h. BW -7 (F2) had a flux of 16.65 ± 1.77 µg/cm^2/h
335 and an enhancement ratio of 14 times compared to the saturated drug solution. Finally, BW-10
336 (F3) had a flux of 9.92 ± 0.7 µg/cm^2/h with 8 times enhancement ratio. Fig. 6B shows that the
337 permeation of F1, F2 and F3 through PDMS was significantly higher ($p < 0.05$) as the surfactant
338 concentration increased compared to the aqueous saturated solution of loratadine. However, when
339 the concentration of the surfactant increased from 7.5% to 10%, there was a significant increase
340 ($p < 0.05$) in loratadine permeation through PDMS membrane, while there was a significant
341 decrease ($p < 0.05$) in permeation when the concentration of Tween 80 reached 15%. A similar
342 observation was reported [43,44] for other compounds and might be due to the interaction between
343 the charged and neutral functional groups with the surfactant head group, as well as with the
344 surfactant micelles' core and outer surface. It was also reported that there may be a peak
345 concentration of surfactant above which the permeation of the drug decreases [15,45]. The
346 difference in the particle size had no effect on the loratadine permeation through PDMS membrane.
347 The cellulose data was also comparable to PDMS membrane ones where BW-5, BW-7 and BW-
348 10 showed the following fluxes 29.75 ± 5.2, 19.69 ± 3.4 and 12.35 ± 1.1 µg/cm^2/h, respectively.
349 Both membranes showed the same behavior when surfactant concentration increased (data not
350 shown). The data also showed that the SLNs release is directly proportional to the zeta potential
351 as it approaches the positive value and similar to that reported by Baspinar and Borchert [46].
352 This behavior could be due to the concentration of Tween 80, which might trigger the release of
353 the drug.
354 The permeation of the different SLNs formulations was also examined using pig skin and Franz
355 diffusion cells and compared to the saturated solution of the model drug, with the latter displaying

356 very low flux across the skin membrane (0.09 ± 0.04 μg/cm^2/h). However, the SLNs exhibited
357 higher permeation, showing enhancement ratios (ER) reaching up to 28 times that of the saturated
358 solution. Cumulative amount of loratadine permeating after 24 hours through pig skin from SLNs
359 prepared with bees wax, (F3) showed the highest value with flux of 2.54 ± 0.23 μg/cm^2/h (ER=28),
360 followed by F2 with flux of 0.87 ± 0.16 μg/cm^2/h (ER= 9.64) and F1 flux of 0.28 ± 0.02 μg/cm^2/h
361 (ER= 3.11) as shown in Fig. 7. It was observed that increasing the surfactant ratio enhanced the
362 permeation significantly ($p < 0.05$) and the data is comparable to that reported by Tavares [47].
363 Once again there was no relation between the particle size and the loratadine permeation. However,
364 there was a clear increase in the drug permeation as zeta potential deceased and approached the
365 positive value as reported by Baspinar and Borchert [46]. It may be attributed to an increased
366 contact and adsorption of SLNs with the negatively charged corneocytes of the skin's layer main,
367 stratum corneum.
368

369 **►Fig. 6 A and B◄**
370 **►Fig. 7◄**
371

372 **Discussion**

373 One major objective in the development of SLNs is the control of physical stability by reducing
374 the particle size. The application of higher shear rate has been reported to reduce droplet size when
375 the continuous phase viscosity is low [35]. Simple shear flow conditions, as applied by Ultra-
376 Turrax homogenizer, were not sufficient to reduce the particle size for a continuous phase of low
377 viscosity [48]. For this type of system, it is indicated that homogenizers such as sonicators that
378 utilize elongational, turbulent, or cavitational flow are used to break down droplets [35]. SLNs
379 with small particle size and narrow droplet size distribution can be formed by the application of

