INTRODUCTION

Neurodegenerative diseases affect memory induced cognitive damages, affects the ability to speak, move and breathing. It is predicted that neurodegenerative diseases will affect 35 million population of world by 2050. The neurodegenerative diseases are age dependent, and going progressively in recent years. There are various examples of neurodegenerative diseases viz. Alzheimer’s disease, Parkinson’s disease (PD), Huntington’s disease and spinocerebellar ataxias. PD disease affects motor dysfunction resulting in rigidity, bradykinesia, and tremor. These types of symptoms are shown due to the low level of dopamine in nigrostriatal pathway. Lewy body formation is one of the reasons for induction of PD.

In recent years PD is the second most common neurodegenerative disease. Around 1-2% of the population those over the age of 65 years go through PD and frequencies of PD are increases with age. The prevalence of PD is likely to double over by 2030. The therapy of PD involves MAO-B inhibitors and combinations of carbidopa and levodopa. Levodopa and carbidopa are the remedy for Parkinson’s disease. In spite of balanced remedy, levodopa therapy gives less response to treatment after couple of months and observed several on-off sequences. In the primary phase of disease, monotherapy of monoamine oxidase B (MAO-B) inhibitors are favoured for controlling the symptoms of PD. The first line treatment for PD is carried out with selegiline which is selective and irreversible inhibitor of MAO-B enzymes. Oral bioavailability of selegiline is low due to extensive hepatic first pass metabolism. It is metabolised in liver by the enzyme cytochrome P450 to N-desmethyl selegiline, levomethamphetamine, levamphetamine. There is no requirement of adjusting the dose of selegiline in patients with liver and kidney failure because its metabolites are quickly appear in urine and faeces. The drug administered by transdermal route of administration, avoids its hepatic first pass metabolism. Moreover transdermal drug delivery provides steady state plasma concentration of drug. Transdermal matrix patches would be the better option regarding the patient compliance, human health benefit in sustain use of drug and favoured by...
health care provider throughout the complete treatment of disease\textsuperscript{18}. The purpose of the release controlling membrane is one of the techniques to modulate the drug release. Among the various numbers of polymers, ethylene-vinyl acetate (EVA) copolymer is heat processible, elastic and cheaper material. The EVA membrane has been used as rate controlling membrane for controlled delivery of drug\textsuperscript{19}.

For the development of a film a plasticizer usually in concentration 10 to 20 % of polymer weight is added to impart flexibility to the film. It provides plasticity to the film resulting in its smooth appearance. Plasticizers promote drug release from polymeric matrix film due to creation of channels in film. The penetration enhancer acts by one or more that one mechanism at intercellular lipidic domain of stratum corneum membrane of skin resulting in enhanced permeation of drug. The disorderliness at intercellular lipids leads to the enhanced permeation of hydrophilic and hydrophobic drugs. The penetration enhancers act on bound water between the charged hydrophilic groups of the lipids of stratum corneum resulting in its expansion and the penetration of hydrophilic drug is increased. The denaturation of alpha keratin present in corneocyte of stratum corneum also results in enhanced permeation of drug.

It was envisaged to develop the SGN embedded transdermal drug delivery system and study the effect of plasticizers and penetration enhancers on the transdermal penetration of drug from film. In present investigation we envisaged to study the effect of dibutyl phthalate and triethyl citrate as well penetration enhancers oleic acid and linoleic acid on transdermal penetration of SGN.

**MATERIALS AND METHOD**

Selegiline Hydrochloride (SGN) was obtained as a gift sample from Intas Pharmaceuticals Ltd., Ahmedabad, India. Ethylene-vinyl acetate was purchased from Sigma-Aldrich Chemicals, Bangalore, India. Di-butyl phthalate, and tri-ethyl citrate were purchased from HiMedia, Mumbai, India. All other solvents and reagents were used of analytical grade.

