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1 **Transdermal delivery of propranolol hydrochloride through chitosan**
2 **nanoparticles dispersed in mucoadhesive gel**

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34 **Abstract**

35 This study aimed at improving the systemic bioavailability of propranolol-HCl by the design
36 of transdermal drug delivery system based on chitosan nanoparticles dispersed into gels.
37 Chitosan nanoparticles were prepared by ionic gelation technique using tripolyphosphate
38 (TPP) as a cross-linking agent. Characterization of the nanoparticles was focused on particle
39 size, zeta potential, surface texture and morphology, and drug encapsulation efficiency. The
40 prepared freeze dried chitosan nanoparticles were dispersed into gels made of poloxamer and
41 carbopol and the rheological behaviour and the adhesiveness of the gels were investigated.
42 The results showed that smallest propranolol loaded chitosan nanoparticles were achieved
43 with 0.2% chitosan and 0.05% TPP. Nanoparticles were stable in suspension with a zeta
44 potential (ZP) above ± 30 mV to prevent aggregation of the colloid. Zeta potential was found
45 to increase with increasing chitosan concentration due to its cationic nature. At least 70% of
46 entrapment efficiency and drug loading were achieved for all prepared nanoparticles. When
47 chitosan nanoparticles dispersed into gel consisting of poloxamer and carbopol, the resultant
48 formulation exhibited thixotropic behaviour with a prolonged drug release properties as
49 shown by the permeation studies through pig ear skin. Our study demonstrated that the
50 designed nanoparticles-gel transdermal delivery system has a potential to improve the
51 systemic bioavailability and the therapeutic efficacy of propranolol-HCl.

52

53 **Keywords:** Propranolol-HCl; Chitosan nanoparticles; gels; transdermal drug delivery.

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67 1. Introduction

68 The transdermal route has been a topic of interest for many years and is generally regarded as
69 a “patient friendly” option due to the avoidance of gastrointestinal side effects which usually
70 entail many oral preparations. Not only it avoids first pass metabolism and varying acidic
71 conditions of the gastrointestinal tract, it can also be used to maintain a constant, prolonged
72 and therapeutically effective drug concentration in the body. Transdermal drug delivery also
73 avoids fluctuations in plasma drug concentration, which helps minimising adverse effects and
74 therapeutic failure (Tanner and Marks, 2008). The main challenge in transdermal drug
75 delivery however, is to overcome the inherent barrier of the skin. It has been reported that
76 the rate limiting step in transdermal delivery is the ~ 30 µm thick stratum corneum which acts
77 as the primary barrier for the diffusion and drug penetration (Cevc and Vierl, 2009). Various
78 strategies have been followed to improve delivery of drugs through skin among these is the
79 use of nanoparticulate carriers based on polymers (Prow et al., 2011).

80

81 Chitosan is a cationic polysaccharide made of 2-acetamido-2-deoxy-d-glucose (N-acetyl
82 glucosamine, GlcNAc), and 2-amino-2-deoxy-d-glucose (glucosamine, GlcNH₂) with β-d-(1
83 → 4) glycoside linkages as shown in Figure 1.a. Types of chitosan are differentiated by the
84 degree of N-acetylation (DA). Chitosan contains free amino groups which render it insoluble
85 in water. However, the amino groups undergo protonation in acid and therefore it becomes
86 soluble in aqueous solution. It has very low toxicity and breaks down slowly to harmless
87 products (amino sugars) which are absorbed by the body (Arai et al., 1968). Chitosan is also
88 recognized as a permeation enhancer due to its mucoadhesive properties. It binds to the
89 epithelial cell membrane and the positive charges result in F-actin depolymerisation and
90 disbandment of the tight junction protein ZO-1, leading to opening the tight junctions. With
91 all these attributes, chitosan is a desirable polymer and therefore has been widely used in
92 preparation of micro- and nanoparticles (Agnihotri et al., 2004).

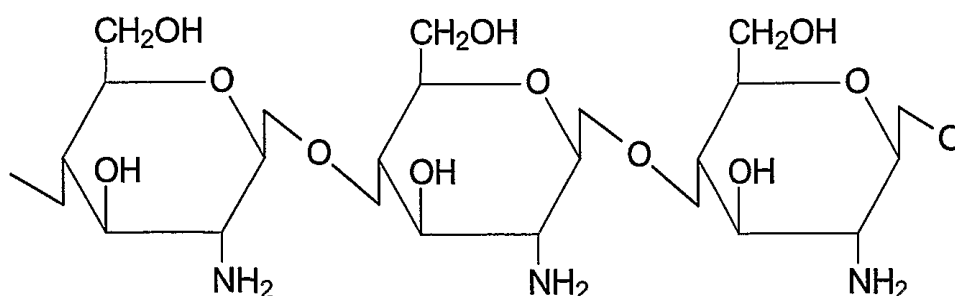
93

94 Nanoparticles are characterised by a mean particle diameter of $\leq 1 \mu\text{m}$ (Gan et al., 2005).
95 These colloidal polymeric drug carriers are used to protect drugs from premature degradation
96 and prevent interaction with the biological environment. Furthermore, they enhance
97 bioavailability, absorption and penetration into the specific target tissues (Budhian et al.,
98 2007). Since drug uptake at the cellular level is size-dependent, smaller particles are taken up

99 to a higher extent (Ubrich et al., 2004). It has been reported that a particle size of less than
100 500 nm is crucial for transdermal delivery (Kholi and Alpar, 2004).

101 For topical application, nanoparticulate systems are needed to be dispersed into suitable
102 semisolid vehicle such as hydrogels to maintain adherence on the skin. However, when
103 dispersed, the characteristics of the dispersed systems as well as the vehicle may be affected.

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Figure 1.a. Structure of chitosan

108

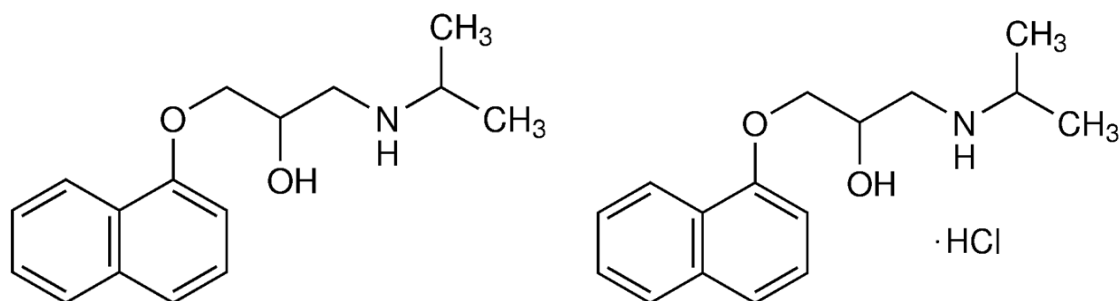
109 Propranolol (PLN) (Figure 1.b), a non-selective β -blocking agent is commonly used for
110 cardiovascular conditions such as hypertension, angina pectoris and cardiac arrhythmia.

111 Propranolol has only an approximate half-life of 4 hours which requires frequent dosing to
112 maintain a therapeutic effect. Although PNL is rapidly absorbed from the gastrointestinal

113 tract, high oral doses are necessary due to an oral bioavailability of less than 23%, extensive
114 first-pass metabolism and susceptibility to enzymatic degradation. It is currently available as

115 an oral preparation and an intravenous formulation which is usually exclusive for hospital
116 use.

117



118

119

Figure 1.b: Structure of PLN and PNL-HCl

120

121 The aim of this study was to develop a transdermal delivery system for propranolol based on
122 chitosan nanoparticles dispersed into gels in attempt to improve the systemic bioavailability of
123 the drug. The properties of the nanoparticles as well the gels before and after dispersion of
124 nanoparticles into gels were evaluated.

125

126 **2. MATERIALS AND METHODS**

127 **2.1 Materials**

128 Propranolol-HCl, medium molecular weight chitosan and pentasodium tripolyphosphate (TPP)
129 were purchased from Sigma-Aldrich Chemical Co. Ltd (New Zealand). Carbopol 940
130 (carbopol) was purchased from Lubrizol Advance Materials, Inc (USA) and Poloxamer 407
131 was purchased from BASF (Germany).

132

133 **2.2 Formulation and characterisation of nanoparticles**

134 **2.2.1 Preparation of nanoparticles**

135 Nanoparticles were prepared by the ionic gelation method (Gan et al., 2005) at room
136 temperature with combinations of chitosan (0.1%, 0.2%, 0.3%) and TPP (0.02%, 0.05%,
137 0.08%) in triplicates. Chitosan was dissolved in acetic acid solution adjusted to pH 4.5 and
138 TPP was dissolved in Milli-Q water. TPP solution was added dropwise to an equal volume of
139 chitosan solution under magnetic stirring at 650 rpm over 60 minutes. The formed
140 nanoparticles were immediately analyzed for particle size and zeta potential in order to obtain
141 appropriate polymer concentrations for further investigation of propranolol-loaded chitosan
142 nanoparticles.

