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

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Abstract

This study aimed at improving the systemic bioavailability of propranolol-HCl by the design of transdermal drug delivery system based on chitosan nanoparticles dispersed into gels.

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

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

1. Introduction

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

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

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

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

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

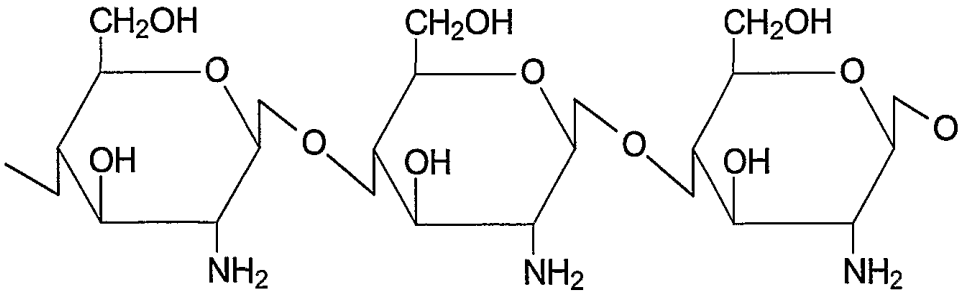

Figure 1.a. Structure of chitosan

Propranolol (PLN) (Figure 1.b), a non-selective β-blocking agent is commonly used for cardiovascular conditions such as hypertension, angina pectoris and cardiac arrhythmia. Propranolol has only an approximate half-life of 4 hours which requires frequent dosing to maintain a therapeutic effect. Although PNL is rapidly absorbed from the gastrointestinal tract, high oral doses are necessary due to an oral bioavailability of less than 23%, extensive first-pass metabolism and susceptibility to enzymatic degradation. It is currently available as an oral preparation and an intravenous formulation which is usually exclusive for hospital use.

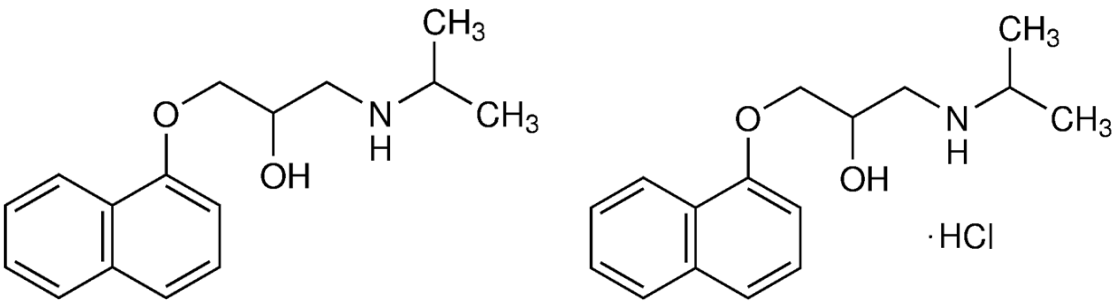

Figure 1.b: Structure of PNL and PNL-HCl

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

2. MATERIALS AND METHODS

2.1 Materials

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

2.2 Formulation and characterisation of nanoparticles

2.2.1 Preparation of nanoparticles

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

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

2.2.2 Particle size and zeta potential measurements:

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

2.2.3 Determination of entrapment efficiency:

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

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

2.2.4 Morphology of propranolol loaded chitosan nanoparticles:

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

2.3 Formulation and characterisation of gels

2.3.1 Formulation of gels

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

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

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

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

2.4 Formulation of nanoparticles/gels transdermal delivery systems:

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

2.4.1 Rheological measurements:

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

2.4.2 Texture analysis

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

2.4.3 Preparation of pig ear skin:

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

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

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

donor compartment contained the following samples; propranolol loaded chitosan nanoparticle suspension, gels and nanoparticles in gels. 0.5 mL samples were withdrawn at different time intervals and replaced with an equal quantity of PBS into the receptor compartment (Parsaee et al., 2002). All samples were analysed for propranolol content by spectrophotometer at 280 nm (UV/Visible Spectrophotometer Libra S32PC).

SEM imaging was taken for pig stratum corneum used in *ex vivo* release study and was compared with untreated pig stratum corneum (Herkenne et al., 2006).

