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Original Article

PREPARATION AND EVALUATION OF FENTANYL TRANSDREMAL PATCHES USING LIDOCAINE AS A MODEL DRUG AND AZELAIC ACID AS A PENETRATION ENHANCER

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ABSTRACT

Transdermal drug delivery offers numerous advantages over the conventional routs of administration; however, poor permeation of most drug across the skin barrier constitutes a major limitation of this rout.

Objective: The possibility of utilizing azelaic acid as penetration enhancer was investigated. And then development of a new transdermal controlled-release device using of non-medicated and lidocaine transdermal patches and then testing the feasibility of loading fentanyl patch.

Methods: DSC, FTIR, X-ray diffraction analysis and skin permeability measurements were done for both skin sample untreated and treated with azelaic acid to prove the possibility of utilizing it as permeation enhancer.

Multilayered lidocaine transdermal patches were prepared by solvent/evaporation casting technique using Eudragit® E100 as transdermal adhesive polymer, and ethyl vinyl acetate as impermeable backing layer. The flexibility of films required for a good compliance and optimum transdermal adhesion of the Eudragit E films was achieved by employing triethyl citrate or dibutylphthalate at concentration of 25% (w/w) of polymer. A physicochemical interaction between azelaic acid and Eudragit E100 (cationic polymer) has been evaluated using FTIR and DSC. Lidocaine as well as fentanyl bilayerd transdermal patches containing triethylcitrate at concentration of 25% (w/w) of Eudragit E100 with and without azelaic acid were selected for further permeation studies

Results: The obtained results indicated that fluorescien permeation through epidermal human skin treated with overnight exposure to saturated aqueous solution of azelaic acid was increased by 8.6 folds while, its permeation through rat skin was increased by 10.89 folds. Additional analysis by FTIR, X-ray diffraction, SEM, and DSC showed that azelaic acid disrupted stratum corneum lipid, which supported its action as promising penetration enhancer.Plasticizers as triethyl citrate or dibutylphthalate at concentration of 25% (w/w) of polymer reduced Tg of Eudragit E100 polymer to about 15.5^{0}C and 26.2^{0}C respectively. A physicochemical interaction between azelaic acid and Eudragit E100 was proven by FTIR study which indicated the present of ionic bonding between them, while DSC showed that azelaic acid may act as non-traditional plasticizer through its reduction in Tg by 7.3^0 C.The results of permeation studies indicated that the presence of azelaic acid was significantly increased ($P < 0.05$) the drug flux as the concentration of azelaic acid increased. As well as; fentanyl transdermal permeability studies revealed similar behavior to lidocaine as drug flux increased by 4.82 folds at AZ concentration of 2mg/cm^2.

Conclusion: the overall obtained data revealed the feasibility of preparing a controlled release fentanyl transdermal patches containing azelaic acid as penetration enhancer.

Keywords: Azelaic acid, Permeation enhancer, Fentanyl and Transdermal patches.

INTRODUCTION

The barrier property of stratum corneum is essential to its protective role but hinder transdermal drug delivery. Penetration enhancers can increase the stratum corneum's permeability and aid the transdermal delivery of drugs by altering its structural organization of lipids [1]. Azelaic acid is a naturally occurring saturated dicarboxylic acid produced by ayeast that lives on normal skin, modifies epidermal keratinisation, and has antibacterial properties against both aerobic and anaerobic bacteria, and possesses anti-inflammatory activity [2].

Physicochemical parameters of drugs as well as their permeation constants are important for determining the best drug candidates for transdermal delivery [3]. The daily dose of drug that can be delivered from a transdermal patch is 5-10 mg, effectively limiting this route of administration to potent drugs [4].

Since fentanyl, synthetic piperidine, is a very expensive controlled drug and can be available for research only with limited quantity, and under certain specification and regulation of the Ministry of Health, lidocaine is used as a model drug for the whole study to select the best condition for preparing bilayer transdermal patch using azelaic acid as penetration enhancer. Then the feasibility of loading fentanyl in the developed patch was investigated. Both of them had approximately the same properties for passive adhesive transdermal patch as: non-ionic, low molecular weight (336.5 for fentanyl and 234 for lidocaine), and log P in range 1-3 (2.8 for fentanyl and 2.5 for lidocaine). In addition they are structurally related.

Aim of the Study is to investigate AZ as transdermal penetration enhancer. And then development of a new transdermal controlled-release device using of non-medicated and lidocaine transdermal patches and then testing the feasibility of loading fentanyl in the developed model patch as well as evaluation of its physicochemical properties.