143 Propranolol-loaded chitosan nanoparticles were prepared by the same method mentioned
144 above, but that the appropriate amount of PNL was dissolved in chitosan solution before the
145 dropwise addition of TPP solution. To study the effect of propranolol concentration on the
146 physicochemical properties of nanoparticles, chitosan 0.3% was used in the ratio of
147 propranolol to chitosan at 1:1, 2:1 and 3:1. Whereas to study the effect of chitosan
148 concentration, 20 mg of propranolol was used in the ratio of propranolol to chitosan at 1:0.5,
149 1:1, and 1:1.5.

150 **2.2.2 Particle size and zeta potential measurements:**

151 Particle size, zeta potential and polydispersity index (PDI) of nanoparticle formulations were
152 measured using a Zetasizer Nano Series Nano-NS (Malvern Instruments, UK). Each sample
153 was measured in triplicate and the average results were calculated.

154 **2.2.3 Determination of entrapment efficiency:**

155 The entrapment efficiency of propranolol was calculated by measuring the amount of free
156 drug left in supernatant after centrifugation. Briefly nanoparticle suspensions were
157 centrifuged (Eppendorf MiniSpin Plus Microcentrifuge) at 13,000 rpm for 30 min. The
158 supernatants were diluted and amount of recovered propranolol was determined
159 spectrophotometric ally at 280 nm. The entrapment efficiency was calculated using the
160 following equation:

161

$$162 \quad \% \text{ EE} = \frac{(\text{Total drug added} - \text{free drug})}{\text{Total drug added}} \times 100$$

163

164 **2.2.4 Morphology of propranolol loaded chitosan nanoparticles:**

165 The morphology of freshly prepared and freeze dried propranolol-loaded chitosan
166 nanoparticles were examined using scanning electron microscopy. Samples were coated in a
167 polaron Sc7640 sputter coater and analyzed by a Phillips x L30SFG with SiLi (Lithium
168 drifted) super ultra Thin Window EDS detector.

169 **2.3 Formulation and characterisation of gels**

170 **2.3.1 Formulation of gels**

171 Poloxamer gels (15% w/v) were prepared using the cold technique. Poloxamer was slowly
172 added to certain volume of cold Milli-Q water (5-10 °C) with constant stirring for 60 min at
173 650 rpm. Additional amount of Cold Milli-Q water was added to the solution at 30 min to
174 make up to the volume. Poloxamer solutions were kept in the refrigerator (4-5 °C) overnight
175 then kept at room temperature for a further 24 hrs (Singh et al., 2009).

176 Carbopol 940 (1% and 2% w/v) gels were prepared by dispersing appropriate amount of
177 carbopol into certain volume of Milli-Q water at room temperature with constant stirring for
178 60 min at 650 rpm (9). Milli-Q water was added to the solution at 30 min to make up the
179 volume to the total amount. Carbopol gels were kept at room temperature for 24 h.

180 15% poloxamer / 1% carbopol combination gels were prepared with similar methods as
181 above. Poloxamer was dissolved in cold Milli-Q water and carbopol was separately dissolved
182 at room temperature in Milli-Q water of the same volume. Both were stirred at 650 rpm for
183 60 min. At 30 min, the two were mixed and Milli-Q water was added to the mixture with
184 stirring to make up the volumes to the total amount. These gels were kept at room
185 temperature for 24 h.

186 Each of the above gels containing 0.6% (w/v) propranolol-HCl was prepared separately.
187 Similar steps as above were followed except propranolol-HCl was dissolved in a small
188 volume of Milli-Q water before the final gels were made up to the volume. pH of all of the
189 gels were adjusted to 5.5.

190 **2.4 Formulation of nanoparticles/gels transdermal delivery systems:**

191 The nanoparticulate system and gel were selected after studying different parameters
192 affecting the formulations. The selected freeze dried nanoparticulate system was incorporated
193 into the gel, and the characteristics of resultant transdermal delivery systems was evaluated
194 and compared with gels containing drug.

195 **2.4.1 Rheological measurements:**

196 The rheological behaviours of the gel and transdermal delivery system formulations
197 consisting of nanoparticles dispersed into gel were measured at 25 °C and 33 °C (Digital
198 Viscometer Brookfield DV-III). Measurement at 33 °C was required to represent the skin
199 temperature. Spindle sizes (CP40 and CP52) were used depending on the thickness of the gel
200 and shear rate adjustments for a torque between 5-100%.

201 **2.4.2 Texture analysis**

202 Adhesive capacity of gels and transdermal delivery systems were measured using (Stable
203 Micro System Texture Analyser TA. XT. Plus). The adhesive test settings were as follows;
204 test speed=0.5 mm/sec, force applied=100 g, contact time=5 seconds, trigger force=5 g.
205 Maximum force (N) was recorded from the texture analysis graph.

206 **2.4.3 Preparation of pig ear skin:**

207 Fresh male pig ears were obtained from the abattoir (Auckland Meat Processors, Auckland,
208 New Zealand). Ears were washed with water and dried. The subcutaneous tissue of the skin
209 was carefully removed using a scalpel to retain the stratum corneum of the skin. The skin
210 specimen was cut into appropriate sizes and washed with normal saline.

211 **2.4.4 *In vitro* and *ex vivo* drug permeation studies**

212 *In vitro* and *ex vivo* drug permeation studies were conducted with Franz diffusion cells (FDC-
213 6 Logan Instruments Corp). Cellulose membrane or freshly excised pig ear skin were
214 mounted between the donor and receptor cell (stratum corneum side facing the donor). For
215 the *ex vivo* permeation study, pig stratum corneum was equilibrated in Franz cells overnight
216 for hydration (El Maghraby, 2009). The receptor compartment was filled with pH 6.8 PBS
217 and its temperature was maintained at 37 ± 1 °C by circulating water bath in order to ensure
218 that the surface membrane temperature was 32 ± 1 °C (Wissing and Müller, 2002). The

219 donor compartment contained the following samples; propranolol loaded chitosan
220 nanoparticle suspension, gels and nanoparticles in gels. 0.5 mL samples were withdrawn at
221 different time intervals and replaced with an equal quantity of PBS into the receptor
222 compartment (Parsaee et al., 2002). All samples were analysed for propranolol content by
223 spectrophotometer at 280 nm (UV/Visible Spectrophotometer Libra S32PC).
224 SEM imaging was taken for pig stratum corneum used in *ex vivo* release study and was
225 compared with untreated pig stratum corneum (Herkenne et al., 2006).

226

227 **3. Results and discussion:**

228 **3.1 Formation and physicochemical properties of chitosan nanoparticles:**

229 Particle size is one of the most important factors in the development of nanoparticles,
230 especially for transdermal delivery as there are evidences of skin penetration of very small
231 nanoparticles into viable tissues (Ryman-Rasmussen et al., 2006; Zhang et al., 2008).

232 Table 1 shows the effect of polymer and TPP concentration on nanoparticle parameters
233 prepared without drug. The smallest drug free nanoparticles were achieved with 0.2%
234 chitosan and 0.05% TPP as they were in the size range of 160-210 nm. This was found to be
235 the optimum polymer to TPP ratio for favourable electrostatic interactions to yield small
236 nanoparticles.

237 The polydispersity index (PDI) which describes the size distribution was also the least for the
238 combination of 0.2% chitosan and 0.05% TPP. Nanoparticles formulated with 0.1% chitosan
239 were significantly larger than those obtained using 0.2% and 0.3% of chitosan with minimal
240 variation between the latter two. de Moura et al. (2009) reported an opposite trend where an
241 increase in particle size was observed with increasing chitosan concentration. They explained
242 the reason for their findings as the solubility of chitosan becomes less at increasing
243 concentrations leaving free particles to aggregate. However the trend observed in this study
244 can be a consequence of the CS/TPP ratio as the ratio is another significant factor that can
245 impact the particle size (Ricci et al., 2005). Table 1 shows that 0.02% and 0.08%
246 concentrations of TPP generally produced larger nanoparticles than 0.05% TPP. Wide PDI
247 was achieved with 0.1% and 0.3% chitosan whereas 0.2% chitosan showed a narrow PDI
248 indicating that nanoparticles with this polymer concentration possessed more uniform
249 nanoparticle size.

250 All nanoparticle formulations reported in the table are suitable for transdermal delivery as
251 they are less than 500 nm in size. Zeta potential reflects the density of the surface charge and

252 is influenced by the composition of the particles and the medium in which they are dispersed
253 (Mohanraj and Chen, 2007). In aqueous solution, chitosan changes its conformation and
254 becomes more flexible even with the presence of TPP, and the overall surface charge
255 becomes positive. Nanoparticles with zeta potential above $\pm 30\text{mV}$ are stable in suspension
256 due to the repulsion of surface charge preventing aggregation of nanoparticles (Mohanraj and
257 Chen, 2007). Table 1 shows the zeta potential increased with ascending chitosan
258 concentration due to its cationic nature. However the zeta potential of nanoparticles prepared
259 with 0.08% TPP was less compared to those prepared with other concentrations which may
260 be due to polyanionic nature of TPP.

