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RESEARCH ARTICLE

Formulation and in vitro evaluation of transdermal drug delivery system for donepezil

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Abstract The effects of different formulation variables on the transdermal absorption of donepezil were investigated. The permeation of donepezil from various pressure sensitive adhesive matrices was evaluated using flow-through diffusion cell system at 37°C. The penetration of donepezil from the matrices was found to be influenced by the nature of adhesives. 1:1 combination of acrylic rubber hybrid adhesives (Duro-Tak® 87-503A and Duro-Tak® 87-504A) provided good adhesion force and high flux of donepezil. Significant increase in flux was obtained using Brij® 30, Brij® 52, and their combination, as penetration enhancers. Manual assessment using thumb test revealed that patches containing combination of enhancers possessed good adhesive properties. The formulation containing combination of Brij® 30 and Brij® 52, each at the level of 5% v/w with 15% w/w drug load in 1:1 combination of Duro-Tak® 87-503A and Duro-Tak® 87-504A matrix was found to be the best. No significant alteration in morphology and assay values were observed during the physical and chemical stability tests conducted for the study period of 3 months.

Keywords Donepezil · Transdermal drug delivery · Percutaneous penetration · Chemical enhancers · Alzheimer's disease

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Donepezil is a centrally acting reversible acetylcholinesterase inhibitor and exerts its therapeutic effect by increasing acetylcholine concentrations and enhancing cholinergic function (Rogers and Friedhoff 1998; Sugimoto et al. 1995). Commercially, donepezil is available in the form of tablet under the trade name Aricept®. Initial dose is 5 mg per day, which can be increased to 10 mg per day after an adjustment period of at least 4 weeks (Rogers et al. 1998). In most of the cases, it is not convenient for patients suffering from Alzheimer's disease (AD) to comply with the self-medication schedule. Moreover, various side effects including diarrhea, nausea, anorexia, and muscle convulsion are reported (da Silva et al. 2006). These adverse effects are mainly due to increase in gastric acid secretion caused by enhanced cholinergic activity through the gastrointestinal tract. Donepezil-nanoclay hybrids have been suggested to reduce the adverse effects of donepezil (Park et al. 2008). It was reported that clay used in the study could reduce the acidity by absorbing proton and control the drug release behavior. As an alternative to oral delivery, microparticles of donepezil as monthly subcutaneous injection has been reported (Zhang et al. 2007). The microparticles were prepared using poly (D, L-lactide-co-glycolide) by an oil–water emulsion solvent evaporation technique. However, due to the better patient compliance, controlled delivery of drug, ease of administration as well as termination, a transdermal product of donepezil would be more appropriate in providing clinical benefit of prolonged response to patients suffering from AD.

However, due to the barrier function of skin, not all drugs can be delivered transdermally (Subedi et al. 2010). In many cases, the absorption may not result in sufficient plasma drug concentration. Various studies have been conducted, along with their pros and cons, to develop transdermal product of donepezil. Matrix based transdermal system has been

reported for donepezil (Kazunosuke et al. 2008). However, to achieve the sufficient transdermal flux through hairless mouse skin, extremely high drug loading (35% w/w) was used. This may lead to crystallization of drug in the polymer matrix and may cause problem with adhesive force. Another study suggested the use of salt form that is converted to the base form in situ within the matrix type delivery system (Terahara et al. 2009). Salt form of donepezil precipitates in the adhesive matrix forming particles in the patch, which reduces the aesthetic value of the patch. Reservoir type patch system was also described for delivery of Alzheimer's pharmaceuticals, particularly donepezil (Valia and Ramaraju 2008). The matrix patches are slimmer and smaller than the reservoir patch, and are preferred both in terms of ease of production and better patient compliance. Therefore, there is a need to explore a commercially viable transdermal matrix based system for donepezil which can give higher flux at lower drug load, through proper selection of formulation and process variables.

The present study was conducted to investigate the feasibility of developing stable matrix based transdermal system for donepezil. In vitro permeation studies were done to characterize passive diffusion with various adhesives and chemical enhancers. Effect of different formulation variables on permeation of donepezil was evaluated.