MATERIALS AND METHODS

Materials

Lidocaine base, fentanyl citrate, and azelaic acid(AMP, Jordan). Eudragit E 100(Röhm, GmbH,Weiterstdt, Germany) Ethylene vinyl acetate copolymer 40 wt% vinyl acetate stabilized and hexane HPLC grade(Acros, New Jersey-USA), Triethyl citrate(Sigma- Aldrich, England), Dibutyl phthalate(Sharlau, Barcelona- Spain), Trypsin enzyme from hog pancreas and Fl Flouresceine(Fluka Biochemik,GmbH, Germany), KBr(Merk, Germany) Acetonitrile HPLC grade, dichlormethane HPLC grade, sodium hydroxide, and ethanol absolute-analytical grade(Sharlau, Barcelona- Spain). Human skin (King Abdulla Hospital, Irbid – Jordan) and Albino dorsal rat skin (Animal House at Jordan University of Science and Technology). All other reagents were of an analytical grade.

Experimental design

Skin samples

Due to the limited sources of human skin (female skin from abdominal surgery), rat skin (dorsal area of albino male rats' weight 350±20g) was used in some parts of this study. Epidermis was prepared using a heat separation method [5], full thickness of human skin pieces were incubated for 90 seconds in hot water (60 ^{0}C), and anatomical forceps were used to peel off the epidermis carefully, while stratum corneum isolation was done by placing the skin pieces were placed in a petri dish, soaked in 1% trypsin in phosphate buffer saline (PBS) pH 7.4 and incubated for 24 hours at 32^0 C [6]. This step was repeated with fresh trypsin solution until the transparent stratum corneum was separated and floated over the solution [6].

Investigation of azelaic acid as penetration enhancer

Treated human SC samples were prepared by soaking several small pieces of SC in saturated aqueous solution of AZ (0.24mg %, w/v) for 24 hours at room temperature and then washed them with PBS, while control human SC samples were prepared by soaking them in distilled water. The SC samples were dried under vacuum at 21± 1 ^{0}C (650 mm of Hg) and stored in desiccators to remove traces of solvent [7-9].

Differential scanning calorimeter

Thermal analysis was carried out for both control and treated human SC by sealed SC samples within an aluminium pans and heating samples from 0 ^{0}C to 120 ^{0}C at rate of 10 ^{0}C / minute.

Fourier transform infra red spectroscopy

A special attention was focused on characterizing the occurrence of peaks near 2850 cm^{-1}, 2920 cm^{-1}, and 1650 cm^{-1} which were due to symmetric, asymmetric C-H and C-O stretching frequencies respectively[10].

X-ray diffraction

The peak that caused by scattering of crystalline cholesterol, which is primary component of SC lipid was measured using X-ray diffractometer for both control and treated SC samples to study any changes in the order of the dominant component of SC lipid bilayer structure that caused by AZ [11-12].

Skin permeability measurement

Both human epidermis and whole rat skin were used. Before measuring skin permeability, epidermal human skin, or whole rat skin, was pretreated with AZ by placing them in a vertical, glass Franz diffusion cell apparatus with 1.76cm^2 exposed skin surface area, the receiver chamber was filled with PBS pH 7.4 and the donor chamber was filled with 1ml saturated aqueous of AZ. After 12-hr exposure to saturate aqueous of AZ at room temperature, the Franz cell was transferred to heater/stirrer at 32 ^{0}C for 3hrs [13] After this pretreatment, the receiver chamber was emptied and filled with fresh PBS pH7.4 and the donor chamber was emptied and filled with 1ml of 1mM fluorescein in PBS pH 7.4. Samples were taken at different interval of times for 5hr and analyzed spectrophotometrically to determine the permeability [14].

Preparation of the backing film backing

Backing layer was prepared by dissolving 3g of EVA in 100 ml dichloromethane. Then casting into glass petri dishes with a diameter of 5.0 cm and drying at room temperature, for about five hours to attain complete dryness [15].

Preparation of transdermal patche

Bi-layered transdermal patches 1-6 (table 1) were prepared by the casting / solvent evaporation technique from plasticizer- containing polymer solution. After preparing the dried backing layer, and the medicated transdermal solution, the plasticized drug / polymer solution was poured onto the dried backing layer and dried at room temperature overnight. After drying, the dried bilaminated films were peeled from glass dish and cut in to circular shape of smaller size for further studies[16]. Then the films were stored at 20 ±1^{0}C in a desiccator containing saturated solution of sodium dichromate ($Na_2Cr_2O_7$) for at least 2 days before testing.