261

262 The concentrations of propranolol-HCl have been varied in our research in order to study its
263 effect on the properties of the nanoparticles (Table 2). Nanoparticles containing 0.2%
264 chitosan and 0.05% TPP were used for this study. Generally, there was an increase in size
265 and zeta potential of the nanoparticles with the addition of propranolol-HCl. However,
266 smaller nanoparticles were achieved with 1:2 chitosan to propranolol-HCl ratio at 266.47 nm.
267 The table shows that the zeta potential decreases as the concentration of propranolol increases
268 in the formulation which was possibly because of interactions between the positively charged
269 chitosan and negatively charged propranolol-HCl.

270 Table 3 illustrates the effect of increasing chitosan content in propranolol loaded chitosan
271 nanoparticles. Nanoparticles consisting of 0.2% chitosan and 0.05% TPP were used for this
272 experiment. The table shows that 1:1 chitosan to propranolol-HCl ratio produced particles
273 with average size of 166.53 nm which is comparable to the size of drug free nanoparticles
274 prepared at the same chitosan and TPP content. This may be due to the favourable ratio of
275 cationic and anionic charges between the polymers, TPP and propranolol-HCl. The zeta
276 potential of this formulation was found to be $> \pm 30\text{ mV}$ indicating good physical stability of
277 nanoparticles. PDI has increased with drug incorporation. This may be again due to the
278 aggregation of nanoparticles as explained above. When propranolol-HCl was increased (table
279 2), an increase in entrapment efficiency and drug loading was observed due to the increased
280 drug available for incorporation into the nanoparticles. However at constant propranolol-HCl
281 concentration (table 3) a decrease in entrapment efficiency and drug loading occurred with
282 increasing chitosan concentrations possibly due to the increase of electrostatic repulsion
283 between chitosan polymers. The high drug loading ability and small nanoparticles

284 demonstrated to provide a positive prospect for the further development of the nanoparticles
285 for transdermal delivery.

286 The SEM micrographs of PNL-HCl loaded nanoparticles (Figure 2) have shown that most of
287 nanoparticles were less than 300nm. However, there was some aggregation of nanoparticles
288 which could be due to unpurified chitosan. After freeze-drying of PNL-HCl-loaded
289 nanoparticles, the fluffy and feathery appearance of nanoparticles was observed (Figure 3).
290 This structure shows hygroscopic properties thus it is readily dispersible in aqueous phase.

291

292 From this part of the study it can be concluded that, 0.2% chitosan and 0.05% TPP were
293 selected as the nanoparticulate system for the final formulation and to be incorporated into
294 the selected gel as this combination provides the smallest size and suitable zeta potential with
295 high entrapment efficiency and PNL-HCl loading into nanoparticles.

296 **3.2 Rheological behaviour of the formulated gels**

297 Nanoparticles suspension has low viscosity and therefore the particles will not remain on the
298 skin surface for drug absorption and penetration to take place. To overcome this problem, the
299 selected propranolol nanoparticles formulation was incorporated into gels made of mixture of
300 poloxamer and carbopol as these are the most widely used polymers in gel formulation.

301 In this study, we have investigated the rheological properties of gels prepared from either
302 poloxamer or carbopol with and without presence of the drug. We also investigated the
303 changes that happen to the rheological properties of these gels when they were combined
304 together before and after incorporation of the nanoparticles.

305 The rheological characterization was conducted at room temperature 25°C and skin
306 temperature 33°C. In order to maintain the consistency of pH and optimum gel viscosity, few
307 drops of 1M NaOH were added for pH adjustment to 5.5, this method is suitable for both
308 poloxamer and carbopol gels as mentioned by Lu et al. (1998). Figure 4 shows shear stress
309 versus shear rate of carbopol gels. The shear stress increased by increasing the shear rate.
310 Figure 5 shows a decrease in gel viscosity by increasing the shear rate indicating shear
311 thinning (pseudo-plastic) behavior of the gel. Poly (acrylic acid) carbopol is a pH sensitive
312 polymer. It changes to stiff gel in aqueous solution when the pH is raised. In our study,
313 addition of NaOH to adjust the pH may have increased carbopol ionization in the aqueous
314 solution and resulted in electrostatic repulsion between the adjacent carboxyl groups and an
315 expansion of the polymer network. From figure 4 it can be seen that 2% carbopol gel
316 possessed higher shear stress than 1% carbopol gel for the same shear rate exerted. This could

317 be due to the increased amount of polymer available leading to increased electrostatic
318 repulsion, polymer swelling and consequently increased elastic solid behavior. No marked
319 difference in the shear stress was observed when the temperature was increased from 25 to
320 33°C. This indicates that pH and polymer concentration are the major factors contributing to
321 gelling properties of carbopol.

322 The flow curves of poloxamer formulations (Figure 6) at the experimental conditions
323 investigated exhibited a Newtonian flow as demonstrated by a linear increase in shear stress
324 with increasing shear rate. Poloxamers are in situ gelling polymers as they perform sol to gel
325 transition by enhancement of the elasticity network when the temperature increases (Santos et
326 al., 2015). It has been reported that when the concentration and temperature of the polymer
327 are above a critical value, poloxamer molecules in aqueous solution will self assemble to
328 form spherical micelles with a dehydrated PPO core surrounded by hydrated swollen PEO
329 chain (Dholakia et al., 2012). Therefore gelation in this case, is the result of micelles
330 entanglement and packing. Thus the results presented in Figure 6 suggest that the poloxamer
331 solutions didn't undergo phase transition to turn into gels and remained as free flowing
332 liquids. This could be due to that the experiments were conducted at conditions below the
333 gelation temperature of poloxamer which is 36°C therefore the molecular structure of the
334 polymer solution didn't change. The flow curves presented in figure 7 confirm these findings
335 and reveal that the viscosity remained constant by increasing the shear rate.

336 When carbopol and poloxamer gel were mixed the rheological properties of the resultant
337 system have changed significantly. The rheograms presented in Figure (8) show thixotropic
338 behaviour of gels consisting of combination of carbopol and poloxamer and containing either
339 propranolol-HCl or propranolol loaded nanoparticles as the downward curve was displaced
340 with regards to upward curve. Thixotropy can be defined as isothermal and a slow recovery
341 upon standing of a material of a consistency lost through shearing. These systems are
342 characterized by a decrease in viscosity when they are subjected to shear stress due to the
343 time dependant reformation of the secondary structure. Figure 8 also shows that hysteresis
344 loop formed by the up and down curves of the rheogram is bigger for combination gel
345 containing propranolol nanoparticles than that for gel containing drug alone indicating greater
346 magnitude of structural breakdown and thixotropy of this formulation. This is a desirable
347 property for a topical formulation as the greater the thixotropy, the lower is the settling and
348 sedimentation rate of the nanoparticles in the system. The viscograms presented in Figure 9

349 show that all combination gels possessed non-Newtonian, pseudoplastic (shear thinning)
350 behaviour.

351 The complex rheological properties of systems consisting of nanoparticles dispersed in gels
352 was also reported by others (Chawla and Saraf, 2012).

353 **3.3 Adhesive capacity of the gels**

354 Transdermal delivery system should possess desirable adhesiveness as weak adhesion may
355 results in incomplete absorption of drug through skin. In this study, the adhesiveness of the
356 designed transdermal delivery systems was investigated and the results are presented in
357 Figure 10. The adhesive capacity is dependent on the type and concentration of bioadhesive
358 polymer used in the formulation. In formulation based on Carbopol, the adhesiveness of the
359 gel increased as the concentration of the polymer increased. This may be attributed to the
360 increased number of the hydrophilic carboxyl functional groups available for binding, but
361 may also be a function of increased tack of the gel. Choi et al. (1998) have reported an
362 increase in the adhesive forces of gels by increasing carbopol concentration. From the figure,
363 it can be seen that poloxamer solution possessed significantly lower adhesiveness than
364 Carbopol gel. However, combining carbopol to poloxamer has increased the adhesive
365 properties of both polymers. These results are in agreement with Qi et al., 2007 who
366 demonstrated an increase in mucoadhesive force of ophthalmic gels when Carbopol was
367 incorporated into poloxamer solution. The possible explanation for these finding is the
368 combined effects of hydrophilic oxide groups of poloxamer and the carboxyl group of
369 carbopol which has improved the binding capacity of the formulation to the underlying
370 surface through electrostatic and hydrophobic interaction.