Materials and methods

Materials

Donepezil hydrochloride was generous gift from Samil Pharmaceuticals (Seoul, South Korea). Polyglyceryl-3 oleate (Plurol olieque® CC497), propylene glycol mono laurate (Lauroglycol), and polyoxy glycerate (Labrafil® 1944) were obtained from Gattefosse (Paramus, NJ, USA). PEG sorbitan monooleate (Tween® 80), sorbitan monooleate (Span® 80), propylene glycol (PG), oleyl alcohol was purchased from Junsei Chemicals (Japan). Isopropyl palmitate (IPP), isopropyl myristate (IPM), PEG-12 palm kernel glycerides (Crovol® PK40), and PEG-20 almond glycerides (Crovol® A40) were obtained from Croda (Parsippany, NJ, USA). Lauryl alcohol (R)-(+) Limonene, Brij® 30 and Brij® 52 were purchased from Sigma Chemical (St. Louis, MO, USA). Acrylic rubber hybrid, polyisobutylene (PIB) and styrene–butadiene–styrene (SBS) pressure sensitive adhesive (PSA) solutions in organic solvents were obtained from National Starch and Chemical Company (Bridgewater, NJ, USA). Silicone PSA was obtained from Dow Corning (Midland, MI, USA). All other chemicals were reagent grade or above and were used without further purification.

Methods

Patch preparation

Since patches prepared using salt form showed very low permeability (data not shown), donepezil hydrochloride was converted to the free base form using equimolar amount of sodium hydroxide. Differential scanning calorigrams showed that the melting point of donepezil hydrochloride (230°C) was reduced to around 90°C after the conversion (Fig. 1). The drug solution was obtained by dissolving donepezil in ethyl acetate, and permeation enhancer(s) were added. Adhesive solution and drug solution were mixed and stirred sufficiently. The mixture was cast on release liner coated with silicone and solvent was removed by evaporation at 80°C for 20 min. Then the dried adhesive layer was laminated onto the backing membrane. The drug and enhancers are expressed as weight % with respect to dry PSA polymer throughout the article.

Measurement of in vitro skin permeation rate

Skin permeation rates of various donepezil/enhancer formulations were determined using flow through diffusion cells. Permeation experiments were done on isolated hairless mouse skin. A system comprising a multi channel peristaltic pump, a fraction collector, a circulating water bath and flow-through diffusion cells was used. Each flow-through cell had two arms, which allowed the receiver cell medium pumped to a fraction collector. The diffusion cell temperature was maintained at 37°C by circulating water through the outer part of jacketed receiver cell. The surface area of receiver cell opening was 2 cm^2, and its volume was 5.5 ml. Skin was excised from hairless mouse that was humanly sacrificed

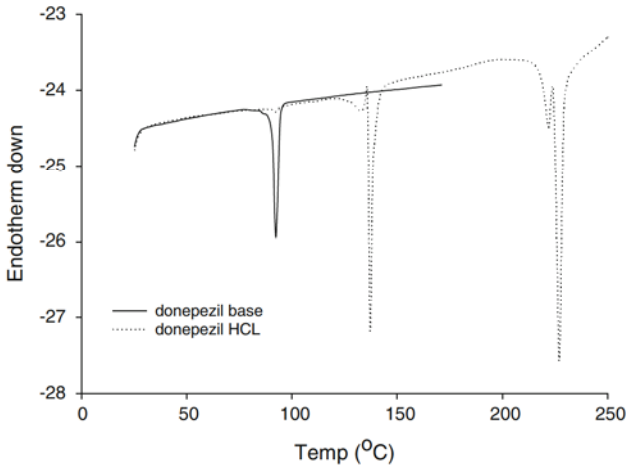

Fig. 1 Differential scanning calorimetric thermogram of donepezil as base and hydrochloride salt form

with diethyl ether. Subcutaneous fat was removed with scissors and scalpel. Each of the flow-through diffusion cell components was connected via silicone rubber tubing with an internal diameter of 0.015 inches. The receiver cell was filled with a pH 6 buffer solution and the media was stirred by Teflon-coated magnetic bar. The prepared patch was placed on the stratum corneum and the excised skin was mounted onto each receiver cell. And O-ring and cell top was placed on the top of each skin. These components were then clamped. The amount of drug permeated across the skin was calculated from the cumulative release. The samples were collected every 4 h for 24 h and assayed by HPLC.

