Drug Development and Industrial Pharmacy, 2012; 38(3): 323–330

ISSN 0363-9045 print/ISSN 1520-5762 online
DOI: 10.3109/03639045.2011.602978

informa
healthcare

RESEARCH ARTICLE

Effect of vehicle systems, pH and enhancers on the permeation of highly lipophilic aripiprazole from Carbopol 971P gel systems across human cadaver skin

Muhammed Anwar Hossain[1,3], Salah U. Ahmed[2], and Fotios M. Plakogiannis[1]

[1]Division of Pharmaceutical Sciences, Arnold & Marie Schwartz College of Pharmacy and Health Sciences, Long Island University, Brooklyn, NY, USA, [2]Abon Pharmaceuticals, LLC, Northvale, NJ, USA, and [3]Teva Pharmaceuticals USA, 223 Quaker Road, Pomona, NY, USA

Abstract

The objective of this study was to investigate the effect of vehicle systems, pH and enhancers on the permeation of a highly lipophilic basic drug aripiprazole (ARPZ) through human cadaver skin. Solubility of ARPZ in single, binary, tertiary, and quaternary vehicle systems of N-methyl pyrrolidone (NMP), dimethyl sulfoxide (DMSO), water, ethanol and isopropyl myristate (IPM) was studied. Gel formulations of 5% ARPZ were developed with 0.5% Carbopol 971P in quaternary vehicle systems consisting of NMP, DMSO, water and ethanol or IPM at optimum ratio of 40/40/5/15. The effect of pH of the gel formulations and fatty acids with different chain lengths on the permeation was studied. The flux of ARPZ from gel formulation with IPM and ethanol was comparable. A four fold increase in APRZ flux was observed when the pH of the gel systems was lowered from pH 8.2 to pH 6 or pH 7. For fatty acids, the order of flux is lauric acid > myristic acid > caprylic acid > oleic acid. In all the cases, *in vitro* permeation rate of ARPZ through human cadaver skin followed zero order kinetics. This study demonstrated that ARPZ in tertiary vehicle system of NMP/DMSO/water/IPM at ratio of 40/40/5/15 and gel system of Carbopol 971P with pH 7 is a promising candidate for transdermal delivery.

Keywords: Psychotropic drug, transdermal delivery system, penetration enhancer, stratum corneum, quaternary vehicle systems, N-methyl pyrrolidone, dimethyl sulfoxide, isopropyl myristate, fatty acids, pH

Introduction

Optimum therapeutic outcome requires not only proper drug selection, but also an effective drug delivery system. In the case of a psychotropic drug, rigorous compliance to a regular medication schedule is of great clinical importance. In many instances, oral administration of psychotropic drugs is considered less than optimal delivery system due to non-compliance. Delivery of psychotropic medication via transdermal patch is one of the potential methods to promote patient compliances[1]. A well designed and successfully developed controlled release transdermal delivery system is able to deliver various drugs across the skin into the systemic circulation with considerable biomedical benefits[2].

The amount of drug that can be delivered through the skin depends on the integrity of the skin barrier, the physicochemical properties of the permeant, the physicochemical characteristics of the vehicles in which the permeant is applied to the skin and the dosing conditions[3]. The relative impermeability of the stratum corneum (SC) provides the principal resistance to percutaneous absorption of most drugs[4]. A drug molecule released from a dosage form has to pass through the skin layers by a multistep sequential process before it reaches systemic circulation. The process includes partitioning and diffusion through the lipophilic SC, partitioning into the aqueous epidermis and finally diffusion into the capillary network of the dermis[5]. In an attempt to overcome the problems arising from skin impermeability

Address for Correspondence: Muhammed Anwar Hossain, Long Island University, Pharmaceutical Sciences, 1 University Plaza, Brooklyn, 11201-5372, USA. Tel: (845) 362–7935. Fax: (845) 362-2660. E-mail: anwar.hossain@tevausa.com

(Received 15 April 2011; revised 20 June 2011; accepted 29 June 2011)

and biological variability, various approaches have been investigated[6]. One possible mechanism is increased skin/vehicle partitioning of the drug. The composition of vehicle systems has been reported to increase the skin permeation and shorten the lag time of several drugs[7–9]. A slight change in vehicle composition could highly influence the skin permeability of yohimbine[10]. The vehicle's composition can affect both drug release and skin permeability properties. Another way to enhance the dermal and transdermal delivery is the addition of penetration modifiers. Two main mechanisms of penetration enhancement can be distinguished[11]. Firstly, the penetration modifier enters the skin, increases the permeant's solubility in the skin and thereby increases the partition coefficient of the active ingredient between skin and vehicle. Secondly, the penetration enhancer diffuses into the skin and interrupts the intercellular lipid packing and hence increases the diffusivity of the permeant in the skin. Highly lipophilic compound showed low skin permeability due to accumulation of lipophilic drugs in SC because of low aqueous solubility[12]. A transdermal candidate must possess both lipophilic and hydrophilic characteristics. A molecule that is too hydrophilic will not partition into SC and if the molecule is too lipophilic, it will not move down to the subsequent aqueous layers in the epidermis[5]. Therefore, an improvement in the transdermal permeability of a highly lipophilic molecule is possible by increasing its hydrophilicity either by salt formation or pH modification.