371 With the addition of propranolol-HCl, the adhesive force of carbopol gels decreased. The
372 effect on poloxamer gels was more substantial with an approximate 75- 80% drop in the
373 adhesive force. Propranolol-HCl causes a decrease in adhesive due to its positive charge
374 which can interact with the negatively charged of carbopol to form a complex. This
375 decreases the negative charge repulsions between carbopol polymers which uncoil and
376 expand, leading to reduction in polymer swelling and gel formation. On the other hand, the
377 hydroxyl group of propranolol molecules can form hydrogen bond with the PEO block of
378 poloxamer molecules (Kim et al., 2002) which may have been responsible for the adhesive
379 force becoming reduced. Interestingly, the contrary was observed for poloxamer and carbopol
380 combination gels. An increase in adhesive force was observed for the combination gels from
381 1.35 to 1.76 N after incorporation of propranolol-HCl. The observed increase can be

382 explained by the steric stabilisation properties of poloxamer which prevents the interaction
383 between propranolol-HCl and carbopol so that the cross-linking, viscoelastic properties of
384 carbopol can be potentiated.

385 The formulation of poloxamer 15% and carbopol 1% containing nanoparticles loaded with
386 propranolol-HCl had an adhesive force of 0.60 N. The chitosan component of the
387 nanoparticles carries a positive charge which can also interact with carbopol. It is observed to
388 cause a significant difference in the adhesion of gels compared with the addition of
389 propranolol-HCl due to the increased positive charge preventing the electrostatic repulsions
390 between carbopol.

391 **3.4 *In vitro* drug release study**

392 The effect of the type of transdermal formulation on the release of propranolol through
393 cellophane membrane was investigated and the results are illustrated in Figure 11. The
394 release profiles followed predictable trends in relation to each other. For propranolol
395 containing buffer solution, the release of propranolol was very rapid and approximately 65%
396 of drug was released in 24 h. However, when propranolol-HCl was dispersed in combination
397 gel system, the release rate has reduced significantly. It was thought that this effect was the
398 result of combining the swollen carbopol with poloxamer solution which has increased the
399 density of the chain structure of the gel and reduced the diffusion of propranolol through the
400 formulation. Figure 11 shows chitosan nanoparticles yielded lowest cumulative mass of drug
401 released. Only 7% and 11 % of propranolol was released in 24 hours from the nanoparticle
402 suspension and nanoparticle/gel. This can be explained by the sustained release properties of
403 cross-linked chitosan and hydrophobic interactions with propranolol-HCl has led to a delayed
404 and an incomplete release of drug from the nanoparticles (Ubrich et al., 2004). It was noticed
405 that the burst effect from these systems was negligible and release profiles were almost
406 linear. Generally, the release of propranolol from each formulation remained steady after 10 h
407 except for propranolol in gel which was shown to increase until at least 24 h.

408 **3.5 *Ex vivo* drug release study**

409 Permeation studies were conducted in an attempt to assess the effect of the nanoparticles-Gel
410 transdermal system on the skin uptake and permeation properties of propranolol. The studies
411 were performed across pig ear skin since it can be considered as a reasonable model for
412 human barrier (Testa et al., 2001). The % cumulative mass of propranolol permeated across
413 the skin of different transdermal formulations over 24 hrs is shown in Figure 12. An initial
414 burst of drug permeation was noticed from the formulations in the first 5hrs, after which the

415 drug continued to permeate slowly and steady. The permeation profiles have exhibited zero
416 order kinetics with r^2 values of 0.9911, 0.9973 and 0.9622 for gel, nanoparticles suspension
417 and nanoparticles in gel respectively. Of all formulations investigated, gel showed the highest
418 permeation rate. This can be explained by the high drug release properties of the gel system
419 which resulted in an increase in drug concentration in the donor compartment and an increase
420 of the concentration gradient towards the skin. Figure 12 shows that the permeation rate of
421 propranolol from nanoparticles in gel was the lowest. It has been reported that both high and
422 low permeation rates are of interest in skin application. Enhanced permeation rate can
423 improve drug permeability through skin whereas; sustained release can provide the skin with
424 drug over long period of time. It was noticed that The Papp values (Table 4) have confirmed
425 the permeation profiles results and they have followed this order which is gel > nanoparticle
426 suspension > nanoparticles in gel. The same trend was also observed from release studies. The
427 fact that trend of Papp values is the same as the release rate from formulations suggests that
428 the mechanism of propranolol permeation through the skin is formulation controlled rather
429 than skin controlled. In an attempt to support this finding, the skin uptake effect was followed
430 using scanning electron microscopy. The SEM micrographs of untreated and treated pig
431 stratum corneum with nanoparticles in gel formulation are shown in Figure 13 (a&b). Figure
432 (a) indicates that there are unblocked and clear pores before treatment. However, after
433 treatment the micrograph reveals no clear pore since the nanoparticles have penetrated
434 through the stratum corneum and blocked all the pores. One of the interesting properties of
435 chitosan is that it can widen the tight junctions between the mucoepithelial cells reversibly by
436 interaction of the protonated CS with anionic components of glycoprotein on the surface of
437 the epithelial cells and with fixed negative charges in the interior of the tight junction, which
438 lead to absorption enhancement of the drug (Yeh et al., 2011). Therefore, presence of
439 nanoparticles in gel have increased their contact time with the skin and the properties of
440 chitosan might have affected the stratum corneum nature and widened the tight junctions and
441 pores in the skin and allowed the particles to be up taken. Although the Ex vivo studies
442 showed slow permeation rate from nanoparticles in gel over 24 hours, however the results of
443 the SEM suggest that nanoparticles uptaken will create a reservoir of drug within the skin
444 where it provide the system over long period of time with small doses of propranolol to
445 control the systemic blood pressure. From these results it can be concluded that the type of
446 formulation and its unique properties have affected and both the permeation rate of drug and
447 its concentration within the skin.

448 **4. Conclusion**

449 The present work showed that transdermal delivery system for propranolol based on chitosan
450 nanoparticles dispersed into gel was successfully prepared and characterized. The novel gel
451 formulation exhibited thixotropic behaviour with a prolonged drug release properties as shown
452 by the permeation studies through pig ear skin. Furthermore, the SEM images showed that
453 the chitosan nanoparticles were uptaken by the skin which may create a drug reservoir to
454 provide the system with propranolol over long period of time to control the blood pressure.
455 Thus, the nanoparticles gel could be a promising transdermal delivery system for propranolol
456 however, in vivo studies are necessary to confirm this conclusion.

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Table 1:

593 Effect of chitosan and TPP concentrations on the physical properties of the nanoparticles

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% Chitosan	% TPP	Particle size (nm) \pm SD	Average PDI \pm SD	Average ZP (mV) \pm SD
0.1	0.02	462.59 \pm 212.26	0.49 \pm 0.27	15.97 \pm 4.67
	0.05	311.72 \pm 111.70	0.34 \pm 0.13	17.91 \pm 2.23
	0.08	421.56 \pm 102.71	0.43 \pm 0.16	3.09 \pm 8.15
0.2	0.02	254.60 \pm 25.91	0.26 \pm 0.10	53.91 \pm 3.49
	0.05	191.30 \pm 18.33	0.19 \pm 26.40	35.48 \pm 26.36
	0.08	253.10 \pm 16.06	0.28 \pm 16.06	7.12 \pm 10.07
0.3	0.02	215.26 \pm 19.08	0.18 \pm 0.08	63.58 \pm 10.83
	0.05	270.03 \pm 141.66	0.40 \pm 0.16	62.76 \pm 2.55
	0.08	247.22 \pm 14.91	0.30 \pm 0.13	57.08 \pm 2.39

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Table 2

610 Effect of chitosan to propranolol HCl ratio on the physical properties of the nanoparticles.

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Chitosan : PNL-HCl ratio	Particle size (nm) \pm SD	Average PDI \pm SD	Average ZP (mV)	Amount (mg)	EE (%)	Drug loading (%)
Drug free particles	191.30 \pm 18.33	0.19 \pm 26.40	35.48 \pm 26.36	0	0	0
1:1	310.63 \pm 69.78	0.41 \pm 0.01	55.53 \pm 2.20	28.31 \pm 0.24	94.38	80.18
1:2	266.47 \pm 14.81	0.21 \pm 0.05	51.30 \pm 1.74	57.32 \pm 0.33	95.53	89.12
1:3	291.43 \pm 22.38	0.29 \pm 0.06	48.40 \pm 2.43	87.40 \pm 0.17	97.12	92.58

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Table 3

Effect of changing chitosan concentration on the physical properties of nanoparticles