Table 1: Composition of transdermal patches loaded with lidocaine (1.76cm^2).

S. No.	First layer	Second layer
1	EVA	56 mg Eudragit E 3.52 mg lidocaine 56 mg Eudragit E
2	**E**VA	3.52 mg lidocaine 3.52 mg AZ 56 mg Eudragit E
3	EVA	3.52 mg lidocaine 14 mg DBP 56 mg Eudragit E
4	EVA	3.52 mg lidocaine 14 mg TEC 56 mg Eudragit E
5	EVA	3.52 mg lidocaine 3.52 mg AZ 14 mg DBP 56 mg Eudragit E
6	EVA	3.52 mg lidocaine 3.52 mg AZ 14 mg TEC

Characterization of the films

Physical characterization

The thickness of the patches was measure by using a micrometer, weight variation by individual weighing 10 randomly selected patches [17], and morphology of transdermal film was observed under a scanning electron microscope.

Differential scanning calorimetric analysis

Samples of 3-5 mg were placed in crimped aluminium pans. Thermal analysis was performed, using an empty crimped pan as a reference, at a scan rate of 10°C per min from 20 to 300°C under nitrogen purge at a constant flow rate of 20 ml/min.[18]. A glass transition temperature (Tg) of the films and the effect of AZ content and plasticizers on Tg were determined using the cooling system of the DSC. Thermal analysis was performed at multi-step runs as follow: the sample was heated at a rate of 20 ^{0}C /min to 100 ^{0}C (holds there for 5 minutes), quench cool the sample, and finally DSC run was carried at a rate of 10 ^{0}C /min from -50 ^{0}C to 100^{0}C [19]. Triplicate samples were tested for each formulation.

Fourier transform infrared analysis

FTIR spectra of azelaic acid and Eudragit E, as well as their films, were measured. Solid samples were prepared as KBr discs.

Method of lidocaine analysis in aqueous samples

High performance liquid chromatography (HPLC) analytical method was used for the analysis of lidocaine [20] using reverse phase column, 15 cm in length and 4.6 mm in diameter. The mobile phase acetate buffer pH of 3.4: acetonitrile(4:1). The operating conditions included mobile phase flow rate of 1.5 ml/min, volume of injection was 30 µl, and detection was carried out at 254 nm. The analysis was conducted under ambient temperature.

Evaluation of *in vitro* permeability of lidocaine

In vitro permeation studies for patch no.4 and patch no.6 were conducted at 32 ± 1^{o}C.. Rat skin membrane was used. For comparison, the permeability of a controlled solution was studied using the same above set up except that a controlled solution composed of 3.5mg of drug in 2 ml of PBS was added to the donor cell.

Evaluation of lidocaine permeation using different amount of az

In order to compare the effect of AZ concentration on the permeation of lidocaine, patches with different concentrations of azelaic acid (2, 3, 4, and 5 mg/cm^2) were prepared.

Preparation of fentanyl patches

Two different types of patches were prepared. Patch A without azelaic acid and patch B with azelaic acid as penetration enhancer (table 2).

Table 2: Formulas of (1.76cm²) bilayer fentanyl patches

Formula	First layer	Second layer
patch A	EVA	56 mg Eudragit E100 14 mg TEC 0.88 mg fentanyl 56 mg Eudragit E100
patch B	EVA	3.52 mg AZ 14 mg TEC 0.88 mg fentanyl

Evaluation of *in vitro* Fentanyl Permeation

For solution permeability study, a control solution composed of 1.0 mg fentanyl in 2 ml PBS was used and the receptor medium consisted of PBS pH 7.4 [21].

RESULTS AND DISCUSSION

Investigation of azelaic acid as penetration enhancer

The DSC thermogram of human SC treated with azelaic acid indicated that the first and second transition temperatures(first one at approximately 73°C which is assigned to lipid structure transformation nd the second one at approximately 92 °C which is assigned to a protein-associated lipid transition from gel to liquid form) were decreased by 9.1 and 12 °C, respectively, relative to untreated skin, as illustrated in figure 1 which is indicative of increased lipid fluidity [22].