3. Results and discussion:

3.1 Formation and physicochemical properties of chitosan nanoparticles:

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

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

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

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

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

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

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

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

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

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

3.2 Rheological behaviour of the formulated gels

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

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

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

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

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

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

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

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

3.3 Adhesive capacity of the gels

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

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

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

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

3.4 *In vitro* drug release study

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

3.5 *Ex vivo* drug release study

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

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

4. Conclusion

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

5. Acknowledgment:

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Table 1:

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

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

Table 2

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

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

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

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}

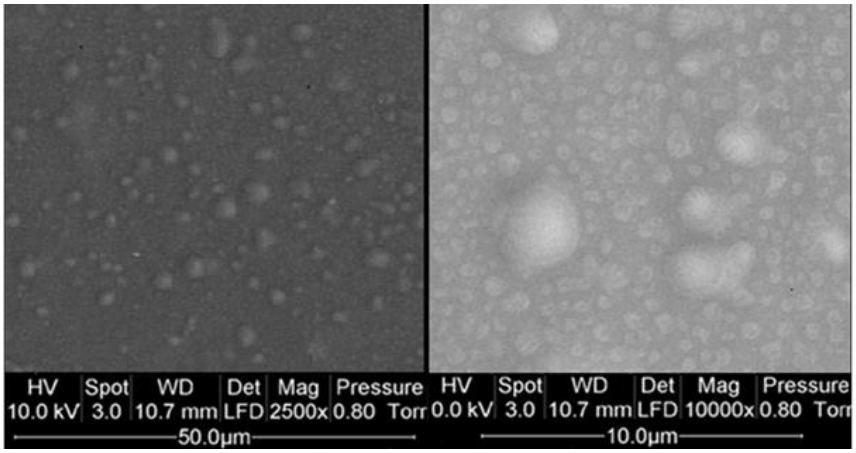

Figure 2. SEM micrographs of wet PNL-HCl-loaded nanoparticles

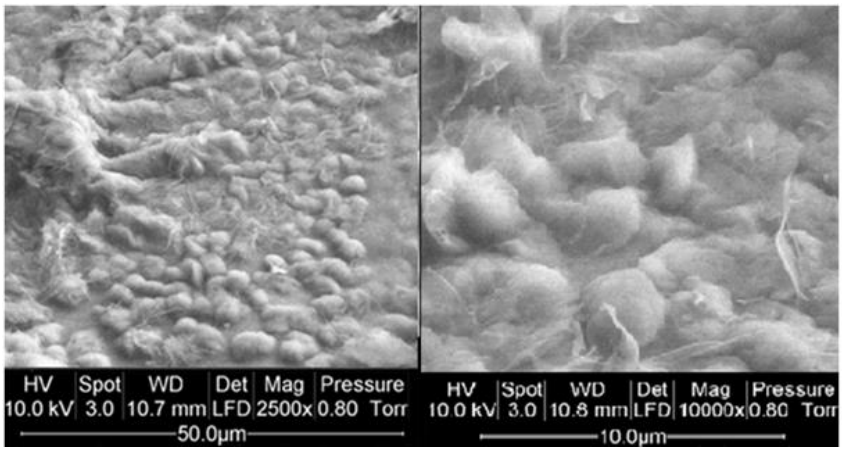

Figure 3: SEM micrographs of freeze-dried PNL-HCl-loaded nanoparticles

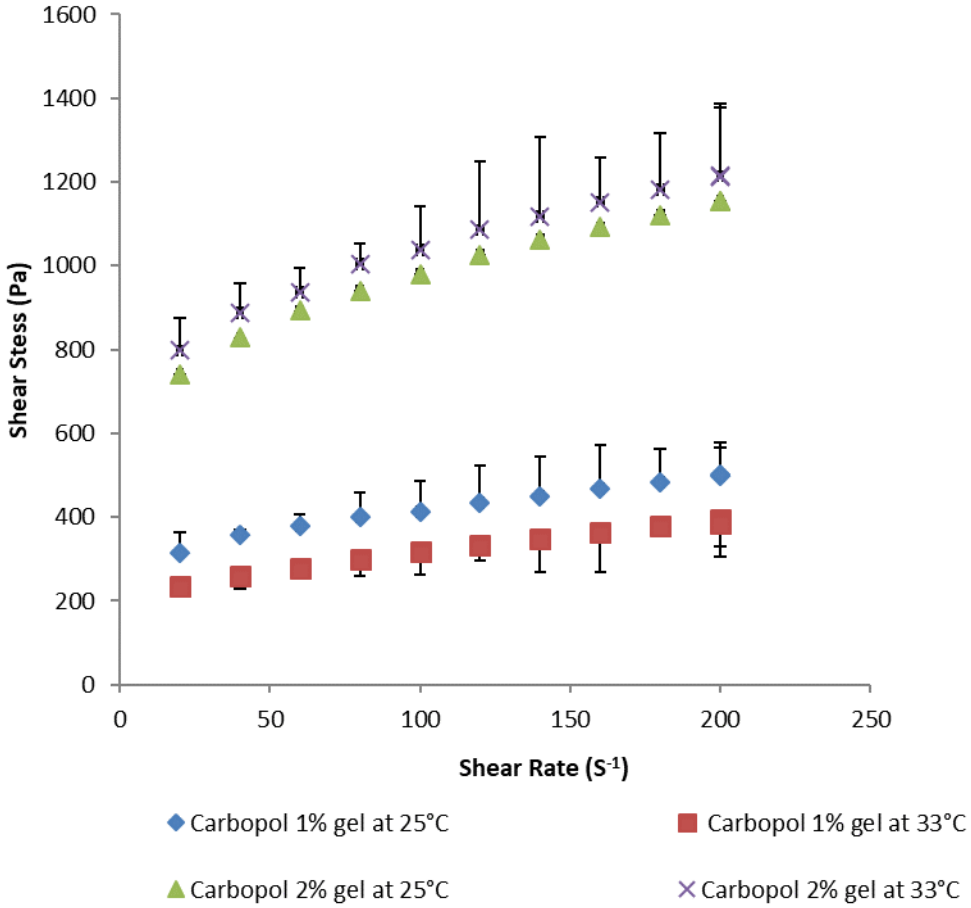

Figure 4: Rheogram of 1% and 2% carbopol gels at 25 °C and 33 °C

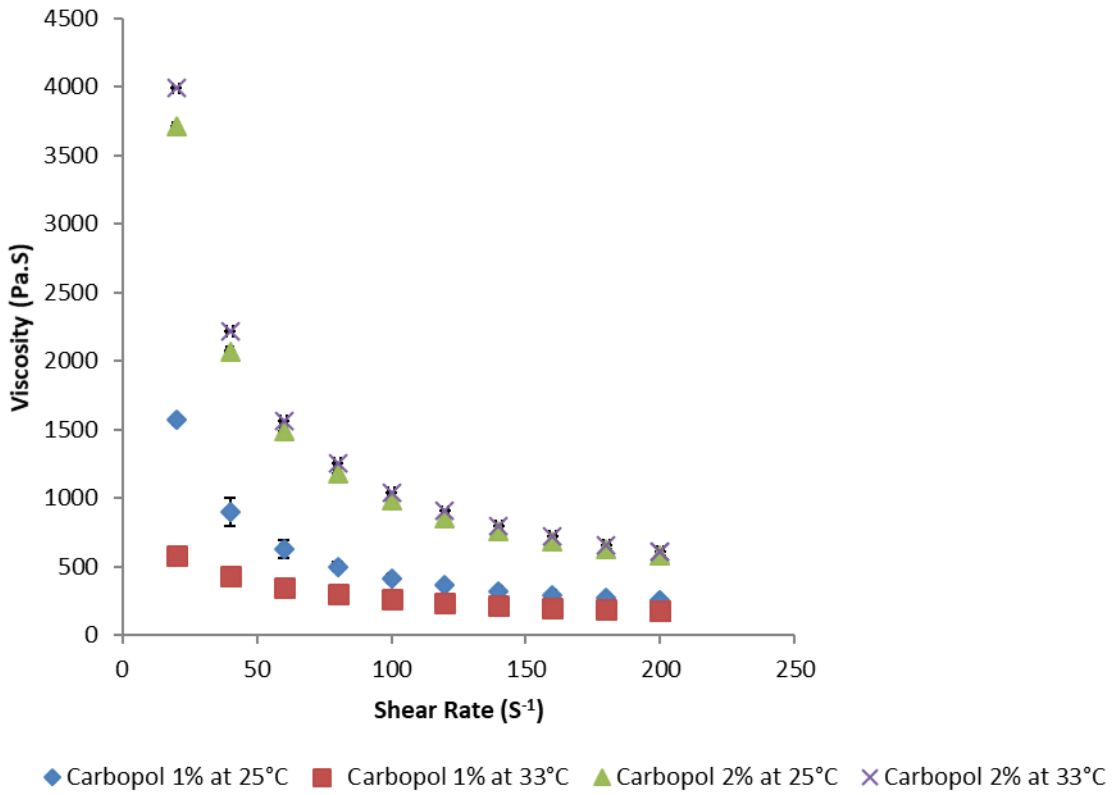

Figure 5: Viscosity as a function of shear rate of 1% and 2% carbopol polymer at 25 °C and 33 °C