PNL-HCl : chitosan ratio	Particle size (nm)±SD	Average PdI±SD	Average ZP (mV) ±SD	Amount (mg)	EE (%)	Drug loading (%)
Drug free particles	191.30 ± 18.33	0.19 ± 26.40	35.48 ± 26.36	0	0	0
1:0.5	644.70 ± 31.24	0.76 ± 0.04	17.77 ± 1.07	17.72 ± 0.09	88.60	85.52
1:1	166.53 ± 5.55	0.58 ± 0.03	41.90 ± 1.15	17.56 ± 0.14	87.78	77.83
1:1.5	311.63 ± 26.52	0.73 ± 0.21	49.43 ± 0.50	17.45 ± 0.11	87.26	71.37

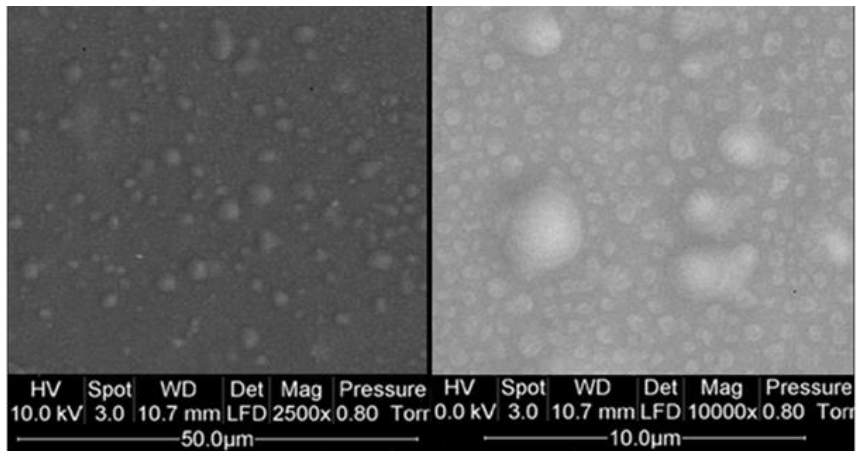
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Table 4

Apparent permeability coefficient of formulations investigated

Formulations	P_{app} (cm/s)
Propranolol-HCL solution	7.702×10^{-7}
Propranolol-HCl gel	1.844×10^{-7}
Propranolol nanoparticle suspension	0.363×10^{-7}
Propranolol HCl nanoparticles gel	0.167×10^{-7}

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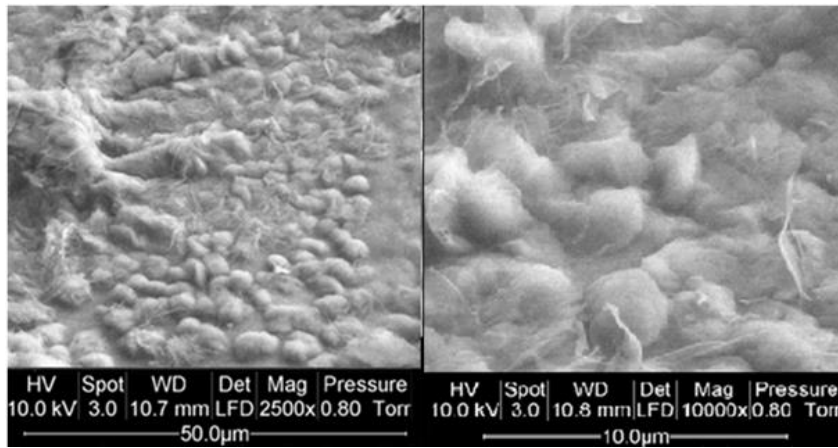
Figure 2. SEM micrographs of wet PNL-HCl-loaded nanoparticles

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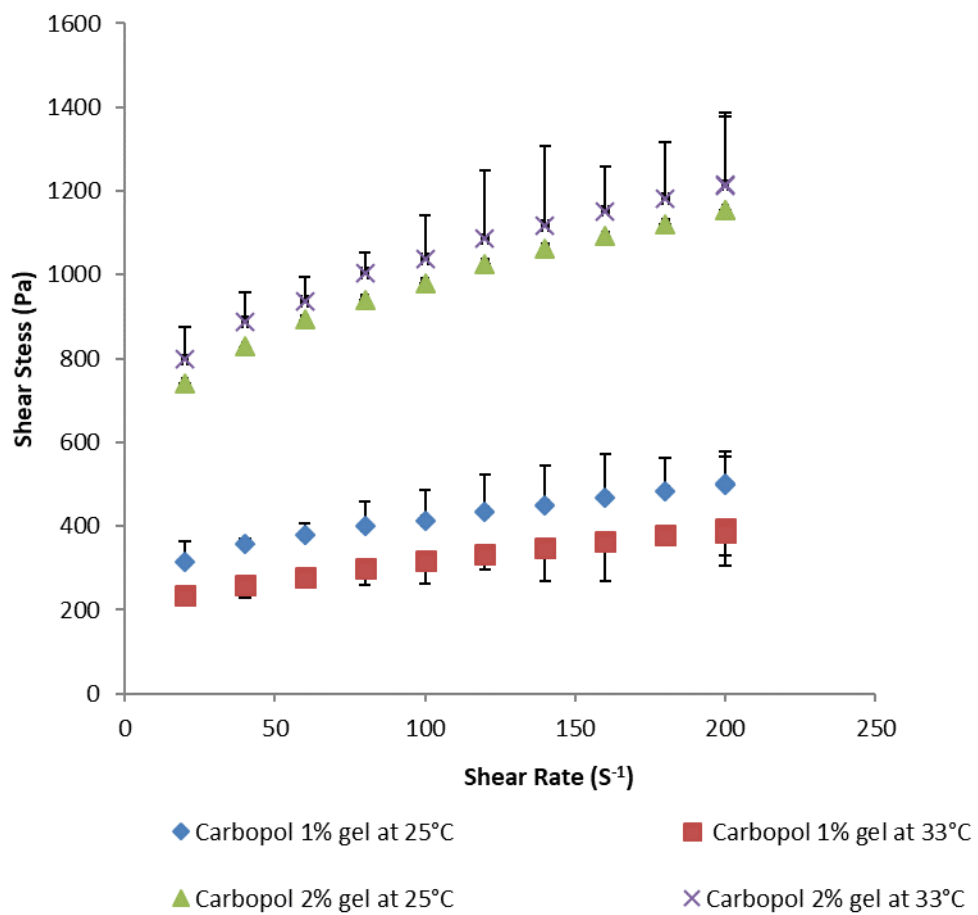
Figure 3: SEM micrographs of freeze-dried PNL-HCl-loaded nanoparticles

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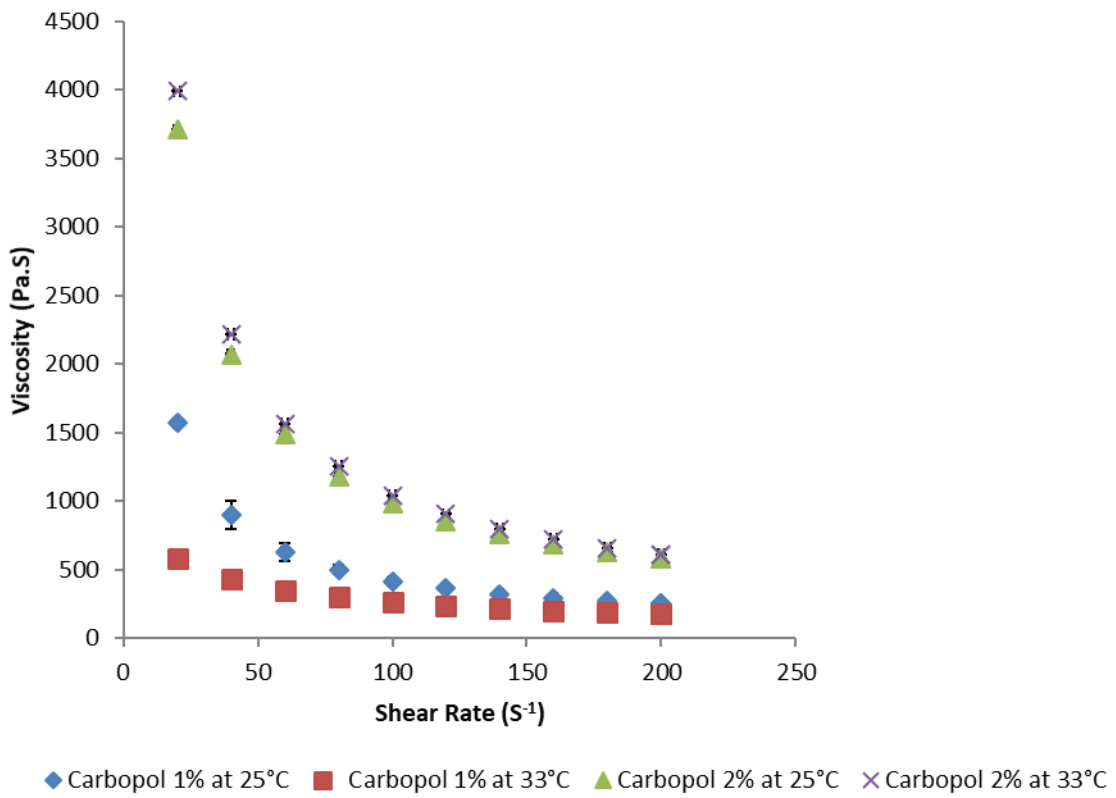
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Figure 4: Rheogram of 1% and 2% carbopol gels at 25 °C and 33 °C