Analytical method

Donepezil was analyzed by HPLC system (Shimadzu Scientific Instruments, MD), consisting of a UV detector (SPD-10A), reversed-phase C_{18} column (4.6 × 100 mm, 5 µm, Gemini), a pump (LC-10AD), and an automatic injector (SIL-10A). Briefly, the wavelength of the UV detector was 315 nm, the column temperature was maintained at 30°C, the flow rate was 1 ml/min and injection volume was 10 µl. Mobile phase consisted of Acetonitrile/phosphate buffer 0.1 M with triethanolamine (0.01% v/v) adjusted to pH 2.7 with 85% phosphoric acid (30/70).

Content analysis

4 cm^2 patch samples were cut, and weighed. Release liner was separated and weighed. Backing membrane containing the matrix was transferred in 50 ml vial with screwed cap (Schott Duran). Then, 50 ml of HPLC grade methanol and teflon coated magnetic bar was added. The container was then capped and sealed with Parafilm®. Then, the samples were sonicated for 30 min followed by stirring for 12 h. The backing membrane was removed from the container, washed with ethyl acetate to remove the PSA matrix, and weighed. The solution was filtered through Whatman® nylon membrane filter (13 mm, 0.45 µm) and analyzed by HPLC.

Differential scanning calorimetry (DSC)

Thermal analysis was carried out to characterize donepezil hydrochloride and base form, using a DSC unit (Pyris 6 DSC, Perkin-Elmer, Netherlands). Indium was used to calibrate the temperature scale and enthalpic response. Samples were placed in aluminum pans and heated at a scanning rate of 5°C/min from 25 to 250°C.

Stability

Stability studies of the optimized formulation were conducted at three different temperature conditions. Physical stability of the patches kept in refrigerator (2–8°C), room temperature (RT) and 40°C oven were monitored visually at different time intervals. Chemical stability was assessed using previously reported stability indicating analytical method (Hanatani et al. 2008). HPLC system (Shimadzu Scientific Instruments, MD), consisting of a UV detector (SPD-10A), reversed-phase C_{18} column (4.6 × 150 mm, 5 µm, Shiseido), a pump (LC-10AD), and an automatic injector (SIL-10A) was used. Briefly, the wavelength of the UV detector was 271 nm, the column temperature was maintained at 25°C, the flow rate was 1 ml/min and injection volume was 20 µl. The mobile phase used consisted of sodium1-decansulfonate aqueous solution/Acetonitrile/70% perchloric acid = 650/350/1 (volume ratio); sodium 1-decansulfonate concentration was 10 mM of total mobile phase.

Results and discussion

Selection of pressure sensitive adhesive matrix

The effect of the PSA matrix on the permeation of donepezil was investigated using silicone, PIB, SBS, acrylic and acrylic rubber hybrid adhesive matrixes. Permeation profile of donepezil from various PSA matrices is shown in Fig. 2. Solubility of donepezil was found to be inadequate in silicone and PIB adhesive matrices and some of donepezil was suspended in the matrix. The glass transition temperature of PSA, interaction between the drug and functional group of PSA, adhesive force and many other properties can influence flux of drug from PSA across the skin (Hai et al. 2008; Venkatraman and Gale 1998). The permeation rate was lowest in the PIB matrix, followed