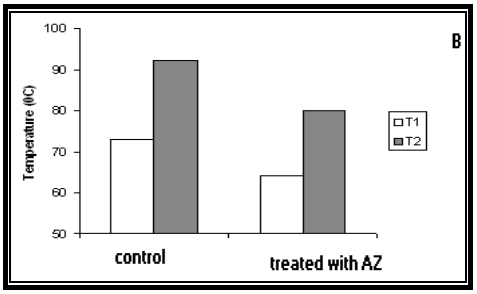

Fig. 1: Changes of two characteristic transition midpoint temperatures

FTIR: It appears from figure2 that the treatment of skin with AZ raised the stretching frequency of asymmetric (CH2) by 2.34cm^{-1}, asymmetric (CH2) by 1.11 cm^{-1}, and (CO) by 2.31 cm^{-1} relative to untreated human skin. These data indicate that AZ significantly increased (p < 0.05) stratum corneum lipid chain disorder and fluidity as reported [23]. Although, these wavenumber shifts are relatively small, they are consistent in magnitude with pervious studies of chemical enhancers [24].

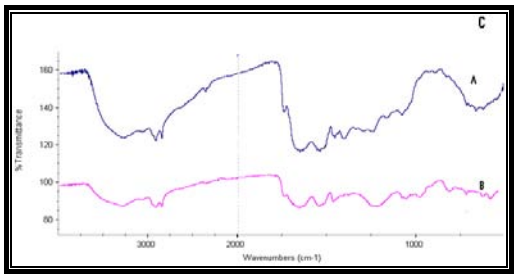

Fig. 2: FTIR spectra of human SC. (A) Control and (B) Treated

X-ray Diffraction

Figure (3) represents X-ray diffraction of control and treated human SC with AZ. The reduction in the peak intensity associated with crystalline form of SC indicated that the AZ reduces lipid bilayer order [25].

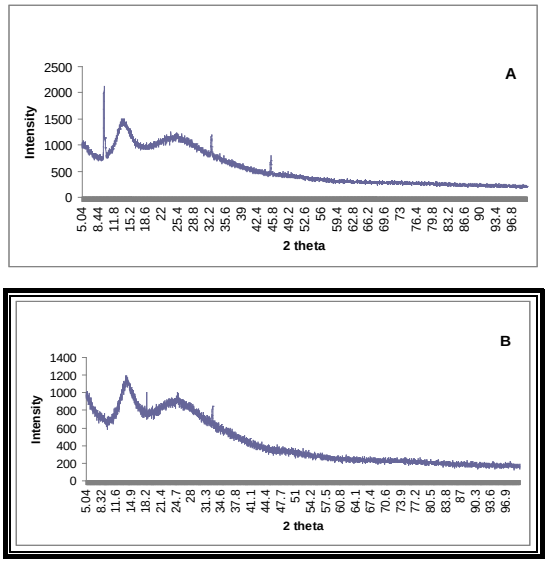

Fig. 3: X-ray diffraction of human sc, a(control) and b: (treated)

Effect of Azelaic Acid on Skin Permeability

The steady state fluxes were calculated from the slope of penetration profiles (figure 4), the flux of fluorescein through treated human SC skin with AZ was found to be 1.3µg/cm².hr., while flux through untreated skin was 0.15 µg/cm².hr., so the enhancement ratio is 8.6, while for rat skin equals to 10.89(figure 5). It appears that AZ significantly ($P < 0.05$) increased fluorescein permeability through human and rat skin but rat skin is often more permeable as reported [26].

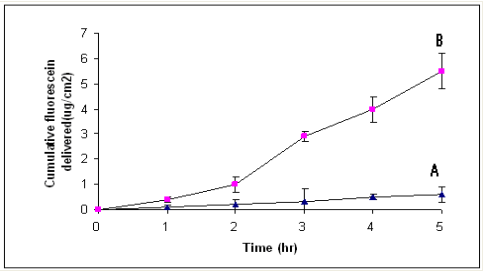

Fig. 4: Cumulative amount of fluorescein permeated at pH 7.4 and temperature of 32 °C through human skin, A (control) and B(treated)

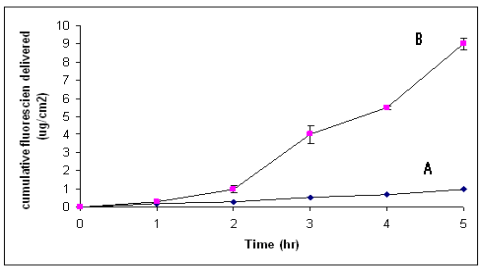

Fig. 5: Cumulative amount of fluorescein permeated at pH 7.4 and temperature of 32 °C through:AControl rat skin,B Treated rat skin with AZ. (Data expressed as mean ± SD)

Physical characterization of lidocaine patches

Low standard deviation (table 3) values in the film weight and thickness measurements ensured uniformity of thickness in each

film indicated that the method of casting solvent technique gives reproducible results with regard to film thickness.