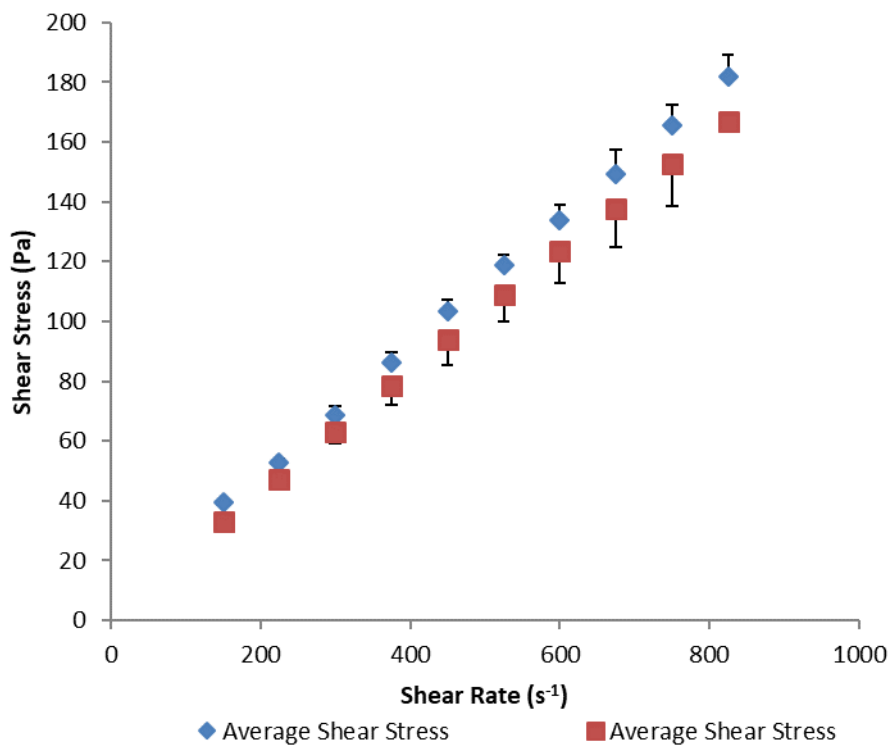
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Figure 5: Viscosity as a function of shear rate of 1% and 2% carbopol polymer at 25 °C and 33 °C

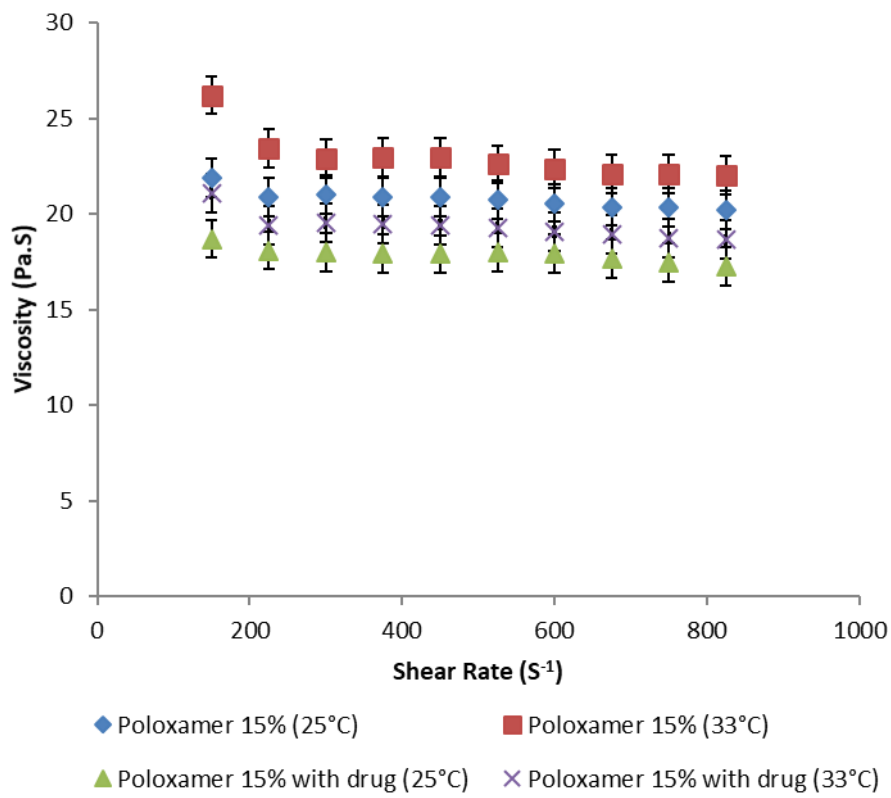
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Figure 6: Rheogram of 15% poloxamer at 25 °C and 33 °C

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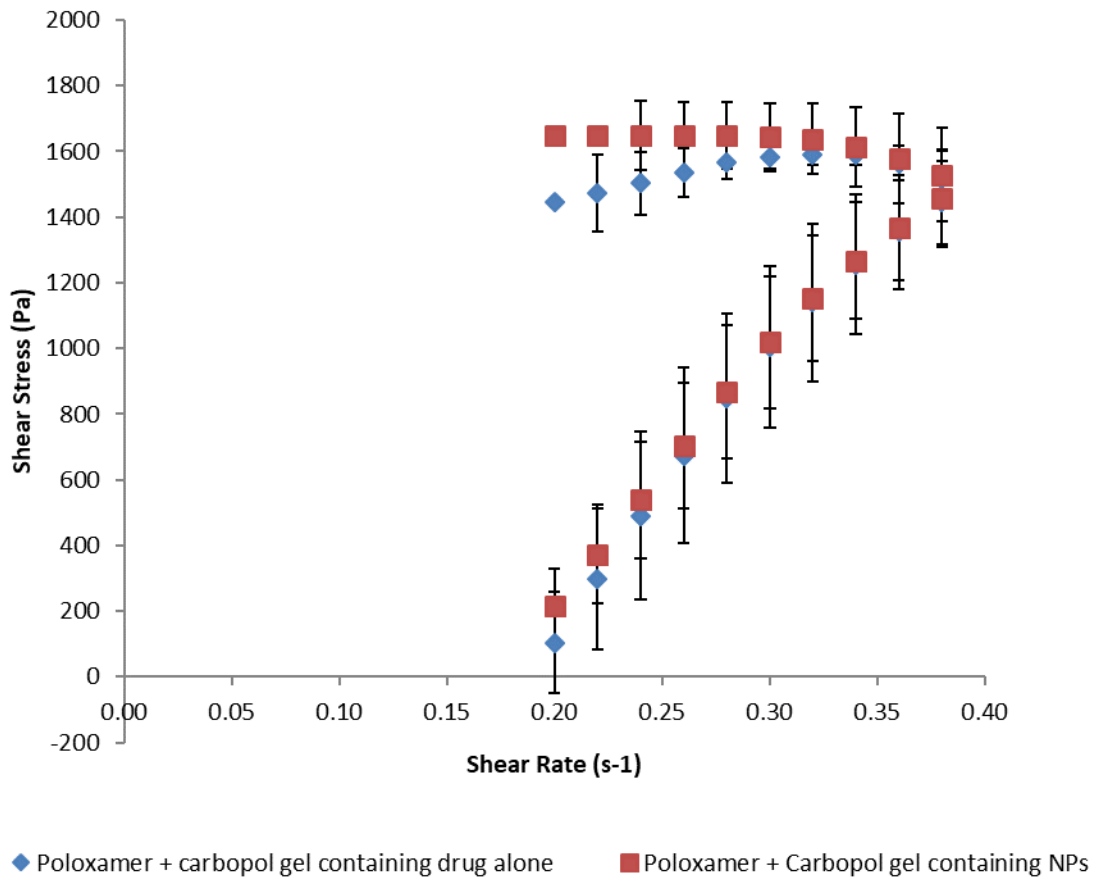


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740 Figure 7: Viscosity as a function of shear rate of 15% poloxamer in presence and absence of
741 PNL-HCl at 25 °C and 33 °C

