Design and Evaluation of Polysaccharide-Based Transdermal Films for the Controlled Delivery of Nifedipine

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It was aimed to develop the matrix type polysaccharide-based transdermal films of nifedipine (NFD) to provide its long term plasma concentration. The mechanical tests were carried out on gel formulations which were utilised in the fabrication of transdermal films to determine the type of polymer (pectin, sodium alginate) and plasticizer (propylene glycol, glycerine) as well as their concentrations. The mechanical strength, elasticity, bioadhesiveness and the drug release characteristics of optimised films containing NFD were evaluated. Permeation of NFD from the films with/without adding an enhancer (nerolidol) was followed through excised rat skin using Franz diffusion cells. Results showed that the gels composed of either pectin or sodium alginate were appropriate for the fabrication of transdermal films of NFD, and the addition of propylene glycol improved mechanical strength, flexibility, and bioadhesiveness of the films. Permeation data showed that nerolidol was an effective permeation enhancer for the polysaccharide-based transdermal films of NFD.

Key words nifedipine; transdermal film; sodium alginate; pectin; texture analysis; nerolidol

Transdermal delivery possesses many advantages over conventional drug administration routes, including avoidance of intestinal and/or hepatic first pass metabolism, reduction in side effects and better patient compliance. Polymers are crucial components for the formulation of transdermal delivery systems. There are some critical parameters for the optimisation of transdermal therapeutic systems including physical or chemical stability of the formulation, compatibility with skin, adhesion to skin, the release characteristics of drug from the vehicle and its permeation through the skin. Therefore, the choice of polymers and plasticizers used in transdermal systems have strong impact on drug release, permeability, elasticity, and wearing properties of transdermal formulations.2,3) Natural polysaccharides such as sodium alginate, chitosan, pectin, guar gum, gellan gum have been widely evaluated as a polymer in the optimisation of transdermal systems due to their non-toxic, biocompatible and potentially biodegradable properties.3,4) Sodium alginate is a mixture of polyuronic acids composed of residues of D-mannuronic acid and L-guluronic acid, which is used in design of controlled drug delivery formulations.3) It has also been used to formulate transdermal films.6,7)

Despite all the advantages of transdermal drug delivery, stratum corneum, the outermost layer of the skin, provides the greatest resistance to entrance of drugs to the systemic blood stream. In order to achieve therapeutic drug concentration following transdermal administration, penetration enhancers which reduce the barrier characteristics of stratum corneum reversibly are added into transdermal formulations.8,9)

Terpenes appear to have good skin penetration characteristics for transdermal systems due to their low cutaneous irritancies, good toxicological profiles.8) Moreover, this interaction with the stratum corneum lipids is reversible.10) Therefore, terpenes have been recommended as promising transdermal permeation enhancers due to their non-toxicity and non-irritant properties. Furthermore, some terpenes are in the list of “Generally Recognized As Safe” (GRAS) agents issued by Food and Drug Administration (FDA).11,12) It has been suggested that terpenes increase the penetration of drugs through the skin by disrupting the intercellular packaging of stratum corneum lipids and by increasing drug diffusivity.13,14) Different types of terpenes have been shown to be effective penetration enhancers for both hydrophilic5,15) and lipophilic drugs.16,17) Nerolidol is an amphiphilic and highly lipophilic sesquiterpene (log P 5.36±0.38).10) It has been reported that larger lipophilic sesquiterpenes such as nerolidol shows their effects over prolonged periods (up to 5 d) in contrast to the monoterpenes that tended to wash out relatively easily from the stratum corneum.9)

Nifedipine (NFD) is a calcium canal blocker utilised in the treatment of ischemic heart diseases and hypertension.18) NFD is absorbed rapidly and completely from the gastrointestinal tract following its oral administration. However, its oral bioavailability changes between 45–75% due to systemic metabolism. NFD should be applied frequently because of its relatively short half-life of 2–5 h.19,20) Therefore, transdermal delivery of NFD can be considered as an alternative to enhance the therapeutic efficacy of NFD, to decrease the frequency of application and side effects and to enable the patient compliance.21) The topically applied o/w microemulsion formulations of NFD22) and its collagen and chitosan-based transdermal films,23) controlling the drug release, were prepared. Propylene glycol, cis-oleic acid, dimethyl isosorbide and d-limonene as permeation enhancer were used in order to enhance its transdermal permeation in literature.24–27)

The aim of the present study was to design matrix-type transdermal films of NFD with pectin and sodium alginate, which are polysaccharide-based polymers, and to assess their mechanical properties such as tensile strength and elasticity, as well as the in vitro permeation of NFD across excised rat skin from transdermal films containing nerolidol as penetration enhancer.