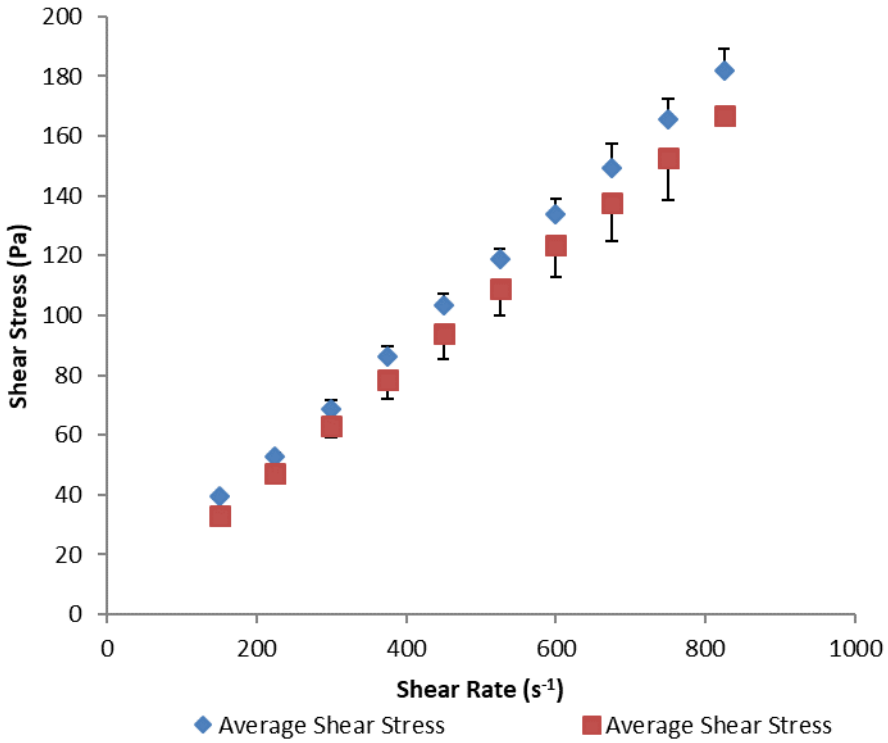

Figure 6: Rheogram of 15% poloxamer at 25 °C and 33 °C

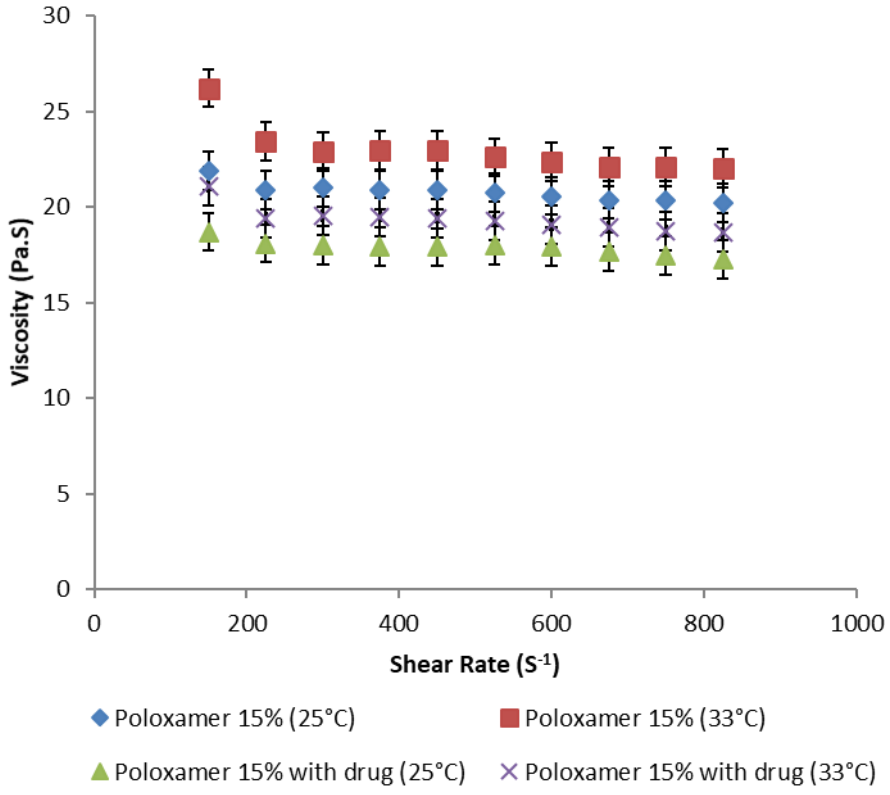

Figure 7: Viscosity as a function of shear rate of 15% poloxamer in presence and absence of PNL-HCl at 25 °C and 33 °C