Aripiprazole (ARPZ), an atypical psychotropic drug, is indicated for acute and maintenance treatment of schizophrenia and manic and mixed episodes associated with bipolar disorders and is available as tablet, oral solution and injection. Although transdermal delivery is a preferred route of administration for a psychotropic drug, there is no ARPZ transdermal delivery system available. Therefore, transdermal delivery of ARPZ, especially with long duration of action, would be helpful in achieving compliance with a rigorous medication schedule and is expected to have a profound impact on patient care.

The purpose of this study was to investigate the effect of vehicle systems, enhancers and pH on the skin permeation of the highly lipophilic drug ARPZ from a matrix transdermal delivery system (TDS) to achieve an optimum delivery of ARPZ through the skin.

Materials and methods

Materials

ARPZ was obtained from Teva Pharmaceuticals, USA (Pomona, NY). N-methyl pyrrolidone (NMP) was obtained from Budrick & Jackson, (Muskegon, MI). Dimethyl sulfoxide (DMSO), isopropyl myristate (IPM), Hydrochloric acid, acetonitrile and trifluoroacetic acid were obtained from J.T. Baker (Phillipsburg, NJ). Ethanol was obtained from Spectrum (New Brunswick, NJ) and Carbopol 971P was obtained from Lubrizol Advanced Materials (Cleveland, OH).

Methods

High-performance liquid chromatography (HPLC) analysis of ARPZ

The analytical separation of ARPZ was performed using a Waters HPLC system equipped with 600 E multistation delivery system, 717 Plus autoinjector, 996 photodiode array detector and 2010 Millennium data management system. A reversed phase Varian Inertsil ODS-3 Column, 100 cm × 4.6 mm, 5 μm particle size was used as analytical column that was maintained at 35°C. Acetonitrile 35% in deionized water with 0.1% Trifluoroacetic acid (TFA) was used as the mobile phase at a flow rate of 2.0 mL/min. With an injection volume of 10 μl and detector wavelength set at 216 nm, the chromatographic peak for ARPZ was detected at a retention time of about 4.5 min.

Solubility in various vehicle systems

The solubility of ARPZ in various vehicles was determined by adding excess amounts of ARPZ into screw-capped centrifuge tubes containing 5–10 mL solvents (NMP, DMSO, water, IPM and Ethanol) and mixtures of various solvents at different ratios. The tightly sealed centrifuge tubes were shaken in a water bath set at 37°C for 48 h. After 72 h of shaking, the tubes were centrifuged at 30,000 RPM for 30 min. The supernatant from each tube was collected after equilibrium at room temperature and analyzed using the HPLC method cited above after appropriate dilution of the analyte.

Preparation of ARPZ Gel Formulations

Appropriate amount of drug was weighed into 20 mL volumetric flask. About 15 mL of vehicle system was added to flask and sonicated for 20 min or until the drug was completely dissolved. Weighed amount of Carbopol 971P was dissolved into the drug solution. Finally, the gel formulation was diluted to 20 mL with the vehicle system. The prepared gel systems were left at least 12 h at room temperature for any trapped air to be removed before using for diffusion study.

Two gel formulations with quaternary vehicle systems of (NMP/DMSO/Water/Ethanol) and (NMP/DMSO/Water/IPM) at ratio of 40/40/05/15 were prepared using 5% ARPZ and 0.5% Carbopol 971P. The pH of the formulations was 8.2. The pH of the gel formulations was adjusted to pH 6 and pH 7 by adding concentrated hydrochloric acid. Four additional gel formulations were made in which 5% caprylic acid, oleic acid, myristic acid and lauric acid were added. Viscosity and pH of all the gel systems were determined before being used for the diffusion study.

Viscosity determinations

The viscosity of gel formulations were measured with a controlled-stress Rheometer (AR 1000, TA Instruments, New Castle, DE, USA). The measurement was performed at constant temperature (25°C) with parallel-plate geometry. The stainless steel parallel plate diameter was 40 mm, and the plate-plate gap was set at 250 μm for all

tests. The apparent viscosity of each gel formulation was evaluated in triplicate.

In vitro *permeation studies*

Permeation of ARPZ across human cadaver skin was evaluated using vertical Franz diffusion cells with an available diffusion area of 1.77 cm2. The frozen human cadaver skin was thawed and hydrated for 1 hour at room temperature before mounting on the vertical Franz diffusion cell. The skin was mounted on the receptor compartment with the SC side facing upwards into the donor compartment. The formulation was gently applied to the donor side of the skin surface. In each of the donor cells, 2 g of test formulation was added and the sample in the donor cell was sealed with parafilm. The receptor cell volume was 7 mL and was filled with receptor medium of NMP/DMSO/water/ethanol at the ratio of 40/40/05/15. The receptor medium was kept at 37°C and stirred by Teflon coated magnetic bar at 500 rpm throughout the run. At predetermined time intervals, 500 µl of receptor solutions were withdrawn and replaced with fresh medium. The amount of ARPZ released from the formulation was determined by HPLC directly. All diffusion studies were carried out in triplicate.