Experimental

Materials NFD was from Sanofi-Synthelabo, Turkey; pectin (from citrus peel, galacturonic acid ≥74.60%) and sodium
alginate (medium viscosity, from brown algae) were supplied from Sigma, Germany; nerolidol, glycerine, propylene glycol, polyethylene glycol 400 (PEG 400) were purchased from Merck, Germany. All the other chemicals used were of analytical grade.

**Preformulation Studies** Gel formulations containing different concentrations of pectin (2.5–4.5%, w/w) and sodium alginate (3.5–5.5%, w/w) were prepared in order to select the polymer to be used in the preparation of the transdermal films and its concentration. The gel formulations were fabricated by swelling polymers in a mixture of ethanol and water (1:1) and by standing at room temperature for 24 h. Glycerine, propylene glycol and PEG 400 in concentrations of 5.0–10.0% (w/w) were added to the formulations as plasticizers.

For the aim of determining the most appropriate gel formulations to be used in the preparation of the transdermal films, their mechanical properties were determined using a software-controlled penetrometer, TA-XTPlus Texture analyser (Stable Micro Systems, U.K.), with a 5 kg load cell at 37 ± 0.5°C. Each formulation was transferred into a universal bottle (25 mL) to a fixed height of 8 cm and kept in the ultrasonic water bath for 20 min to remove air bubbles and the temperature was adjusted to 37 ± 2°C. The Perspex probe of 10 mm diameter was compressed twice into each formulation at a defined rate of 2 mm/s until a depth of 15 mm. A delay period of 15 s was allowed between the two compressions. Data collection and calculation were performed using the Texture Exponent 6.0.7.0 software package of the instrument. From the resultant force–time plot, mechanical parameters such as hardness, compressibility, cohesiveness and elasticity of the gel formulations were defined.28–30

**Preparation of Transdermal Films** Transdermal film formulations containing different concentrations of NFD (1.0–10.0% w/w) were fabricated using the polymers (pectin and sodium alginate) and plasticizers (glycerine and propylene glycol) which were selected as a result of the preformulation studies (Table 1).

In order to fabricate the transdermal films, polymer was dispersed in a mixture of ethanol–water (1:1, v/v) and was kept in room temperature overnight and then the plasticizer was added. NFD was dissolved in the same solvent mixture using ultrasonication for 45 min and then added into the gel. The resulting gel formulation was sonicated to remove air bubbles, dropped into the petri dish (θ: 9.8 cm) and dried in oven at 40 ± 2°C for 24 h. After drying, the films were peeled off and kept in dark desiccator at room temperature to protect the NFD from light until the work. The effect of formulation parameters on the NFD stability was examined by thin layer chromatography.31) NFD in films was extracted with alcohol in a mechanical shaker for 3 h and the obtained solution was applied to chromatography plate coated with silica gel 60F254. The chloroform–methanol–glacial acetic acid (98:2:0.5, v/v/v) solvent system was used as a mobile phase. The chromatography plate was examined under UV light at a wavelength of 254 nm and the retention factor (Rf) value of the NFD spot was compared to that of standard material.

**Studies on Transdermal Films. Evaluation of Film Characteristics** The thicknesses of the films at five different locations (centre and four corners) were measured using a digital micrometer (QLR digit, IP4, PRC) and the mean thickness was calculated. The values were the average of three experiments. In order to determine the weight homogeneity, 6 different incisions having a diameter of 0.8 cm (0.502 cm²) were taken from different regions of the transdermal formulations and weighted, the average weight and standard deviation were calculated.