Table 3: Physical properties of patches with a surface area of 1.76 cm² (values are presented as mean ± sd).

Sample (n=10)	Weight (mg)	Thickness (mm)
Placebo	72 + 0.50	0.40 + 0.01
Mono-layered film	105 + 1.26	0.60 + 0.01
Bi-layered patch		
Lidocaine (2 mg/cm²)	74.70 + 0.53	0.41 + 0.01
Mono-layered film	107.5 + 1.12	0.61 + 0.01
Bi-layered patch		

Morphological analysis

A smooth, compact surface, clear, and colourless film was noted as shown in figure (6). This result agrees with the specification of Eudragit E 100 polymer to form a clear and transparent film and indicates a good distribution of drug within a polymer [27].

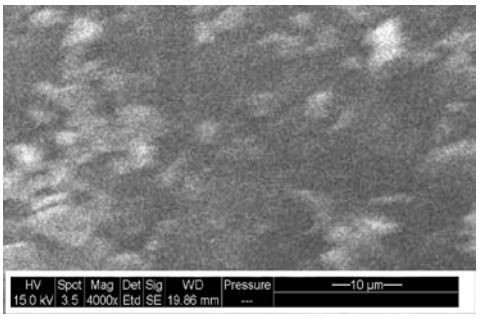

Fig. 6: Scanning electron microscope of Eudrgit E 100 transdermal patch.

Eudragit E100 produces transparent film but it is extremely brittle; therefore, two types of plasticizers including TEC and DBP were used to determine their influence on the flexibility of Eudragit E 100 films. Figure (7) shows the effect of TEC on Tg of Eudragit E100, Eudragit E100 film had Tg of 39.79 °C, while 10%, 25%, and 30% (w/w) polymer content of TEC had Tg of 29.5 °C, 15.5 °C, and 14.9 °C respectively. On the other hand, figure (8) illustrated the effect of DBP on Tg, 10%, 25%, and 30% (w/w) polymer content of DBP had Tg of 31°C, 26.2 °C, and 25.1 °C respectively. The results indicated that both TEC and DBP can reduce Tg of polymer to about room temperature.

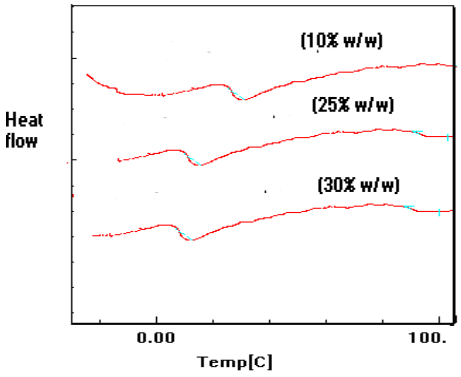

Fig. 7: Effect of triethylcitrate plasticizer concentration on the DSC thermogram of Eudragit E film.

A new broad endothermic peak appeared at 121.5 °C (figure 9) may be formed due to the possible interaction between cationic Eudragit E100 polymer and AZ to form a new complex. Further elucidation of the nature of this interaction will be latter conducted using FTIR.

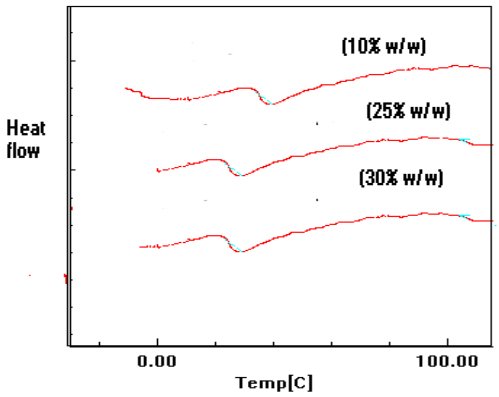

Fig. 8: Effect of dibutylphthalate concentration on the DSC thermogram of Eudragit E film.