Stability of ARPZ solution and gel systems

ARZP solution and gel systems were stored at 40°C/75% RH and tested for assay at 1 month, 2 months and 3 months intervals.

Data analysis

The cumulative amounts of ARZP permeated were plotted as a function of time. The average flux (J_{ave}, µg/cm2/h) was calculated from the slope of the linear portion of the profiles. The lag time (L, h) was the x-intercept of the above linear fit of the permeation profiles[13]. The following equation was used:

$$J_{ave} = \left(1/A\right)\left(\frac{dM}{dt}\right)_{ave} \quad (1)$$

Where, $\left(dM/dt\right)_{ave}$ = slope of the straight line obtained by linear regression of the M vs. t profiles over the experimental time frame

A = diffusional area.

Statistical analysis

All the data were expressed as mean ± SD (n = 3). Where appropriate, statistical comparisons were made using Student's t-test ran on GraphPad Software (GraphPad Software, Inc., CA 92037 USA). Results were considered significant at 95% confidence interval ($p < 0.05$).

Results and discussion

Solubility of ARPZ and selection of vehicle system

The saturation solubility of ARPZ varied among various combinations of binary, tertiary and quaternary vehicle systems of NMP, DMSO, water, Ethanol and IPM. ARPZ is practically insoluble in water, slightly soluble in Ethanol and IPM, but freely soluble in NMP and DMSO. In the presence of water, the solubility of ARPZ decreases dramatically. The maximum solubility of ARPZ was found to be in NMP (45.1% w/v) followed by DMSO (11.7% w/v). As the concentration of NMP and DMSO increased in quaternary vehicle system, solubility of ARPZ increased significantly. The solubility of ARPZ in various single, binary, tertiary and quaternary vehicle systems is presented in Table 1. A general and initial approach to improve transdermal permeation rate is to optimize a suitable solvent system[14]. It is well established that a principal driving force for diffusion across the skin is the thermodynamic activity of the permeant in the donor vehicle[15]. This activity is reflected by the concentration of the permeant in the donor vehicle as a function of its saturation solubility within that medium. Solvents may act as a penetration enhancer (PE) by increasing thermodynamic activity of the drug and/or changing the barrier property of the skin[16]. An optimum and pharmaceutically acceptable quaternary vehicle system of NMP/DMSO/Water/Ethanol or NMP/DMSO/Water/IPM in the ratio of 40:40:05:15 was used for permeation studies. Although the solubility of ARPZ is significantly higher in NMP and DMSO, due to safety concerns, 40% was used in the vehicle system. Only 5% water is selected as it diminishes ARPZ solubility significantly (Table 1).

Table 1. Solubility of ARPZ in single, binary, tertiary and quaternary vehicle systems.

Solvent and composition (%)					
NMP	DMSO	Water	Ethanol	IPM	Solubility % (w/v)
100	—	—	—	—	45.08
—	100	—	—	—	11.71
—	—	100	—	—	0.00
—	—	—	100	—	0.62
—	—	—	—	100	0.16
50	50	—	—	—	24.08
40	40	20	—	—	0.86
40	40	—	20	—	15.14
40	40	—	—	20	21.02
40	40	15	5	—	2.12
40	40	10	10	—	4.83
40	40	5	15	—	8.53
40	40	15	—	5	3.56
40	40	10	—	10	7.56
40	40	5	—	15	12.68

Effect of vehicle systems on the permeation of ARPZ from 5% gel formulation through human cadaver skin

The composition of the vehicle system in which the drug is formulated may have a significant effect on drug delivery across the skin, which may markedly affect therapeutic efficacy. A slight change in vehicle composition highly influenced the skin permeability of yohimbine[10]. Han-Joon et al.[17] showed that apparent diffusion parameters

Table 2. Formulation composition, viscosity and gel systems with their respective average flux and lag time.

Gel ID	Vehicle systems (%)					Fatty Acids	Viscosity cPs	pH	Jave (μg /cm2/h)	T_L (h)
	NMP	DMSO	Water	Ethanol	IPM					
F-1	40	40	5	15	0	—	176.2	8.2	94.51 ± 10.1	6.2 ± 1.24
F-2	40	40	5	0	15	—	172.0	8.1	100.53 ± 11.4	2.2 ± 1.19
F- 3	40	40	5	0	15	—	42.9	6	386.32 ± 16.8	5.2 ± 0.75
F-4	40	40	5	0	15	—	77.9	7	388.50 ± 15.2	5.0 ± 0.90
F-6	40	40	5	0	15	Caprylic	150.6	8.0	60.71 ± 9.7	16.5 ± 3.8
F-7	40	40	5	0	15	Oleic	178.4	8.0	48.70 ± 8.1	17.0 ± 2.9
F-8	40	40	5	0	15	Myristic	114.7	8.1	164.79 ± 13.9	2.5 ± 0.21
F-8	40	40	5	0	15	Lauric	139.1	8.1	178.05 ± 13.1	2.5 ± 0.56

Average permeation flux = Jave and lag time = T_L.
Mean ± SD (n = 3).