In order to determine the content uniformity of transdermal films containing NFD, 3 different incisions having an area of

<table>
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<tr>
<th>Formulation code</th>
<th>Nifedipine amount</th>
<th>Pectin (g)b</th>
<th>Sodium alginate (g)b</th>
<th>Glycerine (g)b</th>
<th>Propylene glycol (g)b</th>
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<tr>
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<td>1.875</td>
<td>—</td>
</tr>
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<td>1.125</td>
<td>—</td>
<td>1.875</td>
<td>—</td>
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<tr>
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<td>1.875</td>
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<td>0.875</td>
<td>—</td>
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<td>1.125</td>
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<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
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<td>1.125</td>
<td>—</td>
<td>1.875</td>
<td>—</td>
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<tr>
<td>A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.250</td>
<td>1.125</td>
<td>—</td>
<td>1.875</td>
<td>—</td>
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</tbody>
</table>

<sup>a</sup>) Amounts for each formulation stated in table were given to prepare total 25 g gel. Ethanol–water (1:1, v/v) mixture was used as a solvent system in gels. <sup>b</sup>) Amounts are equivalent to 3.5% for pectin; 4.5% for sodium alginate and 7.5% for propylene glycol and glycerine.
0.502 cm² were taken from films and dissolved in a mixture of alcohol–water (1:1, v/v) of 15 mL in an ultrasonic bath for 15 min. After that, the absorbance of the solutions was measured by spectrophotometer (Shimadzu, UV 1601, Japan) in a wavelength of 340 nm. The concentration was calculated by using the standard equation.

The surface morphology of transdermal films was examined using scanning electron microscopy (JEOL JSM-5200, Japan) at 20 kV.

Mechanical Properties of Transdermal Films The mechanical properties of transdermal films were measured using the Texture analyser (TA.XT Plus, Stable Micro Systems, Haslemere, Surry, U.K.) equipped with a 5 kg load cell. A film strip in a dimension of 2×1 cm was held between two clamps and pulled by top clamp at a rate of 0.5 mm/s. The force and elongation were measured when the film broke off. The values were the average of three experiments. The mechanical strength and the elongation at break (elasticity) were calculated using Eqs. 1 and 2:

\[
\text{mechanical strength (N/cm}^2) = \frac{\text{breaking force (N)}}{\text{cross-sectional area of sample (cm}^2)}
\]

(Eq. 1)

\[
\text{elasticity (\%)} = \frac{\text{increase in length at breaking point (mm)}}{\text{initial length (mm)}} \times 100
\]

(Eq. 2)

In Vitro Bioadhesion Studies The bioadhesive strength of the films was evaluated using the modified method of Wong et al. The measurement was conducted using the texture analyser equipped with 5 kg load cell and bioadhesion test rig. Rat skin was utilized as a model tissue. As follows, the skin was taken from the freshly slaughtered animal and fitted on the bioadhesion test rig. Then, 100 µL of distilled water was applied on the surface of the tissue before the experiment. The tests were conducted at 37±2°C. The film was cut into a circular shape and attached to the P/10 cylindrical perspex probe with a double-sided adhesive tape. The probe was lowered onto the surface of the tissue with a constant speed of 1 mm/s. The force and elongation were measured when the film broke off. The values were the average of three experiments. The mechanical strength and the elongation at break (elasticity) were calculated using Eqs. 1 and 2:

\[
\text{work of mucoadhesion (WM) (mJ/cm}^2) = \frac{\text{area under the curve (AUC) }}{\pi r^2}
\]

where \( \pi r^2 \); surface area of the mucosal surface that is in contact with transdermal film formulations.

In Vitro Release Studies In vitro release studies were performed to optimize the drug loading capacity of transdermal films. The method in the monograph of “dissolution test for transdermal films” given in the European Pharmacopoeia was utilized to determine the dissolution rate of NFD from all transdermal film formulations (Table 1) containing different concentrations of NFD fabricated using either pectin or sodium alginate as matrix polymer and, either glycine or propylene glycol as plasticizers. The mixture of ethyl alcohol–water (1:1, v/v) (900 mL) that provides the sink condition for NFD was used as dissolution medium in experiments. The test was conducted in 32±0.5°C at a stirring rate of 50 rpm. The transdermal formulation was placed on a glass container with a diameter of 5 cm and a sieve of stainless steel with a pore diameter of 125 µm was placed onto the glass container. Then, the sample was placed into a beaker containing the dissolution medium and the release of the active substance from films was investigated. Samples were taken in a time intervals (0 to 6 h) and NFD concentration was determined by spectrophotometer at a wavelength of 340 nm. Each experiment was carried out at least three times. The cumulative amount of released NFD from transdermal films was plotted against time. Drug release mechanism from each transdermal formulations was evaluated kinetically with zero order and first order models and, Higuchi matrix model which was plotted the amount of drug released versus square-root of time as described below (Eqs. 4–6).

