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Original Article

Influence of chemical enhancer and loading dose on caffeine and Vitamin B_5 skin permeation and adhesion properties of transdermal patches

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ABSTRACT

Evaluated in this study were the effects of the two parameters, namely level of penetration enhancer and drug loading dose, on *in vitro* skin release of Caffeine and vitamin B_5 and adhesion properties of drug in adhesive transdermal systems. In order to characterize *in vitro* drug release, a hydrodynamically Chien permeation system with rat abdominal skin was employed. Caffeine skin release rate followed Higuchi's model with different concentrations of chemical enhancer as well as drug loading doses in transdermal patches; however, vitamin B_5 skin permeation rate as well followed Higuchi's model as it does zero order equation in different time intervals. Ethylene glycol acts as a chemical enhancer in those adhesives which are sensitive to pressure changes. Adhesion properties were reduced as the chemical enhancer concentration and loading dosage were increased. The formulation with the 85 *%w/w* (measured on the basis of dry adhesive weight) Ethylene-glycol demonstrated optimum drug skin release and adhesion properties.

Keywords: Ethylene glycol, Drug loading, Patches, Rat skin, Pressure sensitive adhesives.

INTRODUCTION

As an effective route for drug administration, transdermal drug delivery system (TDDS) is defined as a set of self-contained, discrete dosage forms delivering the drugs through the skin when applied to an intact skin. Avoiding first pass metabolisms transdermal therapeutic systems maintain a more steady concentration of active drug in the blood while improving patient's satisfaction, along with a reduction in side effects [1-4]. Today, patches are usually drug in adhesive systems where a drug is dispersed or dissolved in a pressure sensitive adhesive (PSA) matrix [5-7]. In spite of many advantages of transdermal drug delivery in terms of inherent skin barrier properties, skin permeation of drugs is generally poor in these systems; so that it is very important to select an appropriate penetration enhancer when developing transdermal and topical formulations, as these enhancers can contribute to drug release [8-10]. Drug permeation through the skin can be enhanced via chemical penetration enhancement as well as physical methods [11]. As of now, more than 360 chemicals have been demonstrated to enhance skin permeability including terpenes, sulphoxides, laurocapram, pyrrolidones, fatty acids and fatty alcohols, alcohol and glycol, surfactants, urea, etc [12, 13]. It has known that chemical penetration enhancers and PSAs are used in transdermal systems to improve drug diffusion rates and adhesion properties [14]. Various derivatives of glycol such as propylene glycol and polyethylene glycol are also employed in the course of transdermal delivery [15-19].

The influence of loading dose on drug release from transdermal systems into animal skins has been characterized to predict its performance and achieve a constant rate of drug permeation through the skin [20, 21]. Higuchi's square-root equation describes the drug release as a diffusion process based on the Fick's law which explains the dependency of drug release upon the square root of time [22, 23].

Most of caffeine (CF) delivered to human bodies is sourced from tea leaves, coffee, and cocoa beans. In cosmetics, it has exhibited an inhibitory effect on UV induced skin carcinogenesis by functioning as a sunscreen, while indicating a stimulatory effect on apoptosis in the epidermis of UV treated mice

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[24, 25]. In therapy, CF generates a stimulatory activity suitable for the treatment of neonatal apnea via percutaneous delivery. Numerous transdermal studies have used CF as an agent driven by its hydrophilic character, aiming at evaluation and enhancement of its release [25]. Transdermal delivery of CF as an active ingredient has been well studied [26]. Also, penetration of chemicals particularly glycols were commonly used in skin permeation of CF [26-29].

As a member of the B complex vitamins, Pantothenic acid is found abundantly in almost all food stuffs, though it is easily lost during processing. This water soluble vitamin acts as an antioxidant playing a very important role in decomposition of carbohydrates, fats and proteins. It is found in three forms: calcium pantothenate, panthenol and vitamin B_5 (VB_5). Na^+ or Ca^{2+} salts of panthenol are usually used in pharmaceutical preparations [30]. Vitamins are used as important ingredients of commercial nutritional transdermal products; thus, VB_5 was selected as the model vitamin due to its benefits in terms of nutrition [31-33].

Albeit there are some literatures where in the skin permeation flux of CF and similar drugs in drug in adhesive patches are reported, but to the best of our knowledge, no single report has yet been published on the role of different concentrations of ethylene glycol (EG) as chemical enhancers and of loading dosage of CF and VB_5 in skin permeation flux and adhesion properties of the final patches.

Based on the above mentioned reasons and considering traditional transdermal patches in the market, there was a great deal of interest to investigate the main objective of the present study which is to achieve an optimal formulation which can generate suitable *in vitro* release characteristics and adhesion properties from a novel transdermal device by changing two parameters: the chemical enhancer concentration and loading dose.

MATERIALS AND METHODS

Materials: Used materials were as follows: Acrylic adhesive Duro-Tak®87-2196 (National Starch and Chemical, USA), Cotran 9720 as a backing layer with a thickness of 85 µm (3M, USA), release liner (Scotchpak 1022, 3M, USA), EG and CF (Merck, Germany), VB_5 (Ca^{2+} salt of panthenol) which was prepared from Tolid-Daru Co. (Tehran, Iran). All other materials were of high performance liquid chromatography (HPLC) grades.