**Formulation of transdermal film**

The transdermal film comprising ethylene vinyl acetate and SGN was developed by solvent casting method using film former machine (V.J. Instruments Pvt. Ltd. India.). Accurately 2 g EVA polymer was dissolved in 10 ml dichloromethane (DCM) in volumetric flask. To it SGN (18 mg) was added and kept on rotary shaker for 3 h. The resulting solution either the plasticizer or penetration enhancers was added (Table 1). The solution was dragged on film former platform of machine with help of dragger to cast a thin film. Solvent was allowed to evaporate for 8 hours at room temperature. The thin film formed was removed with the help of sharp cutter knife.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation</th>
<th>SGN (mg)</th>
<th>EVA (g)</th>
<th>DCM (ml)</th>
<th>DBT (%)</th>
<th>TEC (%)</th>
<th>Oleic acid (%)</th>
<th>Linoleic acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>S\textsubscript{1}</td>
<td>18</td>
<td>2</td>
<td>10</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>S\textsubscript{2}</td>
<td>18</td>
<td>2</td>
<td>10</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>S\textsubscript{3}</td>
<td>18</td>
<td>2</td>
<td>10</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>S\textsubscript{4}</td>
<td>18</td>
<td>2</td>
<td>10</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>S\textsubscript{5}</td>
<td>18</td>
<td>2</td>
<td>10</td>
<td>-</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>S\textsubscript{6}</td>
<td>18</td>
<td>2</td>
<td>10</td>
<td>-</td>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>S\textsubscript{7}</td>
<td>18</td>
<td>2</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>S\textsubscript{8}</td>
<td>18</td>
<td>2</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>S\textsubscript{9}</td>
<td>18</td>
<td>2</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>S\textsubscript{10}</td>
<td>18</td>
<td>2</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>11.</td>
<td>S\textsubscript{11}</td>
<td>18</td>
<td>2</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>12.</td>
<td>S\textsubscript{12}</td>
<td>18</td>
<td>2</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
</tbody>
</table>

**Evaluation of Transdermal Films**

**Differential scanning calorimetry (DSC)**

Drug, polymer and formulation were analysed by differential scanning calorimetry (DSC) studies by using Mettler-Toledo DSC apparatus, Zurich, Switzerland. All samples were weighed into aluminium pans and subjected to heating at a rate of 10 °C/min from 0 °C to 400 °C. Nitrogen gas at a flow rate of 40 ml/min was used as a purge gas in DSC analysis. The results were analysed using STAR\textsuperscript{\textregistered} SW 10.00 software. (Mettler-Toledo DSC apparatus, Zurich, Switzerland).

**Fourier transform infrared spectrometry (FT-IR)**

In FT-IR studies, SGN and EVA polymer were individually triturated and mixed well with potassium bromide in 1:1 ratio. The samples were compressed under 10 t/mm\textsuperscript{2} pressure and scanned in 400 to 4000 cm\textsuperscript{-1} stretching frequency range. The FT-IR spectrum of formulation was compared with spectrum of drug and polymer. The shift in the stretching frequency of drug in presence of polymer was investigated to determine physical interactions between SGN and EVA.

**Physical appearance**

The matrix transdermal films were visually inspected for colour, clarity, flexibility and smoothness.

**Thickness of transdermal films**

The thicknesses of the SGN embedded polymeric matrix transdermal films were measured at five different points using a digital micrometer\textsuperscript{20,21}.

**Weight uniformity**

The transdermal films were dried at 40 °C. Three films from each formulation batch were accurately weighed on digital balance\textsuperscript{22}.

**Content uniformity of drug in transdermal films**

To assure uniform distribution of SGN in the transdermal films, a content uniformity test was carried out. Five samples of each 2 × 2 cm representing the different segments of the films were cut and weighed. The SGN was extracted from the film using 10 ml DCM in rotary shaker at 250 rpm for 3 h. It was diluted with distilled water and estimated...
spectrophotometrically on UV-Visible spectrophotometer at 204 nm.

**Tensile strength and percentage elongation**

The maximum force required to break the film was recorded as a tensile strength. The weight was gradually increased so as to increase stretching force to break the matrix transdermal film. The tensile strength and percent elongation were measured using JJ Lloyd instruments limited (Southampton, England).

**Folding endurance**

The specific area of matrix transdermal film was cut accurately and repeatedly folded at a same place till the film was broke. The numbers of counts up to breakage of film were recorded as folding endurance.