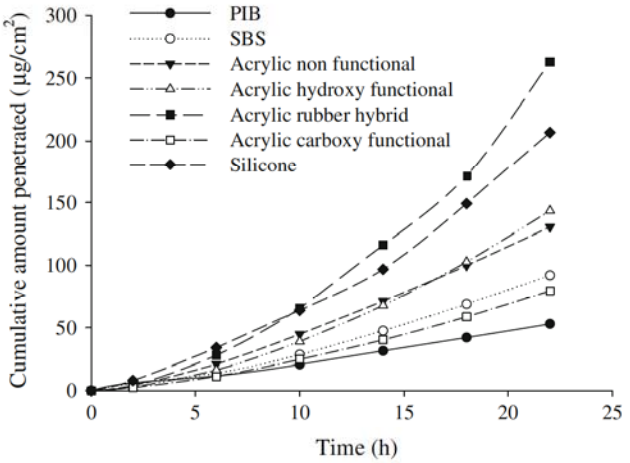

Fig. 2 Screening of different pressure sensitive adhesives at 10% w/w of drug load. Values are expressed as mean ($n = 3$)

by highly crossed linked acrylic adhesive containing carboxyl functional group, Duro-Tak® 87-2677. This could be due to the interaction between amine group of donepezil and carboxyl group of the adhesive. In previous study, low permeation rate of tacrine was observed due to the interaction between the amine group of tacrine and carboxyl group of acrylic adhesive (Kim et al. 2000). Permeation rate of donepezil in the acrylic rubber hybrid adhesive matrix, Duro-Tak® 87-502A was highest followed by silicone, Dow Corning BioPSA® 7-4302. Further study on different kinds of acrylic rubber hybrid adhesives containing hydroxyl functional group revealed that Duro-Tak® 87-504A provided higher flux for donepezil (Fig. 3). Permeation of donepezil from Duro-Tak® 87-502A and 87-503A matrices was similar. Acrylic rubber hybrid PSAs are prepared from an acrylic polymer grafted with a hydrogenated rubber. The hybrid PSA comprises of polymer from ethylene-butylene macromer and hydroxyethyl acrylate monomer (Foreman et al. 2003). Higher flux obtained for donepezil from acrylic rubber hybrid PSAs could be attributed to the suitable polar monomer favorably affecting the thermodynamic behavior of donepezil in the matrix (Cantor and Wirtanen 2002).

Since matrix thickness is an important functional characteristics of matrix based transdermal system, its effect on the permeation of donepezil was also investigated. Permeation profile of donepezil was unchanged when matrix increased from 65 to 85 µm (Fig. 4). However, further increase in matrix thickness resulted in lower permeation profile of donepezil. Matrix thickness of 85 µm was chosen for further experiments based on better adhesive properties as compared to 60 µm matrix.

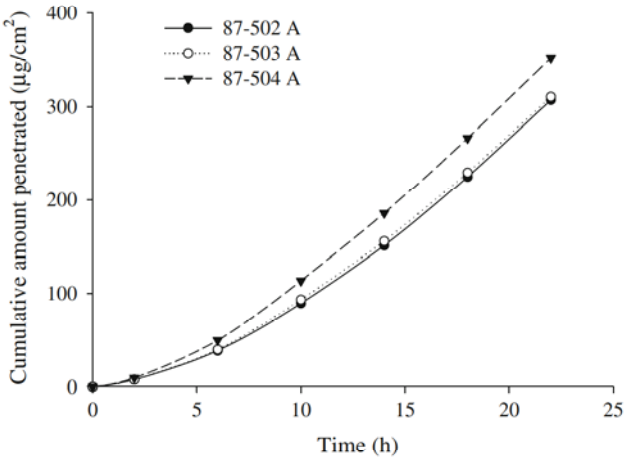

Fig. 3 Screening of different rubber acrylic hybrid pressure sensitive adhesives at 15% w/w drug load. Values are expressed as mean ($n = 3$)