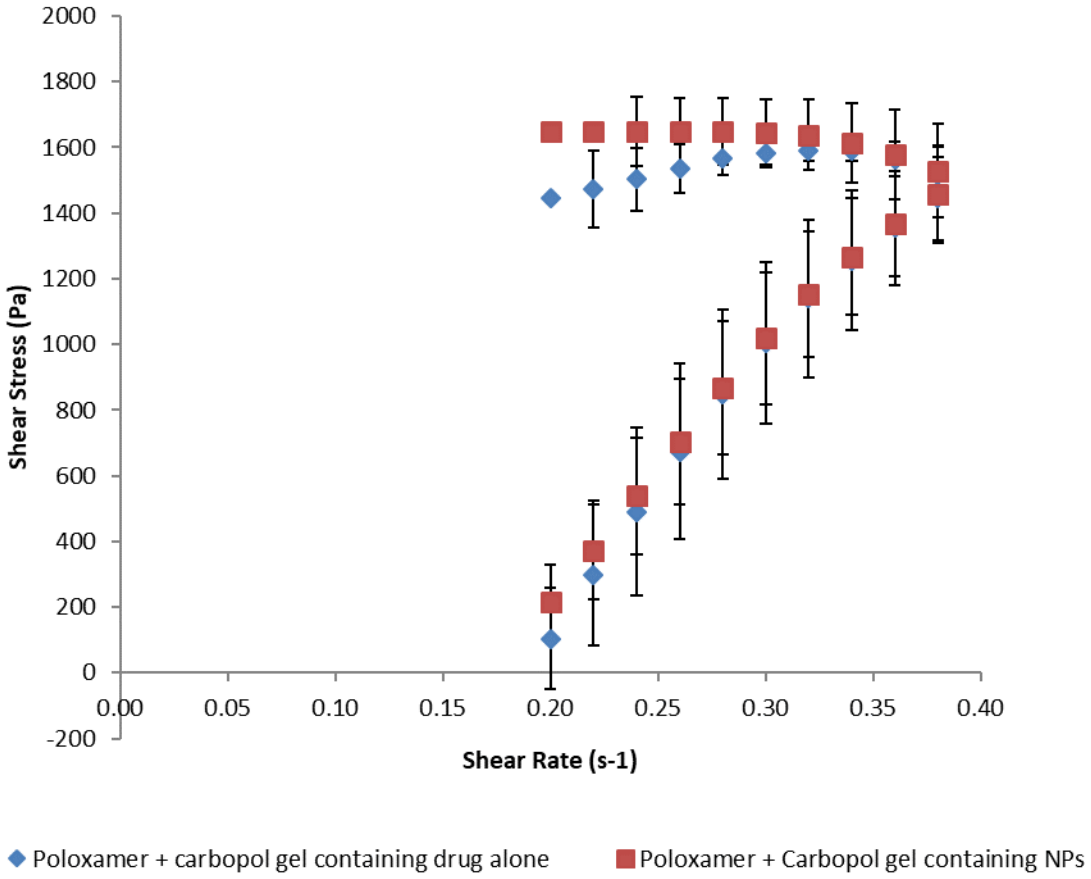

Figure 8: Rheograms of combination poloxamer 15% and carbopol 1% gels in presence of propranolol-HCl or nanoparticles at 33°C.

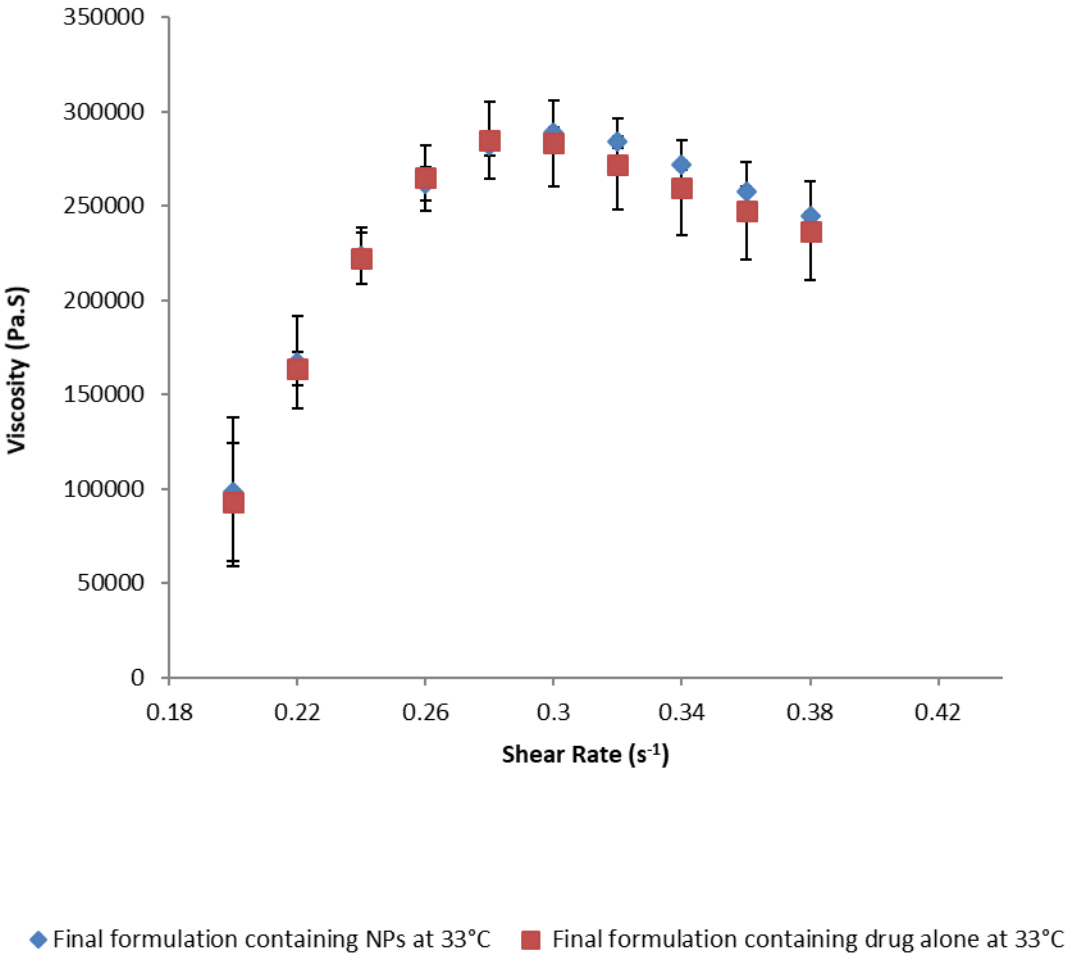

Figure 9: Viscosity as a function of shear rate of the final formulation consisting of combination of 15% poloxamer and 1% carbopol in presence of nanoparticles or PNL-HCl at 33 °C