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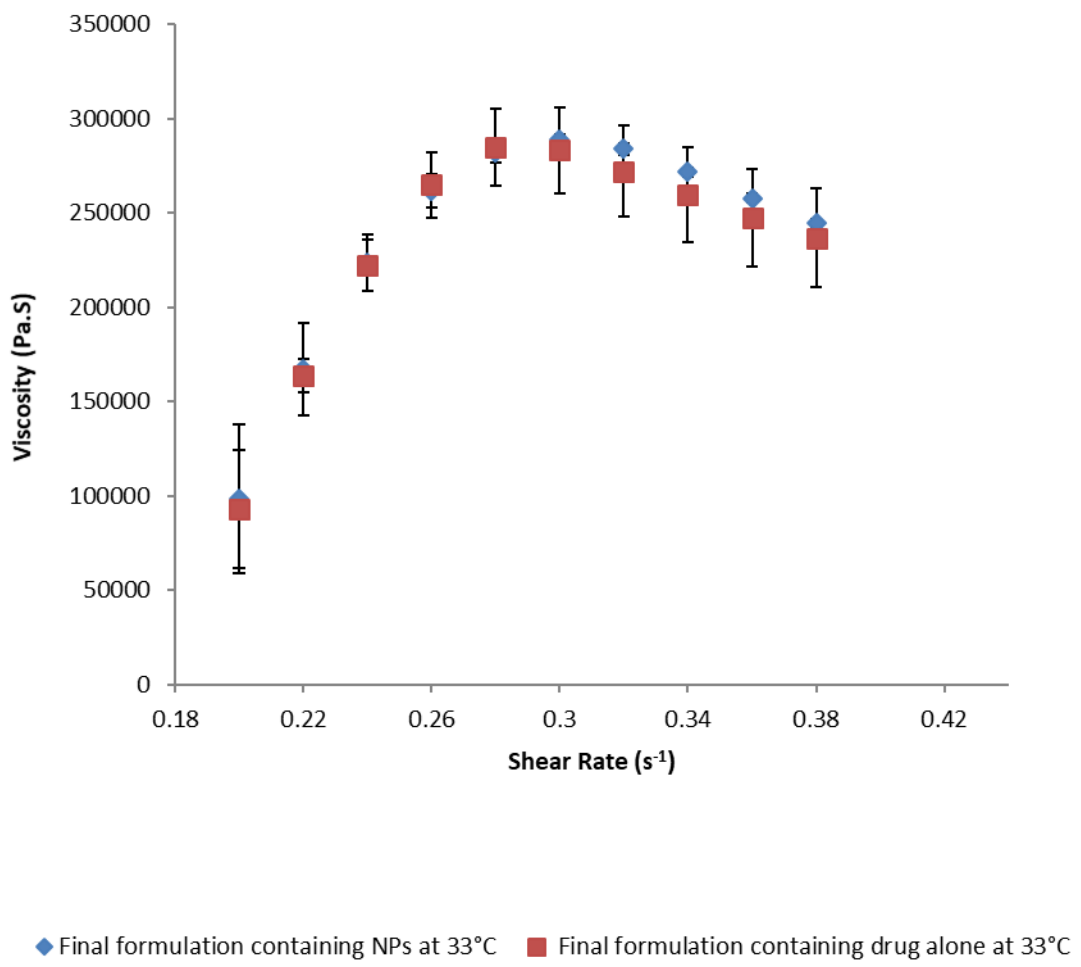
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Figure 8: Rheograms of combination poloxamer 15% and carbopol 1% gels in presence of propranolol-HCl or nanoparticles at 33°C.

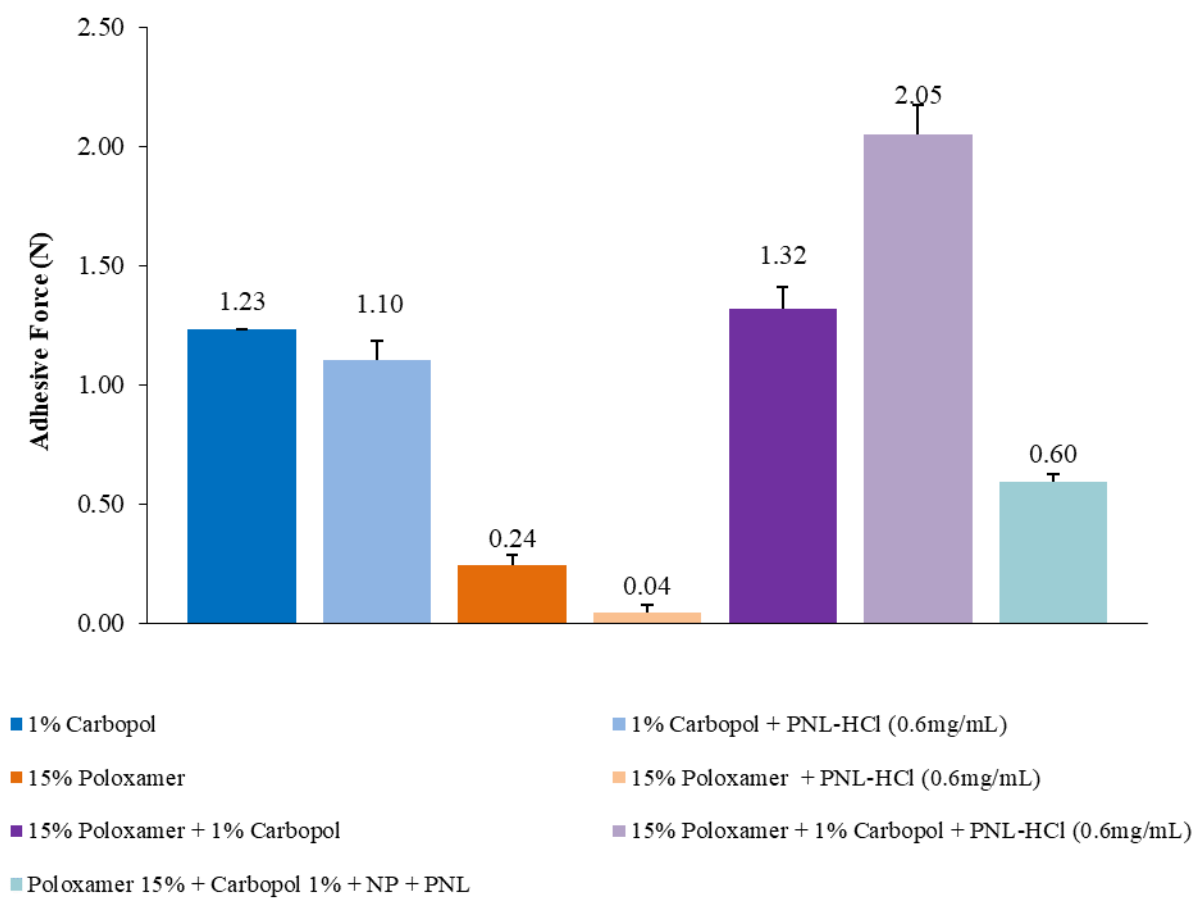
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Figure 9: Viscosity as a function of shear rate of the final formulation consisting of combination of 15% poloxamer and 1% carbopol in presence of nanoparticles or PNL-HCl at 33 °C

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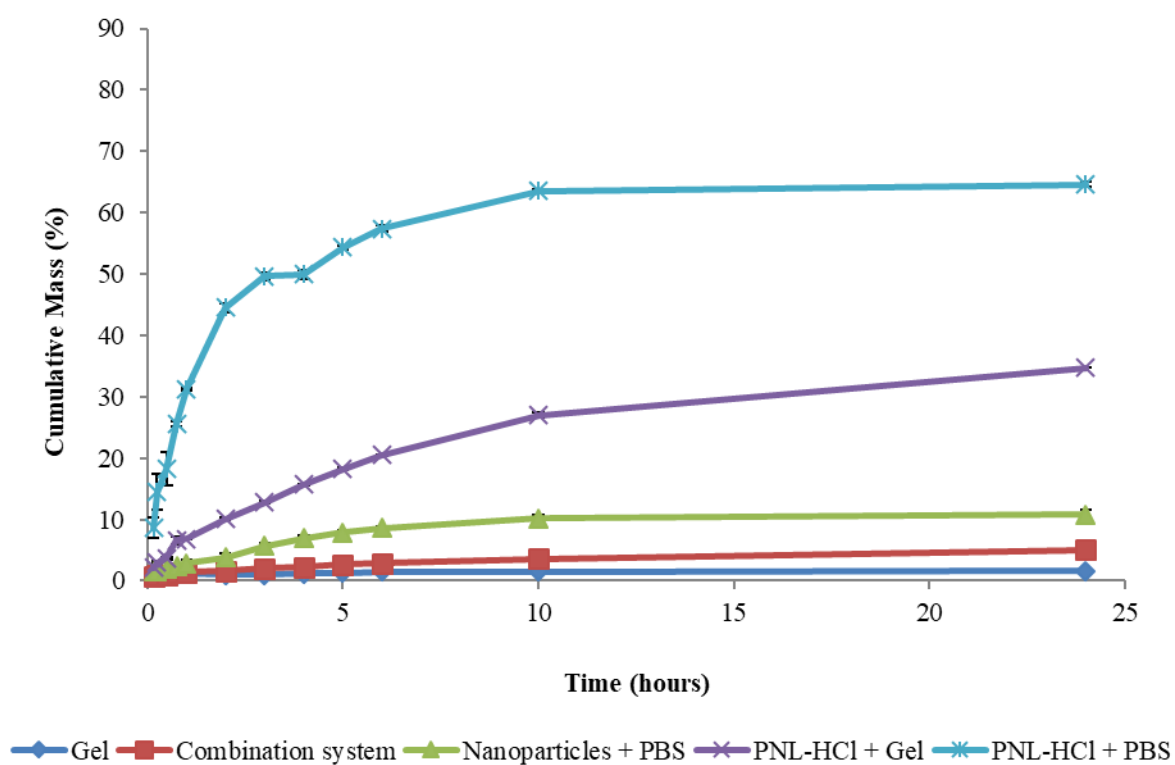
Figure 10: Adhesive capacity of gels at pH 5.5 at room temperature