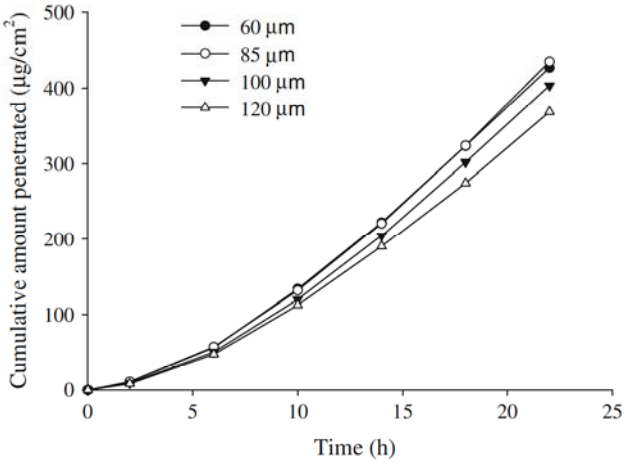

Fig. 4 Effect of acrylic rubber hybrid matrix thickness on the permeation of donepezil. Values are expressed as mean ($n = 3$)

Effect of enhancer

To reversibly overcome the barrier properties of stratum corneum, penetration enhancers are commonly employed in the transdermal systems (Williams and Barry 2004). Enhancer screening was carried out with both Duro-Tak® 87-502A and 87-504A matrices. Table 1 gives the summary of enhancer screening at the level of 5% v/w with 15% w/w drug load in Duro-Tak® 87-502A acrylic rubber hybrid matrix. Due to higher solubility of donepezil in Duro-Tak® 87-502A acrylic rubber hybrid matrix, drug load was increased to 15%. Brij® 30, Plurol olieque®

Table 1 Summary of enhancer screening at the level of 5% v/w with 15% w/w drug load in Duro-Tak® 87-502A acrylic rubber hybrid matrix. Values are expressed as mean ($n = 3$)

S. No.	Enhancer	ER*
1	Control	1.00
2	Brij® 30	2.10
3	Plurol olieque® CC497	1.47
4	Crovol® A 40	1.32
5	Oleyl alcohol	1.37
6	Lauryl alcohol	1.34
7	IPM	1.12
8	Sugar ester P-1670	1.33
9	Limonene	1.06
10	Span® 80	1.25
11	Transcutol®	1.17
12	IPP	1.17
13	Cineole	1.11
14	Labrafil® 1944	1.09
15	Incrocas® 30	1.04
16	Brij® 52	1.29

* *ER* enhancement ratio

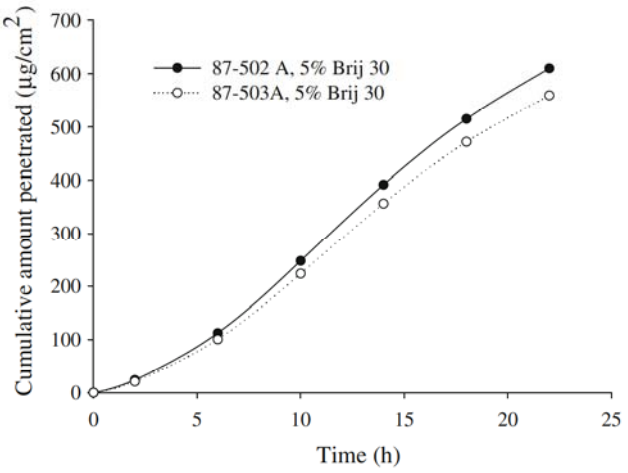

Fig. 5 Permeation profile of donepezil at 15% drug load, in presence of 5% Brij 30®, from Duro-Tak® 87-502A and Duro-Tak® 87-503A matrices. ($n = 3$)

CC497, Crovol® A40, oleyl alcohol, lauryl alcohol, sugar ester P-1670, Span® 80 and Brij® 52 significantly enhanced the in vitro flux of donepezil from Duro-Tak® 87-502A matrix. The enhancing effect of Brij® 30 was compared between Duro-Tak® 87-502A and Duro-Tak® 87-503A. As can be seen in Fig. 5, no significant difference was observed.