The zero order kinetic model:

\[
C = k_d + C_0
\]

(Eq. 4)

The first order kinetic model:

\[
\ln C = \ln C_0 + k_t t
\]

(Eq. 5)

where, \( C \) is the drug concentration released at time \( t \), \( C_0 \) is the drug concentration at time 0, and \( k_d \) and \( k_t \) are zero and first order release rates, respectively.

The Higuchi square root kinetic model:

\[
Q = k t^{1/2}
\]

(Eq. 6)

where, \( Q \) is the drug concentration released at time \( t \), and \( k \) is Higuchi release rate.

Ex Vivo Permeation Studies Ex vivo permeation studies were carried out with the transdermal film formulations containing 1% NFD, which were selected according to results of in vitro dissolution studies. These formulations were composed of propylene glycol as plasticizer and either pectin (Ppg5) or sodium alginate (Apg5) as matrix polymer. In order to increase the permeability of stratum corneum, outermost layer of skin, nerolidol was added into the formulations at two different concentrations (2, 5%; w/w) as permeation enhancer and the effect of that enhancer on permeation rate of NFD through the rat skin was investigated. The abdominal skin obtained from female Wistar albino rats (200–250 g) were used in the studies. After cleaning the hairs on the skin, the parts containing the breast tissue were removed and then skins were kept in a freezer at −35°C until the experiment. The skins were held in the isotonic sodium chloride solution (0.9%, m/v) for 1 h before experiment for hydration. The modified Franz diffusion cell (the receptor phase volume is 33.2 mL and the surface area 3.14 cm²) was used in the diffusion studies. The mixture of ethyl alcohol–water (1:1, v/v) was used as the receptor phase. Hydrated skins were placed on the surface of
the diffusion cell and their upper surfaces were contacted with transdermal films cut into appropriate dimensions (3.8 cm²). The upper surfaces of the cells were closed in order to prevent the drying of the formulations. The receptor phase was stirred in a speed of 600 rpm by using a magnetic stirrer. Samples of 0.5 mL were taken from the receptor phase at predetermined time intervals (1 to 6 h) and medium at same temperature and volume was replaced into the receptor phase. Concentration of NFD in solution was determined spectrophotometrically at 340 nm and the permeation rate of NFD through the rat skin and the determination coefficients were calculated. Each experiment was carried out three times. The cumulative amount of drug permeated through the abdominal rat skin was plotted against time. The permeation rate was estimated from the slope of the straight line portion of the cumulative amount of drug permeated versus time.

**Statistical Evaluation** The findings related to the mechanical properties, the results of the mucoadhesion test and in vitro drug release data were statistically evaluated using one-way ANOVA followed by Newman–Keuls multiple comparison test. A $p<0.05$ was considered to be indicative of significance.

**Results and Discussion**

**Preformulation Studies** Type of polymer and plasticizer as well as their concentrations are important factors that affect the mechanical properties of transdermal films and release characteristics of the drug from the films. Gel formulations which will be used to fabricate films should possess appropriate mechanical properties such as good flowability, pourability and spreadability. In preliminary studies, the gels of different polymers in various concentrations were examined to see their effect on the structural properties of transdermal films. In addition, the effects of plasticizers (glycerine and propylene glycol) to the elasticity and mechanical strength of gels were also investigated in preliminary studies. The optimal gel formulations that were determined as a result of the preliminary formulation studies and that were decided to be used in the preparation of the films were given in Table 1.

Even though the good pourability and the spreadability of gels at concentrations below those indicated in the Table 1 (pectin: 2.5, 3.0%; sodium alginate: 3.5, 4.0%), it was found that the physical strength of the films prepared with these gels were not sufficient because of their low cohesiveness and adhesiveness (data were not given). In case of high polymer concentrations (pectin: 4.0, 4.5%; sodium alginate: 5.0, 5.5%), even though high cohesiveness, adhesiveness and elasticity values were obtained, the pourability and the spreadability of the gels were highly decreased and this fact caused the formation of gels in a heterogeneous structure (data were not given). Since the cohesiveness and adhesiveness of the formulations in which PEG 400 were added as a plasticizer were found to be much lower than the formulations in which glycerine and propylene glycol were added, it was decided that the usage of PEG 400 in film formulations would not be suitable (data were not given).