380 ultrasound and via two mechanisms: (a) droplet disruption and (b) droplet coalescence, which are
381 not provided by other mechanical equipment. Droplet disruption depends on the nature and
382 quantity of the applied shear force and the resistance of the droplets to shear-induced deformation
383 dictated by the surface tension [49]. While, the droplet coalescence is the result of the potential of
384 surfactant adsorption on the surface of newly formed droplets. This is controlled by the surface
385 activity and concentration of the surfactant [49] as they give high surfactant adsorption capacity
386 and prevent coalescing of particles. Thermodynamic equilibrium in o/w emulsions during
387 sonication is influenced by the time of irradiation. For example, longer ultrasonication time (> 5
388 min) was reported to produce larger droplets and could be due to the effect of over-processing,
389 which results in droplets coalescence [49].
390 In this study, the total percentage of solid lipid used to achieve loratadine loaded SLNs (C_{sat}) was
391 0.36% (w/w) after dilution. This helps to ensure effective solubilization capacity, smaller
392 nanoparticles, better stability during storage and maintaining the transparency of the dispersions
393 [26]. Tween 80 was selected based on its ability to achieve an optimal particle size and being non-
394 irritant for safe transdermal application. Tween 80 concentration was determined on the basis of
395 preliminary solubilization studies.
396 Higher HLB surfactants such as Tween 80 are used to form o/w nanoparticles of hydrophobic drug
397 molecules such as loratadine. Tween 80 was reported to produce nanoparticles with smaller
398 particle sizes compared to other Tween surfactants [50], which was in line with the results in the
399 current study data. Initially, as the amount of surfactant adsorbed on the oil–water interface of a
400 droplet increased, it results in reduction of the interfacial tension, which favours the formation of
401 fine droplet nanoparticles. Further increase in the concentration of the Tween 80 could enhance
402 the water penetration into lipid droplets (discussed later in the FTIR section), causing interfacial

403 disruption which will result in larger particle size [28]. Further, the abundance of surfactant in the
404 system could alter the viscosity and give rise to a rigid interface, causing the formation of larger
405 droplets.
406 Zeta potential measurements showed that above given critical surfactant concentration, a sudden
407 expulsion of OH^- groups from the o/w surface can reduce the surface potential and hence the zeta
408 potential [51]. Stability studies demonstrated bimodal distribution and non-linear relationship
409 between $1/r^2$ and t. Bimodal distributions can be attributed to droplet flocculation or coalescence
410 [35]. Therefore, size distribution data was used to differentiate between various destabilization
411 mechanisms in this study. A study [15] showed a non-linear relationship between $1/r^2$ and t and
412 this indicates that droplet coalescence cannot be assumed by the theories relevant to
413 macroemulsions as reported in [34] and also found that the decrease in the average droplet size
414 could be related to Ostwald ripening upon aging. Ostwald ripening progression results in small
415 droplets becoming even smaller especially with the use of high surfactant concentrations, which
416 gives sufficient surface coverage to hinder coalescence. Particle size distribution in Figs. 3 and 4
417 showed peaks broadening with time at both ends, which indicates the occurrence of Ostwald
418 ripening associated with a decrease in the average droplet size (Table 2). Similar results were was
419 reported by Nazarzadeh and co-workers [34].
420 Other reports [52,53], however, suggested that flocculation plays a key role in destabilizing
421 nanoparticles produced by phase inversion method. Cryo-TEM and the appearance of bimodal size
422 distribution showed the presence of aggregates in our study. To confirm this, we used sodium
423 lauryl sulphate to induce deflocculation of aggregates and it did not change the bimodal
424 distribution. Therefore the flocculation by steric interactions was operative due to higher amounts
425 of emulsifier which formed relatively thin interfacial layers [35]. It has also been reported that a