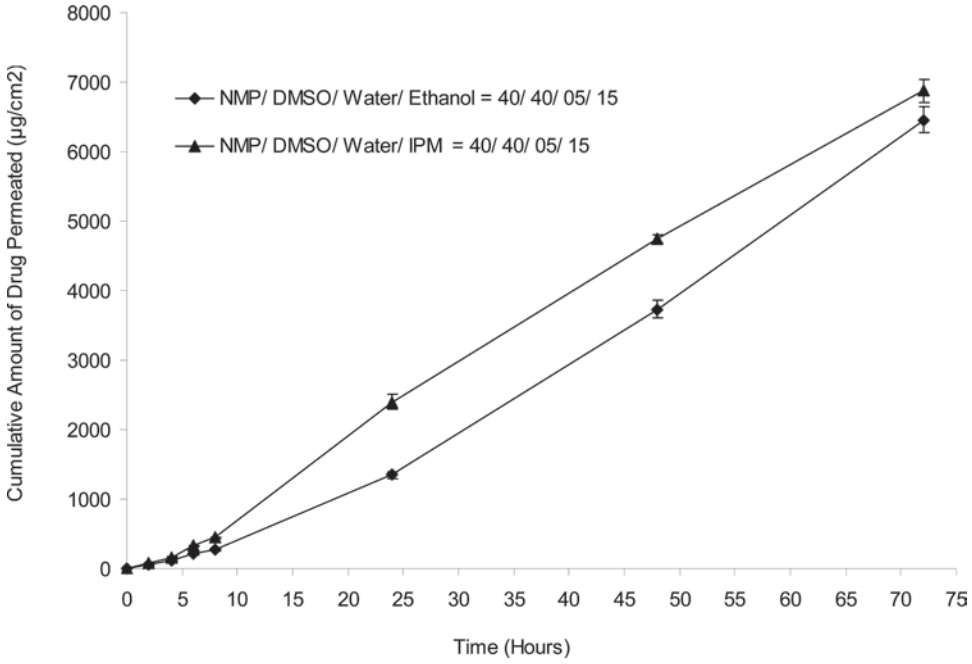

Figure 1. Effect of vehicle systems on the permeation profiles of ARPZ from 5% gel system across human cadaver skin. Each point represents the mean ± SD of three experiments.

of melatonin were affected by the composition of vehicles to different extents. The average flux () of ARPZ from gel systems formulated in a vehicle system of NMP/DMSO/water/IPM and NMP/DMSO/water/Ethanol is 100.53 μg/cm2/h and 94.51 μg/cm2/h, respectively and lag time is 2.2 h and 6.2 h, respectively (Table 2 and Figure 1). This difference in flux is statically insignificant ($p > 0.05$), but the lag time with IPM is significantly shorter ($p < 0.05$). IPM is an aliphatic ester which is widely used as a safe PE in dermatological formulations[18]. IPM probably penetrates between the lipid bilayers of SC and, due to its chain structure, disrupts the order and arrangement of lipid bilayers of the SC thus improving drug penetration into this layer[19]. IPM acts as a vehicle, as well as a penetration enhancer thereby providing higher ARPZ penetration (Figure 1). NMP, DMSO and IPM present in the quaternary vehicle system are well known effective PEs. NMP and its derivatives are widely used chemical enhancers which have produced significant results in the transport of various drugs[20,21]. DMSO is the most important compound belonging to the category of sulfoxides and similar compounds to enhance transdermal permeation of a variety of drugs[22]. It has a long history of being used as a PE, and several reviews have highlighted its ability to enhance the penetration of both lipophilic and hydrophilic drugs[23].

Effect of pH on permeation of ARPZ through human cadaver skin

The major barrier function of skin is provided by the stratum corneum. The stratum corneum is comprised of distinct protein and lipid domains that constituted a highly structured, very stable and effective lipophilic barrier to chemical penetration and permeation. Immediately below the SC is viable epidermis, which is hydrophilic in nature and acts as the rate-limiting step to the absorption of highly lipophilic drugs. Therefore, the optimal characteristic for percutaneous absorption is that the permeant be reasonably soluble in both hydrophilic and hydrophobic media[24]. ARPZ is a highly lipophilic drug