As a result of textural studies accomplished on gel formulations, pectin (3.5%) and sodium alginate (4.5%) as polymers and glycerine (7.5%) and propylene glycol (7.5%) as plasticizers were found to be most suitable at concentrations indicated in brackets for the preparation of film formulations. Results of mechanical characteristics of gel formulations such as hardness, compressibility, adhesiveness, cohesiveness and elasticity were given in Table 2 and Fig. 1.

Hardness is defined as the force required to attain a given deformation or as the maximum peak force during the first compression cycle. The hardness significantly influences the pourability and spreadability of the gel into film molds. Cevher et al. had investigated the hardness of vaginal gel formulations and found that an increase in the polymer concentration raised the hardness value and reduced the pourabil-

**Table 2. Mechanical Properties of Gel Formulations ($n=6$)**

<table>
<thead>
<tr>
<th>Code</th>
<th>Hardness (N)</th>
<th>Compressibility (mJ)</th>
<th>Cohesiveness</th>
<th>Elasticity</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_p$</td>
<td>0.040±0.003</td>
<td>0.398±0.008</td>
<td>0.954±0.006</td>
<td>0.928±0.007</td>
</tr>
<tr>
<td>$P_{pg}$</td>
<td>0.036±0.002</td>
<td>0.352±0.012</td>
<td>0.935±0.008</td>
<td>0.792±0.013</td>
</tr>
<tr>
<td>$A_g$</td>
<td>0.035±0.002</td>
<td>0.284±0.008</td>
<td>0.932±0.005</td>
<td>0.765±0.001</td>
</tr>
<tr>
<td>$A_{pg}$</td>
<td>0.037±0.002</td>
<td>0.279±0.008</td>
<td>0.919±0.007</td>
<td>0.641±0.006</td>
</tr>
</tbody>
</table>

ity of the gel formulations. Ideal gel formulations prepared by polycarboxylic acids had hardness values between 0.033–0.046 N. Their results verify our hardness values. In this study, it is found that the hardness values of gels were between 0.035±0.002–0.040±0.03 N (Table 2). Type of the polymer and the plasticizer did not cause any significant difference in the hardness values of the gels (p>0.05). Results showed that all the gel formulations had appropriate hardness values enabling the gels to flow properly, to be poured into the film molds and to spread homogeneously.

Compressibility defines the work required to deform the gel during the first compression of the probe. This parameter expresses the simplicity of the spreadibility of gel formulations in the film mold affecting homogeneity of formulations.30,36) The compressibility value should be low to allow the easy spread in the film mold. In this study, while the lowest compressibility value was obtained in gels prepared with sodium alginate (0.284–0.279 mJ), the gels prepared with pectin (0.398–0.352 mJ) showed a higher compressibility value (p<0.05). It was seen that propylene glycol decreased the compressibility of gels when compared with glycerine (p<0.05) (Table 2). When compared with other combinations, the gel formulation containing sodium alginate (4.5%) and propylene glycol (7.5%) had the most appropriate compressibility value (0.279±0.008 mJ). These results were in accordance with literature.30,36)

Cohesiveness defines the ratio of the area under the force-time curve produced on the second compression cycle to the curve produced on the first compression cycle, where successive compressions are separated by a defined recovery period.28,30) Cohesiveness is a parameter that significantly affects the strength and the elasticity of the film formulations. It is foreseen that the strength of the prepared films could be increased by using gels with high cohesiveness. As seen in Table 2, the cohesions of the gels prepared with pectin were found to be higher than those of sodium alginate. However, these differences were not statistically significant (p>0.05). In addition, the propylene glycol decreased the cohesion of gels when compared to glycerine, however, this decrease was also not significant (p>0.05). Results showed that the gels prepared with both of the polymers (pectin and sodium alginate) and plasticizers (glycerine and propylene glycol) had sufficient cohesiveness to form films with the appropriate strength. Ceher et al.30) examined the cohesion of the polyacrylic acid gels and indicated that the ideal cohesiveness value for gel formulations should be above 0.900. In this study, it was shown that the cohesiveness of the gel formulations prepared with pectin and sodium alginate ranged between 0.919 and 0.954 and the results were in accordance with literature.30)