**Percent moisture uptake**

The accurately weight matrix transdermal film was kept in desiccators at room temperature for 24 h. To maintain the 65 % relative humidity in desiccators, it was filled with saturated potassium chloride solution. After 24 h, matrix transdermal film was accurately reweighed and calculated the percent moisture using equation (1).

\[
\text{Percent moisture uptake} = \frac{\text{Final weight of film} - \text{Initial weight of film}}{\text{Initial weight of film}} \times 100
\]

**Ex-vivo permeation study**

*The ex-vivo* permeation study was carried out using diffusion cell apparatus (Orchid Scientific Pvt. Ltd, Nasik, India). The male rat abdomen was trimmed and skin samples were obtained. The removed skin was washed with ethyl alcohol to remove excess fat from the skin and soaked in phosphate buffer pH 7.4 before permeation study. The developed transdermal film piece of size 3.14 cm² was placed on epidermal side of skin in the donor compartment of diffusion cell apparatus. Phosphate buffer pH 7.4 was filled in the receptor compartment of the diffusion cell apparatus and after predetermined time interval 2 ml of sample was withdrawn at 0, 2, 4, 6, 8, 10, 12, 16, 20, 24, 36, 48, 60 and 72 h and replaced it with fresh receptor fluid. The samples were analysed for drug content at 210 nm using UV-visible spectrophotometer (UV-1800, Shimadzu, Mumbai, India).

**Skin irritation study**

The skin irritation tests were performed on healthy albino wistar rats weighing between 200 g to 300 g. The wistar rats were kept in cages with free allowance of food and water according to standard protocol of laboratory. The dorsal abdominal skin was carefully trimmed to avoid any damage to skin. A 0.8 % aqueous solution of formalin was used as a standard skin irritant. SGN embedded matrix transdermal film was applied to the rats and compares the score of erythema with a standard irritant solution. The score of erythema was recorded by draize scoring method.

**Stability study**

Stability study was carried out according to the ICH guidelines. Optimized formulation was kept in stability chamber (REMI, Mumbai, India) at a condition 40 °C and 75 % RH (Relative humidity). The formulations were observed for physical changes, weight uniformity, moisture uptake and strength of the transdermal matrix films.

**RESULTS AND DISCUSSION**

**DSC studies**

The DSC endotherm of selegiline HCl, EVA polymer and their physical mixture was recorded from ambient to 400 °C at a heating rate of 10 °C/min. The comparison of thermal transitions has revealed that selegiline HCl has shown the melting transition from 142.77 °C to 148.47 °C as a melting temperature range with a peak at 144.74 °C. The enthalpy of endothermic transition was found to be -149.68 J/g. The polymer EVA has shown the onset at 64.48 °C and end set at 93.75 °C indicating as temperature range with melting peak at 82.83 °C and enthalpy value -81.23 J/g. The physical mixture has not shown any significance change in the melting temperature of drug and polymer as indicated by the melting peak at 148.07 °C for selegiline HCl and 77.94 °C for EVA.

![Figure 1: DSC thermogram of selegiline HCl](image-url)
Figure 2: DSC thermogram of ethylene-vinyl acetate

Figure 3: DSC thermogram of selegiline hydrochloride and ethylene-vinyl acetate polymer physical mixture

Fourier transform infrared spectrometry (FT-IR)

The FTIR spectra of drug, polymer and their physical mixture were recorded from 400 to 4000 cm\(^{-1}\) using KBr press pellets technique. The FTIR stretching frequencies of selegiline HCl was observed at 1093 and 1153 cm\(^{-1}\) representing C-O stretching 1292 cm\(^{-1}\) (C-N stretch), 1434 cm\(^{-1}\) (C=O), 1602 cm\(^{-1}\) (N-H), 1776 cm\(^{-1}\) and 1826 cm\(^{-1}\) (C=O), 2123 cm\(^{-1}\) (C≡C), 2943 cm\(^{-1}\) and 3228 cm\(^{-1}\) (OH stretching) these stretching peaks were not altered significantly in the physical mixture of drug and polymer. It was concluded that no physical interaction was occurred in drug and polymer.

Figure 4: FT-IR spectra of selegiline HCl
Physical appearances

The transdermal films with increasing concentrations of DBT as a plasticizer from 10, 15 and 20 percent as shown decreasing brittleness of films. Also the films was found to be transparent with 20 % dibutyl phthalate and triethyl citrate. The films were found to be transparent, clear, smooth and flexible in appearance.

Thickness

The thickness of transdermal films was found to be 0.78 ± 0.13mm. The weights of 2 × 2 cm unit dimension of transdermal films was found to be uniform.

Weight uniformity

The weight uniformity of randomly selected matrix transdermal films were found to be in the range of 1.9 g to 2.1 g.

Content uniformity

The content uniformity of 2 × 2 cm transdermal films was found to be comply within the acceptable limit of 90 to 110 % selegiline HCl.

Tensile strength

The tensile strength of transdermal film was found to be 0.25 to 0.39 kg/cm². The percent elongation of transdermal films found in the range 17 to 19 %.