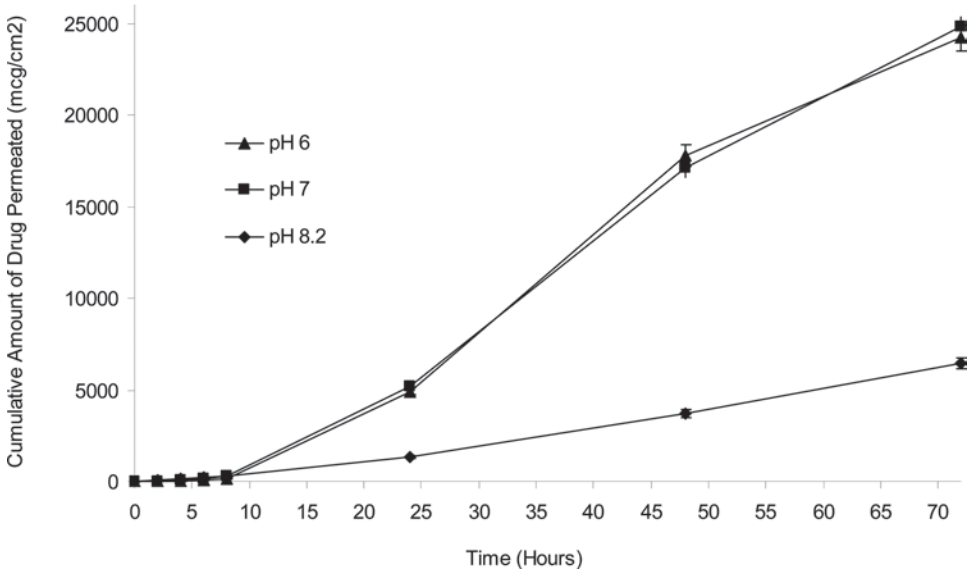

Figure 2. Effect of pH on the permeation profiles of ARPZ from 5% gel system across human cadaver skin. Each point represents the mean ± SD of three experiments.

with a partition coefficient of 5.6 and is basic in nature (pKa = 7.29). When the pH of the gel system is lowered by the addition of hydrochloride acid, the highly hydrophobic ARPZ became reasonably hydrophilic. Both NMP and DMSO present in the vehicle system are excellent PEs for relatively hydrophilic molecules. Large increases in the transdermal penetration of several hydrophilic permeants (up to 450-fold) have been reported using NMP and 2-pyrrolidone, whereas enhancement of lipophilic drugs is less remarkable[25,26]. A vast array of literature describes the penetration enhancing activities of DMSO, and studies have shown it to be effective in promoting both hydrophilic and lipophilic permeants[27]. Therefore, with the addition of hydrochloric acid when ARZP becomes relatively more hydrophilic, the penetration enhancement effect of NMP, DMSO and IPM causes a significant increase in ARPZ permeation though human cadaver skin ($p < 0.05$). The results indicated that flux of ARZP increased by a factor of 4 when the pH of gel system decreased from pH 8.2 to pH 6 or pH 7 (Table 2 and Figure 2). No significant difference in flux was found when pH changed from 7 to 6 ($p > 0.05$).

Effect of fatty acids on the permeation of ARPZ from 5% gel systems through human cadaver skin

Chemical enhancers are thought to enhance permeation through a number of mechanisms, mainly by the disruption of the SC lipid structure and sometimes by the alteration of the SC keratinocytes[28–30]. Among penetration enhancers, various fatty acids have been widely used for transdermal delivery[17]. The mechanism of fatty acids as permeation enhancers was found to be mainly through the enhancement of permeant partitioning into the SC intercellular lipid domain[31]. The effects of fatty acids as permeation enhancers have been shown to be dependent on their structure, alkyl chain length, and degree of saturation[32]. Unsaturated fatty acids have been shown to promote higher magnitudes of permeation enhancement across skin when compared to saturated fatty acids of the same chain length. This has been attributed to the higher disrupting nature of the linked chain of these fatty acids that would result in a higher magnitude of lipid disruption[33–35]. The permeation rate and lag time of ARPZ from 5% gel system are summarized in Table 2. The addition of fatty acids to the gel systems provided mixed permeation results. The permeation of ARPZ from gel systems with 5% oleic acid and caprylic acid is lower compared to the gel system without fatty acid. On the other hand, the permeation is higher from the gel system with 5% lauric acid and myristic acid. The permeation enhancement factor of 1.9, 1.7, 0.62 and 0.52 were obtained with lauric acid, myristic acid, caprylic and oleic acid, respectively (Figure 3). The results indicated a significant increase in flux and shortened lag time ($p < 0.05$) with lauric acid (C12) and myristic acid (C14) compared to caprylic acid (C10) and oleic acid (C18). This finding is in agreement with the work done by Ogiso et al.[36] and Elyan et al.[37], who reported that lauric acid has the optimum chain length (C_{12}) for permeation enhancement. Ogiso et al.[36] suggested that the C_{12} and C_{14} hydrophobic groups have an optimal balance of partition coefficient and affinity for the skin. That is, the short chain fatty acids have insufficient lipophilicity for skin penetration, while long chain fatty acids have a much higher affinity to lipids in the SC, thereby retarding the penetration by hydrophobic interaction.