The elasticity of gels is a parameter that would significantly affect the elasticity of the films to be prepared by using these gels. The measurement of the elasticity is extremely important in the selection of the polymers and their concentrations to be utilised in the preparation of films and the plasticizers that increase the mobility of polymers. When gel formulations are poured into the molds in order to form films, deformation will be shown depending on the force that is applied for enabling the flowing of gels. However, in the case this deformation is reversible (elastic deformation), the gel shows proper spreading in the film mold in which it is poured and a homogenous film is obtained. Therefore, it is important that the elasticity of the gels should be high enough in order to obtain a homogenous film. Elasticity is defined as the direction of reconstruction of the gel after its deformation by compression in a defined period of time. The increase in the numerical value of elasticity obtained during texture profile analysis shows the decrease in the elasticity of the gel.57,58) Our study showed that the gels containing sodium alginate had a higher elasticity when compared to ones containing pectin (Table 2). The fact that plasticizer agents used in formulations increased the mobility and the flexibility of polymer chains. Besides the gels containing propylene glycol as a plasticizer showed significantly higher elasticity than the ones prepared with glycerine (p<0.05). According to these results, it was predicted that the films prepared with gels containing propylene glycol and sodium alginate would show higher elasticity when compared to other formulations. Results of textual analyses of gel formulations demonstrated that pectin and sodium alginate as polymers at concentrations of 3.5% and 4.5%, respectively, and propylene glycol (7.5%) as a plasticizer were more suitable for fabrication of transdermal films.

**Evaluation of Film Characteristics** Transdermal film formulations were prepared by using pectin (3.5%) and sodium alginate (4.5%) as matrix forming polymers and propylene glycol (7.5%) as a plasticizer (Table 1). Studies concerning the stability of the active substance, the mechanical strength, the elasticity and the bioadhesion studies were conducted on the film formulations.

Firstly, the effects of the polymers, the plasticizers and the film fabrication method on the stability of NFD were investigated by thin layer chromatography.50) It was seen that the retention factor (Rf value) of the test samples was similar to the standard Rf value of NFD (0.71) and no degradation product belong to NFD was observed.

The thickness of the transdermal films were between 0.036 mm and 0.052 mm and the differences among the thicknesses of formulations was found not to be significant (p>0.05). The standard weight deviation of the sections taken from films was found to be less than 2% confirming to be compliant with the Pharmacopoeia limits. It was also determined that content uniformity of NFD in the film formulations was more than 97.5%, which also conforms to the pharmacopoeia limits.

The surface properties of the transdermal films prepared with pectin and sodium alginate were investigated by scanning electron microscopy. It was observed that the surface of the films containing sodium alginate was smoother than that of the ones containing pectin (data were not given).

**Mechanical Properties of Transdermal Films** If the films do not have sufficient elasticity or mechanical strength during their administration onto the skin by applying force with hand, some problems affecting the performance of the product such as rupturing and/or disruption could occur. Therefore, the mechanical strength and the elasticity of the films have great importance in order to keep the integrity of films during and/or after their application onto the skin. In our study, while the mechanical strength value of films prepared with pectin were 1.81 N/cm², using of sodium alginate on the preparation of the films increased the mechanical strength value 4 times (6.92 N/cm²) (p<0.05) (Table 3). Results showed that the formulations including sodium alginate had more resistance against breaking.
Results of “Elongation at break” (elasticity) of the film formulation supported the findings of mechanical strength studies. It was found that the elasticity value of the films fabricated with sodium alginate (103.83%) was approximately 2.5 times higher than those of the formulation prepared with pectin (45.15%) \((p<0.05)\) (Table 3). The elasticity values were directly proportional to the mechanical strength values of the formulations. The resistance to breaking or deforming was improved with an increase of the elasticity value. These indications are in accordance with the literature.\(^{39}\) As a result, it was determined that using of sodium alginate as a polymer in transdermal films was more suitable when compared to pectin.