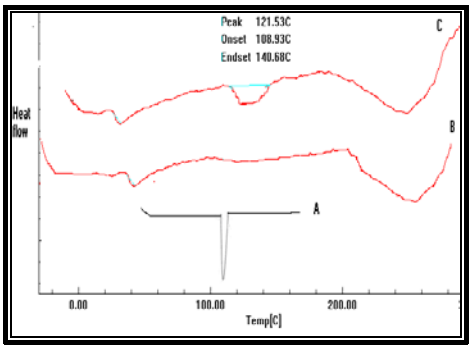

Fig. 9: DSC thermogram A(azelaic acid powder), B Eudrgit E film, and C(Eudrgit E- azelaic acid film)

Fourier transform infrared analysis

Figure (10) represents FTIR spectra of the Eudragit E100-AZ film. A strong band appears at 1557 cm^{-1} which might be assigned to the absorption band of carboxylate group that forms the ionic bonds with protonated dimethylaminoethyl groups of Eudragit E100.

On the other hand, a significant peak broading at approximately 2497 cm^{-1}, might be assigned to the polymer salt absorption band which was brought about by the interaction of the dimethylaminoethyl groups of Eudragit E100 with the carboxyl group of AZ. In addition, the ratio of the bands at 2772 and 2822 cm^{-1} significantly changed. This indicates a difference in the amount of protonated and non- protonated dimethylaminoethyl groups. Finally, changes in the hydroxyl stretching region are observed as well.

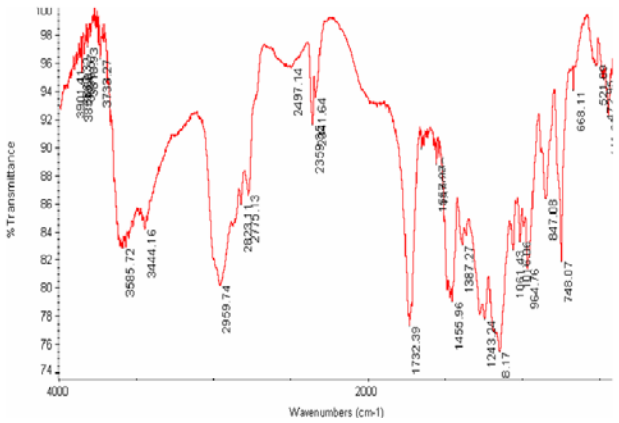

Fig. 10: FTIR spectra of Eudragit E100-azelaic acid film

Evaluation of *In vitro* Permeability of Lidocaine

The effect of different concentration of azelaic acid on permeation rate of lidocaine transdermal patch (2mg/ cm^2) through rat skin was investigated to identify optimum concentration and to compare its effect with the control. Figure (11) illustrates the permeation profile of lidocaine from patches containing different concentrations of azelaic acid,(0, 2, 3, 4, and 5mg/ cm^2) as a donor.

Enhancement ratios (ER) are equal to 2.55, 3.48, 3.77, and 4.0 for patches containing AZ at concentration of 2, 3, 4, and 5 mg/ cm^2 respectively. The results indicate that ER was increased significantly ($P<0.05$) as the concentration of AZ increased in the bi-layer transdermal patch up to 4.0 folds at 5 mg/ cm^2 of AZ.

Based on the overall *in vitro* lidocaine release and its skin permeability results, the component of bi-layer transdermal patch 4 and patch 6 containing AZ at concentration of 2 mg/ cm^2 were selected as the best formulas for loading fentanyl, also its permeation study was done for further supporting the hypothesis states that AZ is a promising penetration enhancer in transdermal fentanyl drug delivery system.

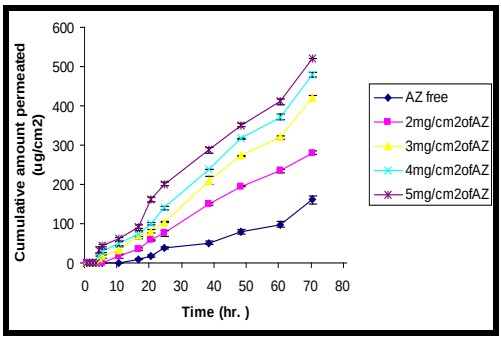

Fig. 11: Cumulative amount of lidocaine (2mg/ cm^2) permeated from bilayer patch6 containing different concentration of AZ as a donor at 32 ^oC. Data expressed as mean ± SD (n=3)

Evaluation of *in vitro* Permeability of Fentanyl:

Sink condition was assured in the receiving solution of Franz cell. Fentanyl solubility in PBS pH 7.4 at 25 ^0C was reported to be 9.8 mg/ml [28]. Therefore a volume of 15 ml is more than enough to solubilize ten times its solubility.