Folding endurance

The folding endurance of films were found in the range of 290 to 345

Percent moisture uptake

The percent moisture uptakes of films were found to be 2 to 3 %.

Ex-vivo permeation study

Effect of plasticizers on ex-vivo permeation

Plasticizers creates pores in the polymer matrix their by increases drug release which in turns increases amount of drug diffusion across skin. The film rigidity gets reduced due to the action of plasticizers on polymeric chains resulting in enhanced flexibility of film. The effect of plasticizers study viz. DBT and TEC has shown enhanced ex vivo drug diffusion across the rat skin.

The diffusion of SGN was found to be enhanced with increasing concentration of TEC from 10 to 20 %. In the ex vivo skin permeation studies transdermal film containing SGN, a lag time of 4 h was observed for the drug to appear in the receptor compartment. TEC in concentrations 10, 15, and 20 % of polymer weight has shown flux values of 37.63, 45.36 and 53.87 (µg/cm²/h) respectively. The hydrophobic plasticizer DBT has shown comparatively lesser drug diffusion than TEC (Figure 7 and 8). DBT in concentrations 10, 15, and 20 % of polymer weight has shown flux values of 30.16, 33.41, and 36.14 (µg/cm²/h). The wicking and channelling action of plasticizer on polymeric chains increases mobility of drug polymer matrix. TEC as plasticizer has shown enhanced permeation of SGN as compared to DBT.
Effect of penetration enhancers on ex-vivo permeation

Penetration enhancers acts on the intercellular lipid of the stratum corneum of the skin resulting in disorderliness in the sphingo lipids, ceramides and other lipidic components their by an increasing fluidity of intercellular lipids. The orderly array of lipid head group and non-polar tails gets disorganise due the action of penetration enhancer leading to enhanced permeation through free space of intercellular lipid domain. The enhanced fluidity permits more drug diffusion across intercellular lipids. Number of carbon atoms in fatty acids and position of double bonds affects the penetration characteristics of fatty acid as penetration enhancers.

Oleic acid is monounsaturated omega-9 fatty acid where as linoleic acid is polyunsaturated omega-6 essential fatty acid with two double bonds in cis configuration. In the ex-vivo skin permeation studies of transdermal film containing SGN, lag time of 4 h was observed for the drug to appear in the receptor compartment. Oleic acid in concentrations 0.5, 1, and 2 % concentrations has shown flux values of 42.04, 47.83, and 59.12 (µg/cm²/h) respectively. Linoleic acid in concentrations 0.5, 1, and 2 % has shown flux values of 49.87, 58.79, and 69.11 (µg/cm²/h) respectively in ex-vivo skin permeation studies. Linoleic acid was found to show enhanced diffusion of SGN as compared to oleic acid.
Skin irritation study
The SGN embedded matrix transdermal films which showed the maximum drug diffusion in ex-vivo studies were applied to healthy albino wistar rats. Score of draize test were 0 in every determination of erythema and edema, this indicates that the primary irritation were absent in rats.

Stability study

Effect of temperature
All the optimized SGN matrix transdermal films showed the high stability upon storage at 40 °C and there were observed no physical change, weight uniformity and strength of the matrix films as compared to freshly formulated matrix transdermal films.

Effect of humidity
All the optimized formulations were showed the slight increase in their weight at 75 % Relative humidity. There were no physical changes observed in matrix transdermal films.

CONCLUSION
Transdermal film formulation containing selegiline HCl were developed by film casting method using ethylene-vinyl acetate as film former. The effect of plasticizer dibutyl phthalate and triethyl citrate and penetration enhancers oleic acid and linoleic acid were studied in increasing concentration on ex-vivo permeation across skin using phosphate buffer 7.4 pH as receptor medium.

Triethyl citrate has shown enhance drug diffusion across skin from transdermal film as compared to dibutyl phthalate. Linoleic acid as penetration enhancer showed better penetration across the skin than oleic acid. Transdermal drug delivery systems provide the better control on drug release resulting in predictable steady state blood plasma concentration of SGN for the management of parkinson’s disease.
CONFLICT OF INTEREST
All contributing authors declare no conflicts of interest.

REFERENCES
23. Heng PW, Chan LW, Ong KT. Influence of storage conditions and type of plasticizers on ethylcellulose and acrylate films formed from aqueous dispersions, J Pharm Sci 2003; 6:334-44.