**In Vitro Bioadhesion Studies** The results of the bioadhesion study of transdermal film formulations were given in Fig. 2. The formulation prepared with sodium alginate \((56\pm1\ \mu J/cm^2)\) showed approximately 4 times higher bioadhesion when compared to that prepared with pectin \((16\pm1\ \mu J/cm^2)\) \((p<0.05)\). The carboxyl groups \((–COONa)\) of sodium alginate and the hydroxyl groups \((–OH)\) of pectin are negatively charged strong hydrogen bonding groups which form hydrogen bonds with negatively charged mucosal components and show much more bioadhesion to mucosa than neutral polymers.\(^{36,40}\) Otherwise, the charge sign of polymer is important element for bioadhesion and pH influences the formal charge of certain ionisable bioadhesive polymers.\(^{41}\) The strength of mucoadhesion of polymers with ionisable carboxyl groups was found to be much stronger than that of those with neutral groups or non-ionisable hydroxyl groups especially at lower pH values than physiological one.\(^{42}\) pH of skin is generally slightly acidic (around 5.5). The carboxyl groups of sodium alginate are non-ionized at lower pH values and react with negatively charged skin component, presumably through numerous hydrogen bonds. However, at higher pH, the chains are fully expanded due to electrostatic repulsion of carboxylate anions and that reduce the bioadhesiveness. Hydroxyl groups of pectin are non-ionisable groups and pH does not change their bioadhesiveness to mucosa. Results obtained in our study were also in compliance with this theory and the films prepared with pectin containing hydroxyl groups presented weaker bioadhesion than those prepared with sodium alginate containing carboxyl groups.

**In Vitro Release Studies** Based on these results, NFD at different concentrations \((1.0–10.0\%\ \text{w/w})\) was added into the film formulations prepared with sodium alginate \((4.5\%)\) and propylene glycol \((7.5\%)\) which showed the highest work of bioadhesion and had high mechanical strength and elasticity, and then \textit{in vitro} dissolution and \textit{ex vivo} permeation stud-

<table>
<thead>
<tr>
<th>Code</th>
<th>Mechanical strength (N/cm²)</th>
<th>Elasticity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₅₀</td>
<td>0.37±0.07</td>
<td>42.13±1.65</td>
</tr>
<tr>
<td>P₇₅</td>
<td>1.18±0.19</td>
<td>45.15±1.04</td>
</tr>
<tr>
<td>A₅₀</td>
<td>3.47±0.09</td>
<td>63.56±0.93</td>
</tr>
<tr>
<td>A₇₅</td>
<td>6.92±0.16</td>
<td>103.83±2.41</td>
</tr>
</tbody>
</table>

Table 3. Mechanical Strength and Elasticity Values of (a) Pectin and Glycerine \((P_g5)\), (b) Pectin and Propylene Glycol \((P_pg5)\), (c) Sodium Alginate and Glycerine \((A_g5)\) and (d) Sodium Alginate and Propylene Glycol \((A_pg5)\) Formulations Containing 1% NFD \((n=6)\)

![Fig. 2. Work of Bioadhesion Graphs of (a) Pectin and Glycerine \((P_g5)\), (b) Pectin and Propylene Glycol \((P_pg5)\), (c) Sodium Alginate and Glycerine \((A_g5)\) and (d) Sodium Alginate and Propylene Glycol \((A_pg5)\) Formulations Containing 1% NFD \((n=3)\)](image)
ies were conducted with these formulations. According to in vitro dissolution data, it was determined that approximately 23% of the drug from formulations containing 10% of NFD (P_{pg1}, A_{pg1}) was released at 6 h (Fig. 3a). Drug release from formulations containing 7.5% (P_{pg2}, A_{pg2}) and 5% (P_{pg3}, A_{pg3}) of NFD at same time was 32% and 37%, respectively (Figs. 3b, c). It was also found that the released amount of NFD from formulations containing 3% of NFD (P_{pg4}, A_{pg4}) were between 73–78% and that the percentage of formulations containing 1% of NFD (P_{pg5}, A_{pg5}) were between 86–101% (Figs. 3d, e) at 6 h.

Results indicated that there was a significant difference ($p < 0.05$) in the quantity of drug dissolved at 6 h among formulations containing different concentrations of NFD (10, 7.5, 5, 3, 1%). Besides, the release rate of NFD increased with the decrease of the concentration of the active substance in the formulations. Results showed that there was no significant difference ($p > 0.05$) with regards to the % concentration of the dissolved NFD between transdermal formulations prepared with pectin and sodium alginate containing same concentration of active substance (Fig. 3).

The kinetics of drug release from all the formulations was analysed by using the zero order, first order and Higuchi kinetics models and it was observed that the release of the drug from all the formulations followed Higuchi’s kinetic model, indicating matrix drug release mechanism of the transdermal films developed (data were not given).