426 of bimodal particle size distribution is observed when one population of droplets is flocculated
427 while the other is non-flocculated [35,54,55]. However, flocculation did not overcome Ostwald
428 ripening due to the high concentration of surfactant [34] but could counteract coalescence as shown
429 because of the extremely strong repulsion at short-range [35]. Due to their small droplet sizes, all
430 SLNs dispersions showed excellent stability to creaming throughout the storage period (6 months),
431 however, further studies are required to understand this phenomenon.
432 The bimodal particle size distribution could result from the use of relatively high surfactant-to-oil
433 (SOR) ratios. The presence of excess surfactant molecules in the continuous phase is associated
434 with high SOR, which may increase the viscosity of the emulsion making it difficult to
435 spontaneously split the oil-water interface and decrease the diffusion of emulsion droplets resulting
436 in larger droplets [28]. Other causes for the bimodal distribution are related to the process-based
437 parameters such as the holding processing temperature and the application of ultrasound rather
438 than an aging process [34]. Temperature is applied in PIT method to form nanoparticles with small
439 particle size by decreasing the viscosity, changing the molecular geometry of non-ionic surfactant,
440 increasing oil solubility of Tween 80 and/or reducing interfacial tension as the PIT is reached
441 [35,55,56]. However, at high surfactant levels, the effect of temperature negatively affects the
442 particle size when it is close to PIT due to rapid droplet coalescence and it results in appearance of
443 a small population of large droplets. The same observation was reported by An and co-workers
444 [28]. Over-processing due to ultrasound application can also be responsible for the bimodal
445 distribution and increase in droplet size [57]. This effect was noticeable in the SLNs with lipid-to-
446 surfactant ratio of 1:7 and 1:10 and to a lesser extent with 1:5 (w/w). Possible reasons include the
447 relatively short residence time of droplets in the disruptive zone and slow rate of adsorption of the
448 emulsifier adsorption compared to the higher coalescence frequency [35].

449 FTIR analysis can also be used to evaluate the emulsion stability via molecular structure of water
450 at the interface of o/w nanoparticles [14,58]. According to Whittinghill and co-workers [41], a
451 stable emulsion is a system where the eventual separation process has been reduced to the point
452 where it is of no practical significance for 2 or 3 years. The OH vibrational stretching modes can
453 be used to determine the stability by sensing the molecular environment of hydrogen bonding in
454 the emulsion and it was found that the weakening of the hydrogen bonding network in the
455 interfacial water layer is responsible for the stabilization mechanism of emulsifiers in emulsions.
456 Therefore, water droplet is stabilized with a resultant decrease in coalescence of water droplets
457 [14,58].

458 There are various possible release mechanisms of loratadine from SLNs include: (i) increased drug
459 solubility in the SLNs used; and (ii) improved uptake of the drug carrier into the SC [59].

460 In addition, the rate at which supersaturated vehicles penetrate the skin membrane is generally
461 very high, bearing in mind that these SLNs were loaded with high concentrations of loratadine,
462 which may induce supersaturation during occluded application and represents a further driving
463 force [11]. It has also been observed that after dilution, drugs had a tendency towards
464 supersaturation in the microenvironment and eventually growing to form crystals [25] which could
465 also explain permeation enhancement of loratadine through the pig skin. Another important factor
466 is the hydration effect of SLNs on the skin due to the presence of water as shown in FTIR studies.
467 SLNs components adjust the water gradient in the upper layers of the skin by preventing
468 evaporation and influencing permeation of the skin [60]. FTIR studies suggested that increasing
469 the quantity of water within the emulsions would slowly increase the ratio of the amide I/II band
470 and this implies an increase in SC hydration [60].

Loratadine permeability across pig skin and PDMS membranes in vitro is weakly correlated, with the PDMS showing substantial overestimation of permeability compared to pig skin and could be attributed to the very hydrophobic nature of loratadine [61]. It is worth to note that this study has a small number of skin permeation studies due financial constraints.

Conclusion

The use of the ultrasound-assisted two-step emulsification process produced SLNs with ideal properties in terms of mean size and long-term stability under ambient temperature. The developed technique was simple and reproducible for the preparation of nanoparticles without any organic solvents or any specialized equipment and has the potential for large-scale processing. SLN dispersions were stable for approximately 6 months and TEM images showed the presence of monodispersed systems and in agreement with the results obtained by DLS. FTIR showed the stability of emulsions via molecular structure of water at the interface of the o/w nanoparticles. The drug permeation across the PDMS and the skin was enhanced significantly compared to the saturated solution of the drug, which makes the formulation promising for transdermal delivery systems.