Figure (12) illustrates the permeation profile of fentanyl from PBS pH7.4 containing 1mg / 2 ml fentanyl as a control solution, patch A, and patch B through rat skin. An average flux of 0.563 µg/cm^2.hr, $Q_{24\ of}$ 7.5 µg, and T_{lag} of 3.2 hr were obtained from control solution, while the average diffusion parameters values for fentanyl from patch A were; flux of 0.399 µg/cm^2.hr, $Q_{24\ of}$ 5.5 µg, and T_{lag} of 3.7 hr (table 4). It can be inferred from the data that flux of fentanyl from PBS pH 7.4 was significant higher as compared to fentanyl in bi-layer transdermal formulation due to its hydration effects on skin [29]. On the other hand, inspection of the permeation parameters of fentanyl from patch B (table 5) illustrates that fentanyl flux is of 1.924µg/cm^2.hr, Q_{24} is of 26.1 µg, and T_{lag} is of 1.2 hr. It can be concluded that the presence of AZ in the formulation of fentanyl bi-layer transdermal patch significantly ($P<0.05$) increased drug flux by 4.82 folds.

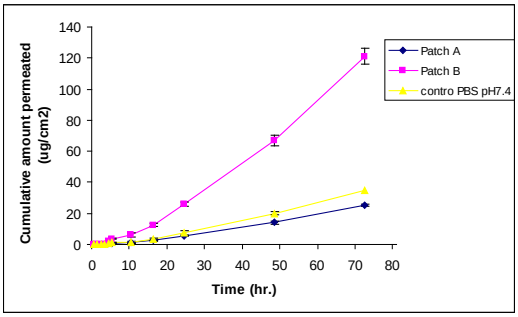

Fig. 12: Cumulative amount of fentanyl permeated from control PBS pH 7.4 (1.0 mg /2ml), patch A, and patch B as donors at 32 ^oC. Data expressed as mean ± SD (n=3)

Table 4: Diffusion Parameters for Fentanyl from Control PBS pH7.4, Patch A, and Patch B. Reading Represent the Mean (SD), n=3

Donor	T_{lag} (hr)	Flux (µg/cm^2.hr)	Permeation Coefficient (cm/hr)×10^{-3}	Cumulative amount at 24hr (µg)	Eenhancement factor
PBS(pH7.4)	3.2(0.09)	0.563(0.04)	1.126(0.06)	7.5(0.53)	-
Patch A	3.7(0.18)	0.399(0.03)	0.798(0.02)	5.5 (0.2)	1.0
Patch B	1.2(0.04)	1.924(0.02)	3.848(0.12)	26.1(1.9)	4.82

CONCLUSION

The present study demonstrates that the azelaic acid confirm as a promising skin permeation enhancer since it significantly increased the permeation of skin to fluoresciene, lidocaine, and fentanyl.

CONFLICT OF INTERESTS

Declared None

REFRENCES

1. Sonia Dhiman, Thakor G S, Ashish K R, Transdermal patches:arecent approach to new drug delivery system. Int J of Pharmacy and Pharm Sci 2011;3 (5):0975-1491.
2. Martindale. the Extra pharmacopoeia,.33ed 2002:1110.
3. Arun Raj R, Formulation evaluation and *in vitro* permeation studies of transdermal nifedipine from matrix type patches. Int J of Pharmacy and Pharm Sci 2014;6 (1):0975-1491.
4. Mohammad Wais, Abudus Samad, Iram Nazish, Anubha Khale, Mohd Agil, Mohib Khan, Formulation development ex-vivo and *in-vivo* evaluation of nanoemulsion for transdermal delivery of glibenclamide. Int J of Pharmacy and Pharm Sci 2013;5 (4):0975-1491.
5. Peter N, Bennett, Marris J. Brown, Clinical Pharmacology, 10thed. J Churchill Livingstone 2008:274.
6. Kligman AM, Christophers E. Preparation of isolated sheets of human sratum corneum. J Arch Dermatol 1963;88:702-5.
7. Wagner Ky, Cho J, Choi HK. Enhancement of percutaneous absorption of ketoprofen:effect of vehicles and adhesive matrix. Int J Pharm 1998;169:95-104.
8. Yui N, Okuhara M, Okano M, and Sakurai Y. Change in water structure in the stratum corneum of hairless rat skin by subcutaneous enhancers and its effect on indomethacin permeation. J Drug Deliv Syst 1992;7:1199-203.
9. Bhatia KS, Gao S, and Singh J. Effect of penetration enhancers and iontophoresis on FT-IR spectroscopy and LHRH permeability through porcine skin. J Control Rel 1997;47:81-9.
10. Krishnaiah YSR, Satyanarayana V, and Karthikeyan RS. Effect of the solvents system on the *in vitro* permeation of nicardipine hydrochloride through exised rat epidermis. J Pharm Pharmaceut 2002;5(2):124-30.
11. Goodman M, Barry BW. Differential scanning calorimetry of human stratum corneum:effects of penetration enhancers Azone and dimethyl sulphoxide. J Anal Proc 1986;23:397-8.
12. Lopez O, Cocera M, Campos L, de la Maza A, Coderch L, Parra JL. Use of wide and small angle X-ray diffraction to study the modifications in the stratum corneum induced by octyl glucoside. J Physicochem Eng Asp 200;162:123-30.