**Ex Vivo Permeation Studies** It was indicated in the literature that the permeation rate of NFD through the skin should be 33.0–37.5 µg/cm$^2$/h in order to provide a therapeutic response.\(^{26,27}\) In this study, it was observed that the drug release rate from the film formulations containing NFD in concentrations of 3 to 10% was between 792 µg/cm$^2$/h and 1189 µg/cm$^2$/h. These values were very high when compared

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**Fig. 3.** *In Vitro* Drug Release Profiles of NFD from the Formulations Containing NFD in Concentrations of (a) 10%, (b) 7.5%, (c) 5%, (d) 3% and (e) 1% ($n=3$)
with the value predicted for the permeation of NFD through the skin. The drug release rates from formulations (Ppg5, Apg5) containing 1% of NFD were also found to be 331 and 424 µg/cm²/h, respectively. Since the stratum corneum is the main barrier of the skin against drug permeability, the release rates which were obtained from in vitro studies will be slowed down in ex vivo skin permeation studies. Therefore, even though the in vitro release rate of NFD was higher than its permeation rate through the skin (33.0–37.5 µg/cm²/h) to reach a therapeutic level, the formulations (Ppg5, Apg5) containing 1% of NFD were selected for ex vivo studies. The results belong to permeation rate of NFD through the rat skin in film formulations with and without penetration enhancers are given in Fig. 4 and Table 4.

The permeation rates of NFD through the rat skin in formulations with pectin (Ppg5, Apg5) and alginate (Apg5, Apg5) were calculated to be 9–11 µg/cm²/h and 23–26 µg/cm²/h, respectively (Table 4). The type of the plasticizer (glycerine and propylene glycol) did not cause any significant change in the permeation rate of NFD (p>0.05). As mentioned above, target flux of NFD through the skin should be 33.0–37.5 µg/cm²/h to reach therapeutic plasma levels. However, since the permeation rate of NFD through the rat skin did not reach to target flux with selected formulations. Nerolidol as permeation enhancer (2, 5%) was added to Ppg5 and Apg5 formulations which were more resistant against breaking and of which the elasticity and the bioadhesiveness were preferable (Table 4).

Nerolidol is an amphiphilic and highly lipophilic sesquiterpene (log P 5.36±0.38). It is frequently used for increasing the permeation of both lipophilic and hydrophilic drugs through the skin due to its amphiphilic structure which is suitable for alignment within the lipid lamellae of the stratum corneum and disrupting its highly organised packing. The lipophilicity of the drug molecule, as well as the enhancer permeant is thought to play an important role in determining the enhancers promoting activity on the permeation of the drug across the skin. It has been reported that larger lipophilic sesquiterpenes such as nerolidol shows their effects over prolonged periods (up to 5 d) in contrast to the monoterpenes that tended to wash out relatively easily from the stratum corneum.

In this study, the addition of nerolidol significantly increased the permeation rate of NFD through the rat skin (p<0.05). While the permeation rate of NFD through the rat skin increased to 26.90 µg/cm²/h and 25.40 µg/cm²/h respectively in Ppg5 formulation containing 2% and 5% of nerolidol, it was increased to 42.30 µg/cm²/h and 31.70 µg/cm²/h, respectively in Apg5 formulation containing 2% and 5% of nerolidol (Table 3). Nerolidol was found to be an effective enhancer in increasing the permeation rate of NFD, which is moderate lipophilic compound (log P: 2.5), as previously described in literature for drugs with similar lipophilicity like carbamazepine. The increase in nerolidol concentration increased the cumulative amount of NFD permeated through the rat skin, however, this increase was found not to be statistically significant (p>0.05).

**Conclusion**

It was shown in the study that the matrix type transdermal films of NFD, which is a suitable drug for transdermal delivery in terms of its pharmacokinetic and physicochemical properties, could be prepared with the polysaccharide polymers. Transdermal films of NFD composed of both pectin and alginate as matrix polymer, and propylene glycol as plasticizer had good mechanical strength, flexibility and high bioadhesiveness and proper drug release characteristics. Based on the in vitro skin permeation data, nerolidol also can be considered as an effective permeation enhancer in the optimisation of polysaccharide-based matrix type transdermal films of NFD. In the next step of the study, it is planned to evaluate the in vivo performance of film formulations by animal experiments.

**References**

4) Prajapati V. D., Jani G. K., Moradiya N. G., Randeria N. P., *Carbo-