Effect of viscosity on the permeation of ARPZ from 5% gel systems through human cadaver skin

Viscosity is an important physical property of topical formulations which affect the rate of drug release. In general, an increase of the viscosity would cause a more rigid structure with a consequent decrease in the

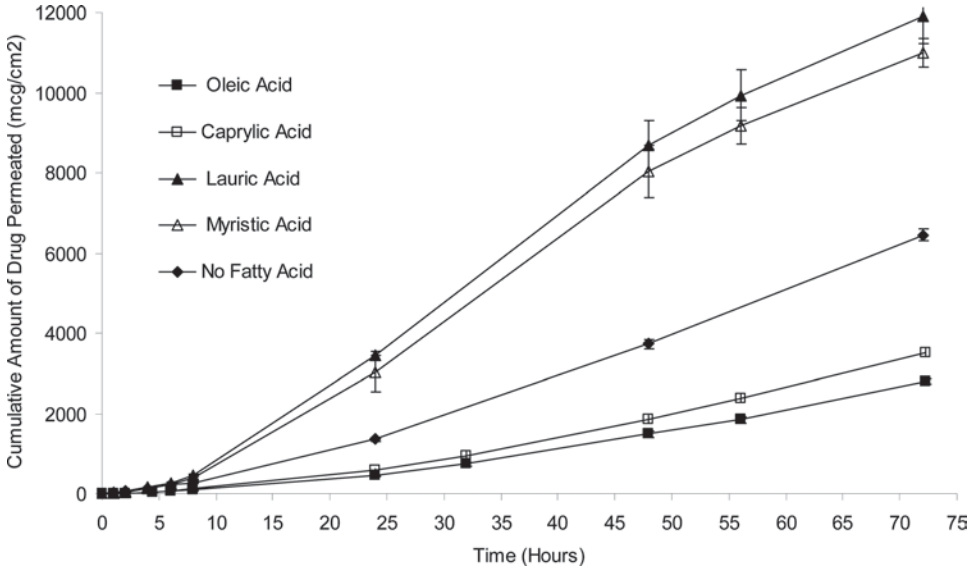

Figure 3. Effect of fatty acids on the permeation profiles of ARPZ from 5% gel system across human cadaver skin. Each point represents the mean ± SD of three experiments.

Table 3. Stability of ARPZ solution and gel systems at 40°C/75% RH.

	% Assay		
Time points	Solution pH 9.8	Gel system pH 8.2	Gel system pH 7.0
Initial	99.1	99.6	99.2
4 weeks	97.6	97.8	98.8
8 weeks	97.1	96.7	99.1
12 weeks	96.9	96.5	99.2

Table 4. Statistical comparisons of average flux and lag time of different ARZP gel systems.

		P-value	
Gel ID	Vehicle systems (%)	Flux	Lag time
Vehicle systems	Ethanol vs. IPM	0.5312	0.0147
pH	8.2 vs. 6.0	0.0001	0.0209
	8.2 vs. 7.0	0.0001	0.0314
	7.0 vs. 6.0	0.8757	0.7822
Fatty acids	Caprylic vs. oleic	0.1715	0.8650
	Myristic vs. lauric	0.2792	0.9856
	Caprylic vs. myristic	0.004	0.0031
	Caprylic vs. lauric	0.0002	0.0032
	Oleic vs. myristic	0.0002	0.0010
	Oleic vs. lauric	0.0001	0.0010

Results considered significant at 95% confidence interval ($p < 0.05$).

rate of drug release[38,39]. Das et al.[40] observed an inverse relationship between in vitro drug release/ex vivo permeation rate and viscosity of the gel formulation. In the present study, Carbopol 971P was used as a gelling agent, which is a hydrophilic polycyclic acid polymer. The carboxyl groups of Carbopol become highly ionized after neutralization forming a gel due to electrostatic repulsion among charged polymers chains interconnected by cross-link[41,42,43]. The viscosity and bioadhesive strength of Carbopol is highly dependent upon the pH[43]. The viscosity of ARPZ gel system decreased from 172 cPs to 77.9 cPs and 42.9 cPs when the pH of the system changed from 8.2 to 7 and 6, respectively. The flux of ARPZ from the gel system was increased from 100.5 µg/cm2/h at pH 8.1 to 388.5 µg/cm2/h and 386.3 µg/cm2/h at pH 7 and pH 6, respectively (Table 2).

Stability of ARPZ solution and gel systems

Stability data of aripiprazole solution and gel systems at pH 8.2 and pH 7.0 are presented in Table 3. Data indicated that ARPZ solution and gel system are stable for 3 months at 40°C/75% RH. However, maximum stability was observed at pH 7.0.

Statistical analysis

Statistical comparisons of flux and lag time of different ARZP gel systems were made using Student's t-test ran on GraphPad Software. Results were considered significant at 95% confidence interval ($p < 0.05$) and p values are presented in Table 4.