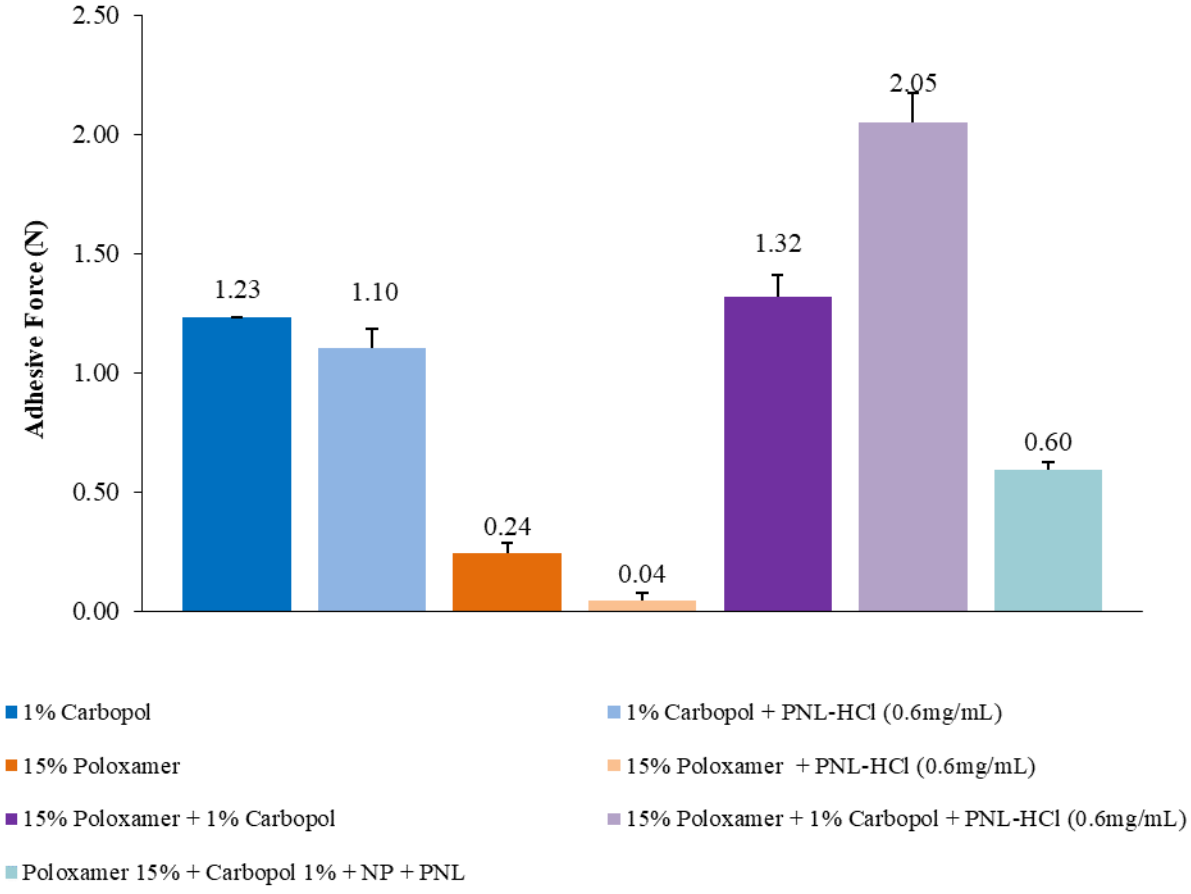

Figure 10: Adhesive capacity of gels at pH 5.5 at room temperature

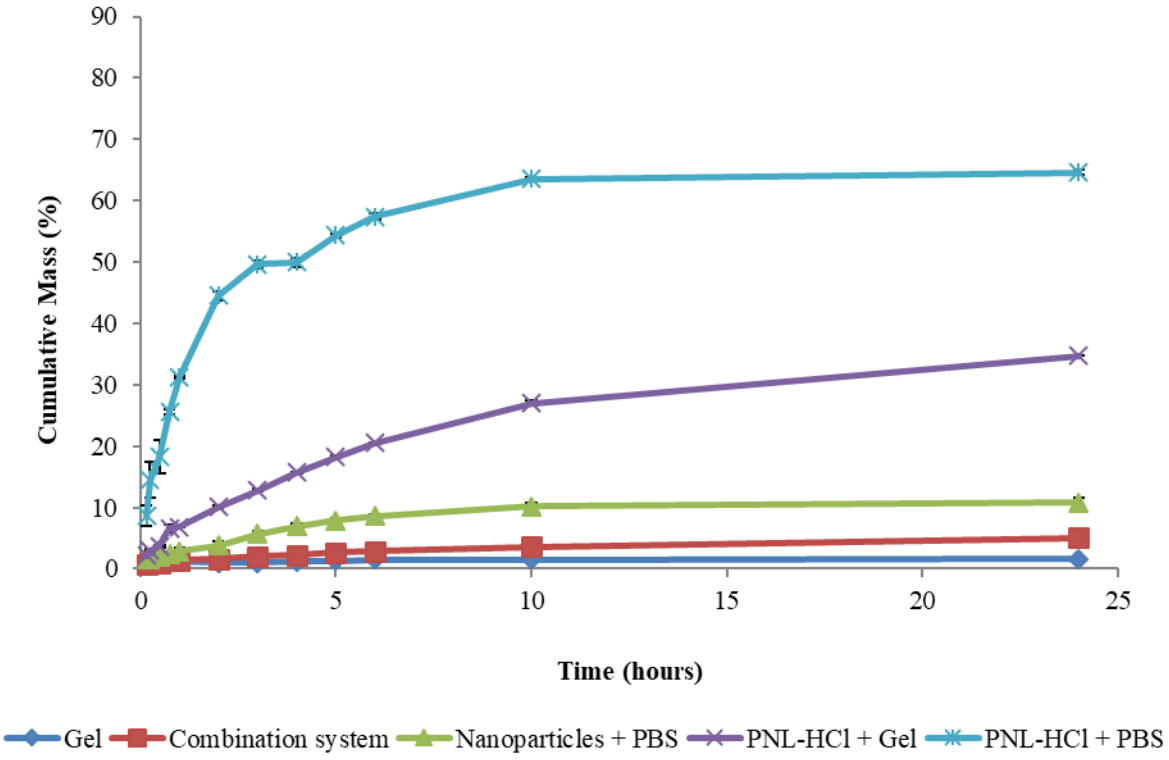

Figure 11. Cumulative percentage of propranolol release from different formulations at 37°C in