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789 Figure 11. Cumulative percentage of propranolol release from different formulations at 37°C
790 in phosphate buffer pH 6.8 across artificial membrane

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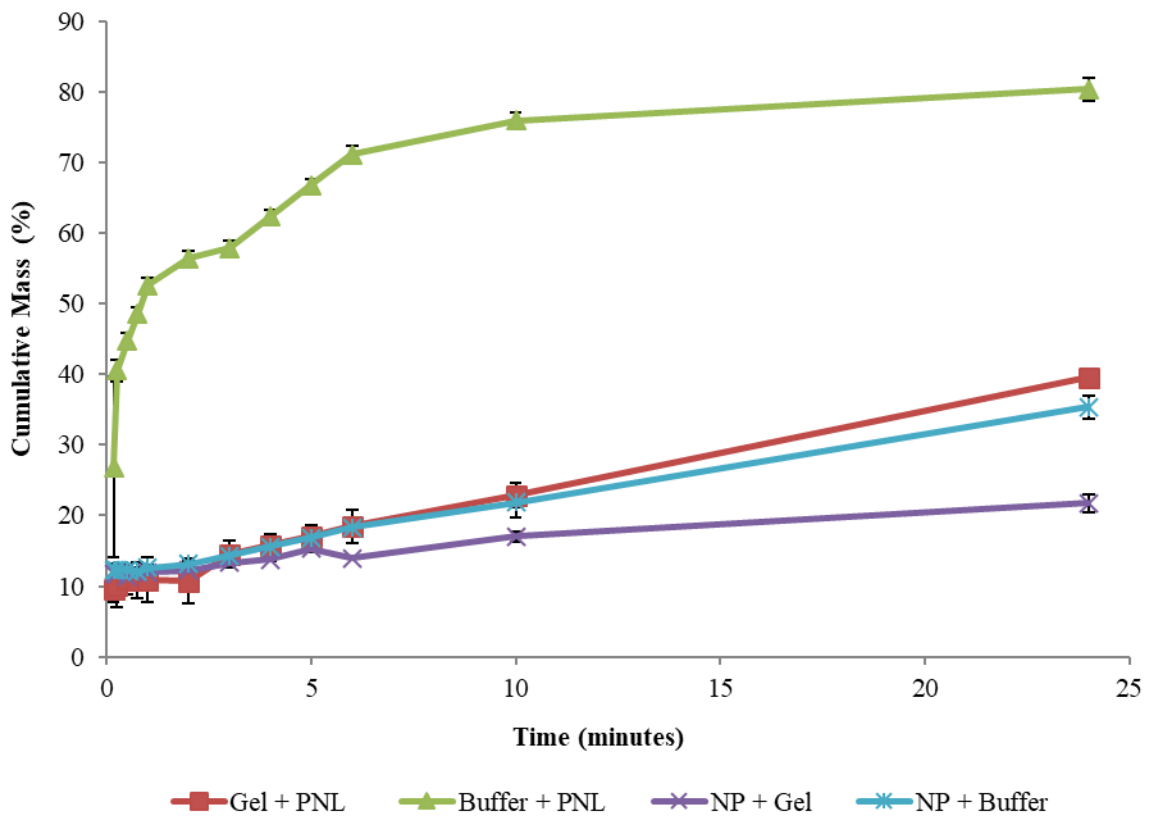
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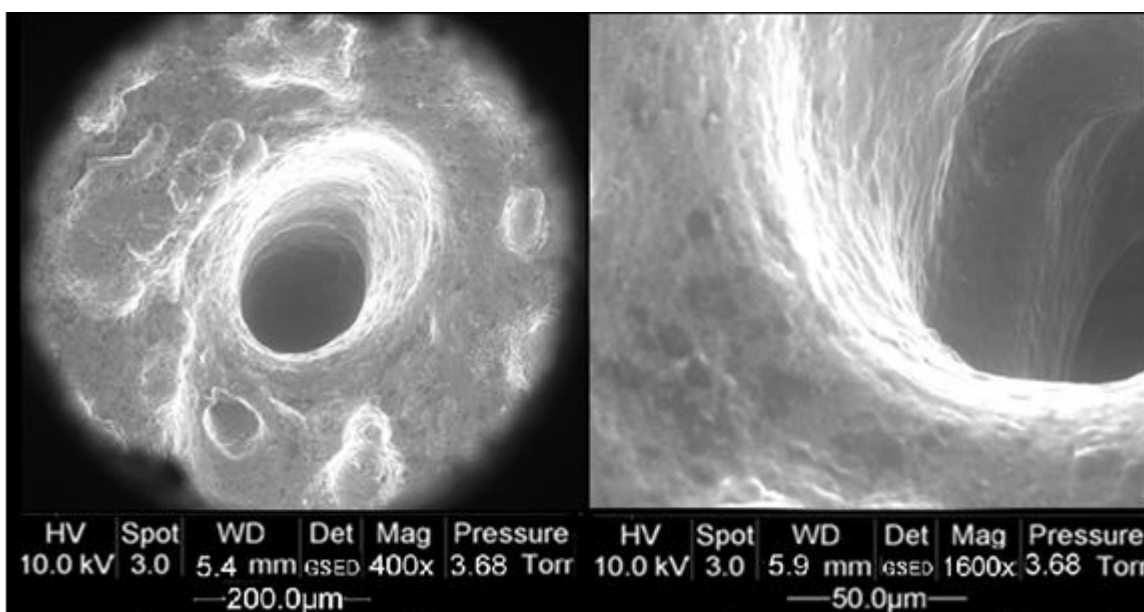
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Figure 12. Cumulative percentage of propranolol release from different formulations at 37 °C in phosphate buffer pH 6.8 across pig ear stratum corneum

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Figure 13a. SEM micrographs of pore of untreated pig stratum corneum

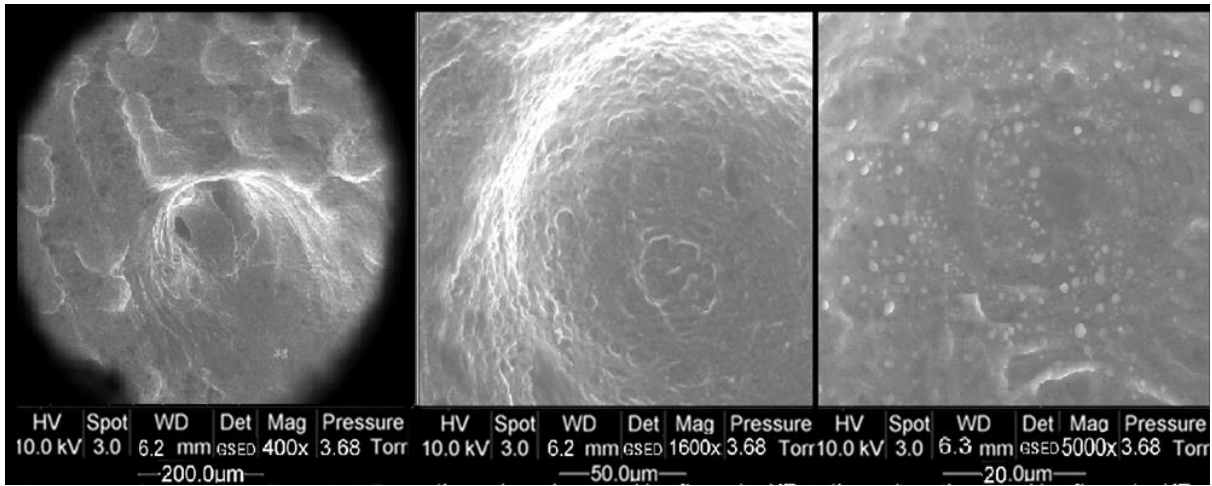


Figure 13b. SEM micrographs of pore of treated pig stratum corneum

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