Table 2 gives the summary of enhancer screening at the level of 5% v/w with 10% w/w drug load in Duro-Tak® 87-504A acrylic rubber hybrid matrix. Among the enhancers screened, Brij® 30, Brij® 52, IPM, glycerol and diethoxyethyl succinate were associated with the significant enhancing effect. Brij® 30 provided highest enhancement ratios in both Duro-Tak® 87-502A and Duro-Tak® 87-504A matrices and was chosen for further experiments. Brij® 30 is a surfactant which belongs to the class of polyoxyethylene (POE) alkyl ethers. The EO chain length and HLB value of Brij® 30 is 4 and 9.7 respectively. Studies have shown that POE alkyl ethers containing EO chain length of 2–5 and HLB value 7–9 are effective promoters for the percutaneous absorption of drug molecules (Park et al. 2000). Brij® 30 could efficiently disrupt the lipid arrangements in SC via both hydrophilic and lipophilic molecular mechanism, thereby enhancing the penetration of donepezil (Breuer 1979; Walters et al. 1987).

Table 2 Effect of penetration enhancers, at the level of 5% v/w, with 10% w/w of drug load in Duro-Tak® 87-504A matrix. Values are expressed as mean ($n = 3$)

S. No.	Enhancer	ER	S. No.	Enhancer	ER
1	Control	1.00	14	Diisopropyl adipate	1.18
2	Brij® 30	1.70	15	Oleyl oleate	1.11
3	Plurol oleique® CC497	1.02	16	Labrasol	1.19
4	Crovol® A 40	1.20	17	Tween® 80	1.09
5	Oleyl alcohol	1.11	18	Limonene	0.97
6	Lauryl alcohol	0.94	19	Glycerol	1.24
7	IPM	1.22	20	Diisopropyl dirrerate	1.13
8	Span® 80	1.17	21	Crovol® PK40	1.11
9	Transcutol®	0.94	22	Hexyl Laurate	1.17
10	IPP	1.00	23	Octyl dodecyl ester	0.99
11	Cineole	1.07	24	Isotridecyl isononanoate	0.99
12	Brij® 52	1.42	25	2-ethylhexyl hydroxystearate	1.05
13	Alkyl 2-ethyl hexanate	1.20	26	Diethoxylethyl succinate	1.32

Effect of combining enhancers

To further increase the transdermal flux of donepezil, effect of combining selected enhancers at the level of 2.5% v/w with 5% v/w of Brij® 30 was studied. Especially for drug in adhesive type of TDDS, presence of additives can modify the mechanical characteristics of PSA, and might make the adhesive more susceptible to creep/cohesive failure. Hence, adhesive properties of the patches containing combination of enhancers were also assessed manually using thumb test. Table 3 provides the summary of results obtained using combination of Brij® 30 with selected enhancers in Duro-Tak® 87-502A matrix at 15% w/w drug load. Enhancement ratios were calculated using flux from Brij® 30 as control. Only combinations of Brij® 30 with Brij® 52, Crovol® A40 and Plurol olieque® CC497 were found to have higher enhancement ratio as compared to Brij® 30 alone. Adhesive properties of patches containing combination of Brij® 30 with Plurol olieque® CC497 or Span® 80 were found to be unsatisfactory. Brij® 52 could be added up to 5% in addition to 5% Brij® 30 without impairing the adhesive property of the patch. Based on the flux and adhesion properties,

Table 3 Summary of the results obtained using combination of Brij® 30 at the level of 5% v/w with selected enhancers at the level of 2.5% v/w in Duro-Tak® 87-502A matrix containing 15% w/w drug load. Values are expressed as mean ($n = 3$)

Combination of enhancers	ER	Adhesive property
Brij® 30	1.00	Good
Brij® 30, Plurol olieque® CC497	1.20	Unsatisfactory
Brij® 30, Span® 80	0.78	
Brij® 30, Oleyl alcohol	0.83	Good
Brij® 30, Brij® 52	1.37	
Brij® 30, Crovol® A40	1.27	
Brij® 30, IPP	0.80	
Brij® 30, Lauryl alcohol	0.75	
Brij® 30, Transcutol®	0.70	
Brij® 30, Cineole	0.81	

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