Conclusion

The studies showed that an effective TDS of highly lipophilic atypical psychotropic agent, ARPZ, can be formulated by using a quaternary vehicle system of NMP/DMSO/water/IPM at the ratio of 40/40/5/15. The pH of the delivery system significantly affects the permeability of ARZP and maximum permeability was observed at pH 6 and pH 7. Fatty acids affect the permeation of ARPZ differently; lauric acid and myristic acid increase the permeation, whereas, caprylic acid and oleic acid decrease the permeation. The addition of IPM and lauric acid along with the adjustment of pH to 7 effectively increased the flux and shortened the lag time of APRZ from TDS. Therefore, ARPZ in quaternary vehicle system of NMP/DMSO/water/IPM at ratio of 40/40/5/15 and gel system

of Carbopol 971P with lauric acid enhancer and pH 7 is a promising candidate for successful development of an effective TDS.

Declaration of interest

We acknowledge the support from Tony (Wei-Qin) Tong, VP R&D, Teva Pharmaceuticals, Pomona (NY) for the encouragement and support for this work.

References

1. Aggarwal G, Dhawan S. (2010). Psychotropic Drugs and transdermal Delivery: An Overview. International J of Pharma and Bio Science, 1(2),
2. Chein YW. Novel Drug Delivery systems. New York: Marcel Dekker, Inc; 1992: 1–42.
3. Wiechers JW. (1989). The barrier function of the skin in relation to percutaneous absorption of drugs. Pharm Weekbl Sci, 11: 185–198.
4. Arellano A, Santoyo S, Martín C, Ygartua P. (1999). Influence of propylene glycol and isopropyl myristate on the *in vitro* percutaneous penetration of diclofenac sodium from carbopol gels. Eur J Pharm Sci, 7:129–135.
5. Subedi RK, Oh SY, Chun MK, Choi HK. (2010). Recent advances in transdermal drug delivery. Arch Pharm Res, 33: 339–351.
6. Marjukka Suhonen T, Bouwstra JA, Urtti A. (1999). Chemical enhancement of percutaneous absorption in relation to stratum corneum structural alterations. J Control Release, 59:149–161.
7. Kim JJ, Chi SC, Shim CK, Kim CK. (1992). Effect of Tween 20 on the penetration of ketoprofen through excised rat skin. J Kor Pharm Sci, 22:163–166.
8. Kim CK, Kim JJ, Chi SC, Shim CK. (1993). Effect of fatty acids and urea on the penetration of ketoprofen through rat skin. Int J Pharm, 99:109–118.
9. Lee CK, Uchida T, Noguchi E, Kim NS, Goto S. (1993). Skin permeation enhancement of tegafur by ethanol/panasate 800 or ethanol/water binary vehicle and combined effect of fatty acids and fatty alcohols. J Pharm Sci, 82:1155–1159.
10. Carelli V, Di Colo G, Nannipieri E, Serafini MF. (1998). Effect of vehicles on yohimbine permeation across excised hairless mouse skin. Pharm Acta Helv, 73:127–134.
11. Otto A, Wiechers JW, Kelly CL, Hadgraft J, du Plessis J. (2008). Effect of penetration modifiers on the dermal and transdermal delivery of drugs and cosmetic active ingredients. Skin Pharmacol Physiol, 21:326–334.
12. Adrian PF, Roman S, Hans WM, Clemens G, Rainer HM, Ralph L. Transdermal delivery of highly lipophilic drugs: In vitro fluxes of antiestrogens, permeation enhancers, and solvents from liquid formulations. Pharmaceutical Research, Vol. 19, No. 5, May 2002.
13. Chisty MNA, Plakogiannis MF, Bellantone RA, Taft DR. "In vitro evaluation of the release of albuterol sulfate from polymer gels: Effect of fatty acids on drug transport across biological membranes". Drug Development and Industrial Pharmacy, Vol 28, No. 10, pp. 1221–1229, 2002.
14. Narisetty ST, Ramesh P. (2003). Transdermal delivery of zidovudine: Effect of vehicles on permeation across rat skin and their mechanism of action. European J of Pharmaceutical Sciences, 18:71–79.
15. Ostrenga J, Haleblian J, Poulsen B, Ferrell B, Mueller N, Shastri S. (1971). Vehicle design for a new topical steroid, fluocinonide. J Invest Dermatol, 56:392–399.
16. Møllgaard B, Hoelgaard A. (1983). Vehicle effect on topical drug delivery. II. Concurrent skin transport of drugs and vehicle components. Acta Pharm Suec, 20:443–450.
17. Han-Joon O, Yu-Kyoung O, Chong-Kook K. (2001). Effects of vehicles and enhancers on transdermal delivery of melatonin. Int J Pharm, 212:63–71.
18. Seyed AM, Reza A. (2003). An investigation into the effect of various penetration enhancers on percutaneous absorption of piroxicam. Iranian J Pharm Research, 2:135–140.