phosphate buffer pH 6.8 across artificial membrane

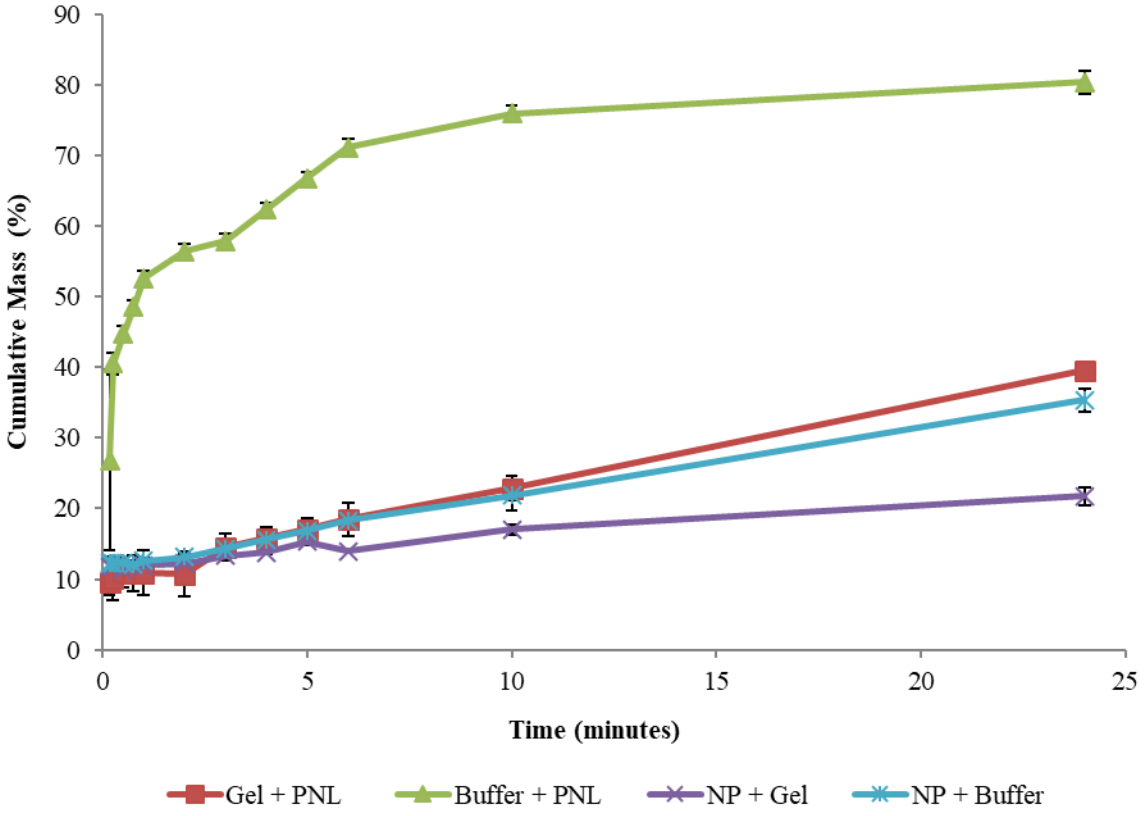

Figure 12. Cumulative percentage of propranolol release from different formulations at 37 °C in phosphate buffer pH 6.8 across pig ear stratum corneum