Future perspective

Solid lipid nanoparticles (SLNs) have been a topic of interest for the encapsulation of both water and lipid soluble compounds to improve their skin permeation. Transdermal drug delivery has witnessed significant growth during the past few years driven by the presence of large number of drugs that have low solubility and poor intestinal permeability as well as some have lower potency

493 due to the first pass effect. SLNs will have a positive future in transdermal drug delivery due to the
494 use of safe lipids and ease of preparation which is feasible for production scale-up.
495

496 **Summary points**

497 - Stable SLNs were successfully prepared with the use of the ultrasound-assisted two-step
498 phase inversion and cooling-stepwise dilution process.
499 - The oil and surfactant used ensured effective solubilization capacity for loratadine.
500 - All SLNs dispersions showed excellent stability to creaming throughout the storage period.
501 - The particle size distribution was bimodal which could result from the use of relatively
502 high surfactant-to-oil (SOR) ratios.
503 - FTIR was employed to investigate the stability of o/w nanoparticles. It showed stability of
504 SLNs via molecular structure of water at the interface of o/w nanoparticles.
505 - Synthetic membranes such as cellulose and silicone did not demonstrate significant
506 resistance to the diffusion of loratadine from the SLNs.
507 - Loratadine permeation across the PDMS and pig skin was enhanced significantly.

508
509

510 **Figure legends**

511 **Fig. 1**: Cryogenic-transmission electron micrograph (Cryo-TEM) of solid lipid nanoparticles of
512 beeswax and Tween 80 at 1:7 ratio (BW-7).

513

514 **Fig. 2**: Representative particle size distribution for freshly prepared BW-7 SLNs.

515

516 **Fig. 3**: Particle size distribution determined by dynamic light scattering of SLNs after storage
517 for 6 months at 25 °C: (A) BW-5 SLNs, (B) BW-7 SLNs and (C) BW-10 SLNs.

518

519 **Fig. 4**: Particle size distribution determined by dynamic light scattering of SLNs after storage
520 for 6 months at 8 °C: (A) BW-5 SLNs, (B) BW-7 SLNs and (C) BW-10 SLNs.

521

522 **Figure 5**: IR spectra of SLNs prepared using beeswax: (A) Loratadine, (B) Tween 80, (C) beeswax
523 and (D) BW-10 SLNs.

524

525 **Figure 6**: Release of loratadine through various membranes: (A) cellulose and (B) PDMS,
526 following release from SLNs. F1, F2 and F3 represent BW-5, BW-7 and BW-10, respectively.
527 Mean ± SD (n ≥ 6).

528 **Figure 7**: Permeation of loratadine through pig skin, following release from SLNs. F1, F2 and F3
529 represent BW-5, BW-7 and BW-10, respectively. Mean ± SD (n ≥ 6).

530

531 **Table Legends**

532 **Table 1:** Preparation of preconcentrates (% w/w) loaded with 0.3% w/w of loratadine and initial
533 surface coverage of drops.

534 **Table 2:** Mean droplet diameter with aging time (six months storage) for solid lipid nanoparticles

535 at different storage conditions.

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703
704

705 Tables

706

707 Table 1

SLN	Bees wax	Tween 80	Water	Surface coverage (%)[a]
BW-5	1.5	7.5	91	100
BW-7	1.5	10.5	88	100
BW-10	1.5	15	83.5	100

708 [a] Surface coverage of 100% implies that water is also saturated with surfactant and excess
709 surfactant exists as micelles
710

711 Table 2

SLN[a]	Fresh PS[b]	Fresh PDI[c]	PS at 25 °C	PDI at 25 °C	PS at 8 °C	PDI at 8 °C	Encapsulatio efficiency (%
BW-5 (F1)	25 ± 2.8	0.36 ± 0.04	24 ± 3.4	0.16 ± 0.02	26 ± 3.1	0.32 ± 0.02	77.63
BW-7 (F2)	391 ± 1.2	0.45 ± 0.01	358 ± 2.5	0.42 ± 0.02	120 ± 2.7	0.24 ± 0.03	73.25
BW-10 (F3)	358 ± 2.3	0.41 ± 0.01	307 ± 2.9	0.24 ± 0.01	29 ± 2.9	0.20 ± 0.01	92.32

712 [a]SLN: solid lipid nanoparticles

713 [b]PS: particle size of solid lipid nanoparticles in nm

714 [c] PDI: polydispersity index

715 *Values are mean diameter by DLS (nm ± SD)
716

717