19. Sato A, Friend O, Nagai I. (1988). Effect and mode of action of aliphatic esters. Int J Pharm, 43:31–40.
20. Sasaki H, Kojima M, Nakamura J, Shibasaki J. (1990). Enhancing effect of combining two pyrrolidone vehicles on transdermal drug delivery. J Pharm Pharmacol, 42:196–199.
21. Phillips CA, Michniak BB. (1995). Transdermal delivery of drugs with differing lipophilicities using azone analogs as dermal penetration enhancers. J Pharm Sci, 84:1427–1433.
22. Sinha VR, Kaur MP. (2000). Permeation enhancers for transdermal drug delivery. Drug Dev Ind Pharm, 26:1131–1140.
23. Asbill CS, El-Kattan AF, Michniak B. (2000). Enhancement of transdermal drug delivery: Chemical and physical approaches. Crit Rev Ther Drug Carrier Syst, 17:621–658.
24. Kenneth AW, Keith RB, Darren MG, Valerie JJ, Adam CW, Robert HS. (1998). Comparison of the transdermal delivery of estradiol from two gel formulations. Maturitas, 29:189–195.
25. Williams AC. Transdermal and topical drug delivery. Lotion: Pharmaceutical Press; 2003.
26. Koizumi A, Fujii M, Kondoh M, Watanabe Y. (2004). Effect of N-methyl-2-pyrrolidone on skin permeation of estradiol. Eur J Pharm Biopharm, 57:473–478.
27. Williams AC, Barry BW. (2004). Penetration enhancers. Adv Drug Deliv Rev, 56:603–618.
28. Chantasart D, Sa-Nguandeekul P, Prakongpan S, Li SK, Higuchi WI. (2007). Comparison of the effects of chemical permeation enhancers on the lipoidal pathways of human epidermal membrane and hairless mouse skin and the mechanism of enhancer action. J Pharm Sci, 96:2310–2326.
29. Ogiso T, Paku T, Iwaki M, Tanino T. (1995). Percutaneous penetration of fluorescein isothiocyanate-dextrans and the mechanism for enhancement effect of enhancers on the intercellular penetration. Biol Pharm Bull, 18:1566–1571.
30. Goates CY, Knutson K. (1993). Enhanced permeation and stratum corneum structural alterations in the presence of dithiothreitol. Biochim Biophys Acta, 1153:289–298.
31. Ibrahim SA, Li SK. (2010). Efficiency of fatty acids as chemical penetration enhancers: Mechanisms and structure enhancement relationship. Pharm Res, 27:115–125.
32. Kandimalla K, Kanikkannan N, Andega S, Singh M. (1999). Effect of fatty acids on the permeation of melatonin across rat and pig skin *in vitro* and on the transepidermal water loss in rats *in vivo*. j Pharm Pharmacol, 51:783–790.
33. Aungst BJ. (1989). Structure/effect studies of fatty acid isomers as skin penetration enhancers and skin irritants. Pharm Res, 6:244–247.
34. Aungst BJ, Rogers NJ, Shefter E. (1986). Enhancement of naloxone penetration through human skin *in vitro* using fatty-acids, fatty alcohols, surfactants. sulfoxides and amides. Int J Pharm, 33:225–234.
35. Ongpipattanakul B, Burnette RR, Potts RO, Francoeur ML. (1991). Evidence that oleic acid exists in a separate phase within stratum corneum lipids. Pharm Res, 8:350–354.
36. Ogiso T, Shintani M. (1990). Mechanism for the enhancement effect of fatty acids on the percutaneous absorption of propranolol. J Pharm Sci, 79:1065–1071.
37. Elyan BM, Sidhom MB, Plakogiannis FM. (1996). Evaluation of the effect of different fatty acids on the percutaneous absorption of metaproterenol sulfate. J Pharm Sci, 85:101–105.
38. Pose-Vilarnovo B, Rodríguez-Tenreiro C, Rosa dos Santos JF, Vázquez-Doval J, Concheiro A, Alvarez-Lorenzo C et al. (2004).

Modulating drug release with cyclodextrins in hydroxypropyl methylcellulose gels and tablets. J Control Release, 94:351–363.
39. Wang YY, Hong CT, Chiu WT, Fang JY. (2001). *In vitro* and *in vivo* evaluations of topically applied capsaicin and nonivamide from hydrogels. Int J Pharm, 224:89–104.
40. Das MK, Ahmed AB. (2007). Formulation and ex vivo evaluation of rofecoxib gel for topical application. Acta Pol Pharm, 64: 461–467.
41. Zhou M, Donovan MD. (1996). Intranasal mucocilliary clearance of putative bioadhesive polymer gels. Int J Pharm, 135:115–125.
42. Shin SC, Kim HJ, Oh IJ, Cho CW, Yang KH. (2005). Development of tretinoin gels for enhanced transdermal delivery. Eur J Pharm Biopharm, 60:67–71.
43. Shin SC, Kim JY, Oh IJ. (2000). Mucoadhesive and physicochemical characterization of Carbopol-Poloxamer gels containing triamcinolone acetonide. Drug Dev Ind Pharm, 26:307–312.