13. Kitagawa S, Sawada M, Hirata H. Fluorescence analysis with diphenylhexatriene and its ionic derivatives of the fluidity of liposomes constituted from stratum-corneum lipids-contribution of each lipid component and effects of long-chain unsaturated fatty-acids. Int J Pharm 1993;98:203-8.
14. Chun Y, Peter J. Ludovice, Mark R. Transdermal delivery enhanced by maganin pore-forming peptide. J Control Rel 2007;122(3):375-83.
15. Arora P, Mukherjee B. Design, development, physicochemical and *in vitro* and in vivo evaluation of transdermal patches containing diclofenac diethylammonium salt. J Pharm Sci 2002;91:2076-89.
16. Abu-Huwaiji R. Development and *in vitro*/ in vivo testing of mucoadhesive buccal patches releasing fentanyl citrate. Ph.D. Thesis, Faculty of Pharmacy-Jordan University of Science and Technology (2004).
17. Gristina P, Gaia C, Sara N, *et al*. Bioadhesive film for the transdermal delivery of lidocaine:*in vitro* and *in vivo* behavior. J Control Rel 2003;88:277-85.
18. Al-Akayleh F. Investigation of drug-excipient interaction phenomena in relation to enhancement of transdermal drug delivery. Ph.D. Thesis, Faculty of Pharmacy-University of Baghdad (2004).
19. Claudia L-Salva, Pereira J, Ramalho A, *et al*. Films based on chitosan polyelectrolyte complexes for skin drug delivery:development and characterization. J Memb Sci 2008;320:268-79.
20. Laboratory 3:Thermal transitions in polymeric materials. Polymer Characterization Lab. J Spring 00-3.
21. British Pharmacopeias, 2007.
22. Roy SD, Gutierrez M, Flynn GL, Gary WC. Controlled transdermal delivery of fentanyl:characterization of pressure-sensitive adhesive for matrix patch design. J Pharm Sci 1996;85(5):491-5.
23. Claudia S, Lippold BC. An attempt to clarify the mechanism of the penetration enhancing effect of lipophilic vehicle with differential scanning calorimetry (DSC). J Pharm Pharmcol 1995;47:276-81.
24. Takeuchi Y, Yasukawa H, Yamaoka Y, *et al*. Effects of fatty-acids, fatty amines and propylene-glycol on rat stratum-corneum lipids and proteins *in vitro* measured by Fourier-transform infrared attenuated total reflection (FT-IR/ATR) spectroscopy. J Chem Pharm Bull 1992;40:1887-92.
25. Potts RO, Golden GM, Francoeur ML, *et al*. Mechanism and enhancement of solute transport across the stratum-corneum. J Control Rel 1991;15:249-60.
26. Yeagle PL. Cholesterol and the cell-membrane. J Biochim Biophys Acta 1985;822:267-87.
27. Pappinen S, Hermansson M, Kuntsche J, *et al*. Comparison of rat epidermal keratinocyte organotypic culture (ROC) with intact human skin:lipid composition and thermal phase behaviour of stratum corneum. J Biochim Biophys Acta-Biomembranes 2008;1778:824-34.
28. Rohm Pharm Information Sheets. Rohm Pharm GmbH, Weiterstadt;info. J EudragitE 100.
29. Barry WB. Novel mechanisms and devices to enable successful transdermal delivery system. Eur J Pharm 2001;14:101-14.
30. Yamashita F, Koyama Y, Kitano M, Hashida. Analysis of *in vivo* skin penetration enhancement by oleic acid based on two-layer diffusion model with polar and nonpolar routes in the stratum corneum. Int J Pharm 1995;117:173-9.