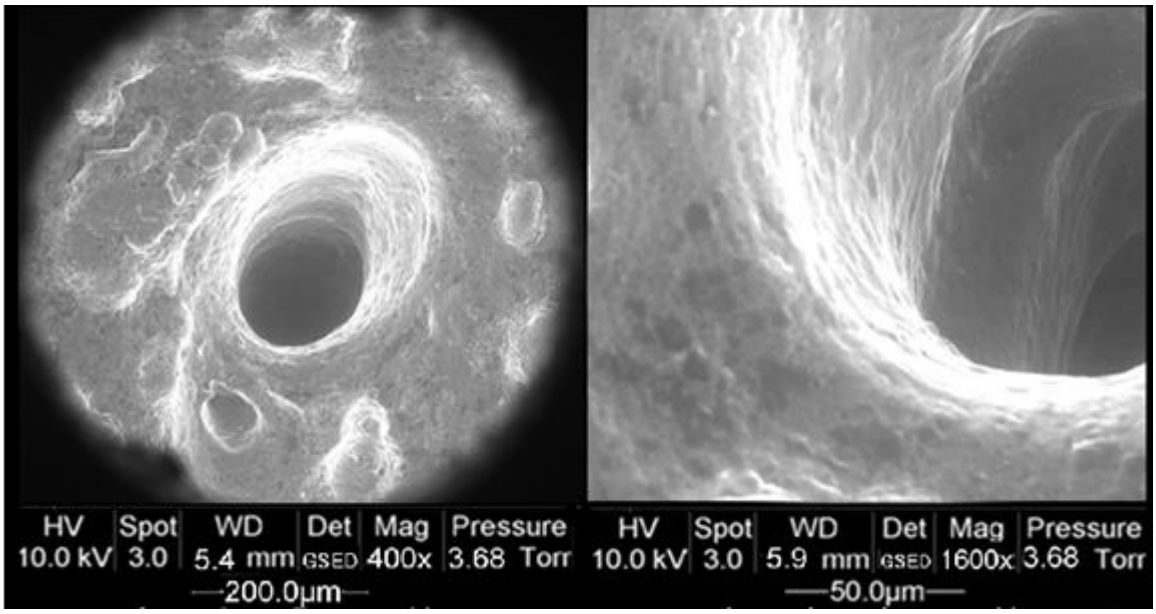

Figure 13a. SEM micrographs of pore of untreated pig stratum corneum

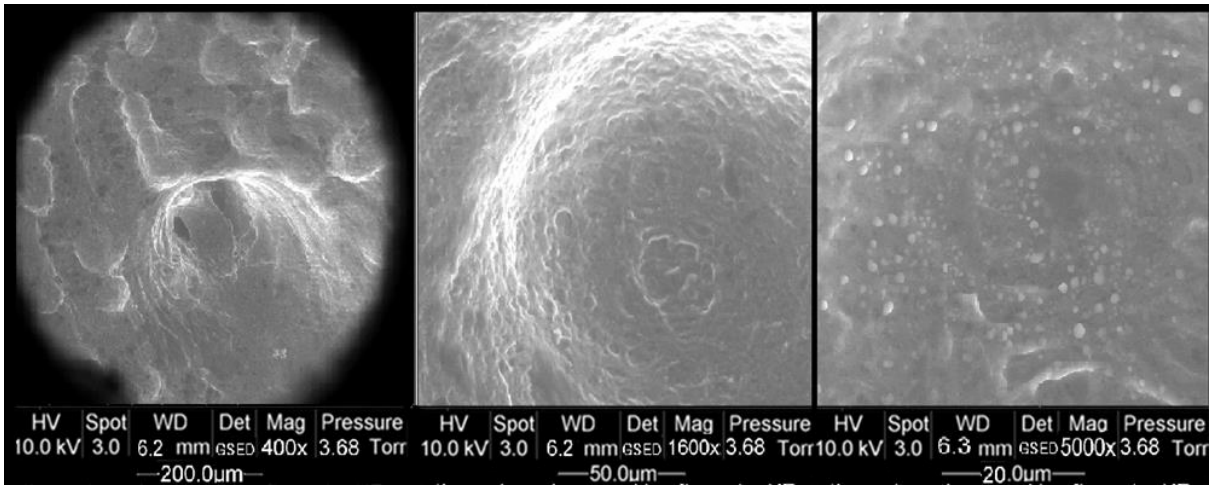

Figure 13b. SEM micrographs of pore of treated pig stratum corneum

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