Sample preparation: Formulation batches for each sample were prepared as depicted in Table 1 where the ingredients are expressed in % of dry weight. In formulations 1 to 4, different concentrations of chemical enhancer were used: 75, 80, 85 and 90 %*w/w*, measured on the basis of dry weight of adhesive. The therapeutic concentration of drugs was kept constant per patch (12 mg / 16 cm^2). The values of loading dose in formulations 5 and 6 were risen up to 15.5 and 20 %*w/w*, respectively, measured on the basis of the dry weight of adhesive. The right amount of acrylic adhesive (Duro-Tak®87-2196), EG, CF and VB_5 were mixed together in a rotary mixer at 30 rpm and room temperature for 6 h. Then the mixture was spread on the backing layer to form a film by using a film applicator (Elcometer 3580). The samples were allowed to stand at room temperature before being further dried in an oven at 50 °C for 45 min [23, 34]. Final specific thickness was found to be 100 ± 5 µm.

Preparation of rat abdominal skin: Male Sprague Dawley rats (150–170 g) were obtained from Razi Vaccine & Serum Research Institute. These animals were sacrificed by excessive chloroform inhalation. The hairs on the abdominal region were carefully removed with subsequent removal of subcutaneous fat layer with a scalpel, so that a 5 cm ×5 cm full-thickness skin sample was excised from the mentioned region on each sacrificed rat. The dermis side was wiped with isopropyl alcohol to remove the residual adhering fat. The skin was dipped and soaked in a normal saline solution. It was then washed with distilled water, wrapped in an aluminum foil and stored in a deep freezer at -20 °C for further use. Skin sample was visually inspected for any damage before being loaded on the penetration cells [35]. One hour prior to the experiments, the samples were thawed.

In vitro transdermal delivery: Permeation investigation was carried out using an excised rat abdominal skin in a well characterized Chien permeation system with an effective diffusion area of 1 cm^2 at 37 °C. Receptor compartment of the diffusion cell was completely filled with 3 mL of filtered degassed phosphate buffer solution (PBS) of pH 6.0 as the receiver medium. A 1.5 cm ×1.5 cm device was applied, with slight pressure, to the epidermal side of the rat skin which then was mounted over the receptor compartment. The remaining air bubbles in the receptor compartment and below the skin were carefully removed by tilting the diffusion cell gently. The receptor medium was stirred by a magnetic stirrer [23]. The receptor medium was completely withdrawn from the receptor compartment and replaced by fresh PBS at predetermined time intervals (10, 20 and 30 min, 1, 2, 3, 4.5, 6 and 24 h). The concentrations of CF and VB_5 were measured via a fully validated

HPLC method, so that the cumulative amounts of CF and VB_5 could be calculated.

The permeation parameter including the permeation rate or skin flux (J_{ss}) was determined from Fick's law of diffusion as follows:

$J_{ss}= (VdC/dt)/ A$

where, J_{ss} is the skin flux ($\mu g/cm^2h$), C is the cumulative drug concentration in the receiver fluid at time t, V is the receiver volume (mL), and A is active diffusion area (cm^2) [23, 36, 37]. Steady-state flux is calculated from permeation profiles.

Drug release: The release of CF and VB_5 were measured in a hydro-dynamically well characterized Chien permeation system at 37 °C. Sodium phosphate buffer (pH 6.0) was prepared and stored at 37 °C before being used as the release medium. For the determination of CF and VB_5 release profiles, each 1.5 cm ×1.5 cm sample was mounted over the orifice of a half-cell of the Chien permeation system [23]. The receptor medium was completely withdrawn and immediately replaced by a fresh solution and assayed by an HPLC-UV method at different time intervals (10, 20 and 30 min, 1, 2, 3, 4.5, 6 and 24 h).

HPLC analysis: CF and VB_5 were assayed by HPLC (Younglin, SDV30) with a UV detector at 275 nm and 205 nm. HPLC separation system was composed of a PerfectSil Target ODS (150 mm ×4.6 mm, 5 µm) equipped with a guard column (10 mm ×4.0 mm, 5 µm). The mobile phase was composed of methanol/ K_2HPO_4 10 mM with a pH value of 3.0 (30:70) which was adjusted to 6.0 ± 0.1 by addition of H_3PO_4. The flow rate and injection volume of mobile phase were 1 mL/min and 20 µL. In order to prepare the standard curve, solutions of 0.1, 0.5, 1, 5, 10, 20, 40 and 100 µg/mL of the two drugs in sodium phosphate buffer 50 mM were prepared and the linear calibration curve was drawn on the basis of these 8 concentrations which were injected into HPLC (R^2 =0.999). The specificity for assay was established using the three sequential replicates of solution which were used in the standard curve. Table 2 shows system suitability parameters, such as plate count, repeatability (RSD of retention time and area), accuracy and precision of the system.

Adhesion performance: Essential performance properties for characterization of PSAs are tack and peel strength of adhesion. The experiments were performed in an air-conditioned room where temperature was kept at T = 21.0 ºC. The first property represents the adhesive's ability to adhere quickly, while the other property expresses its ability to resist removal by peeling. Generally, these two properties are directly related to each other [5].

Probe Tack Test: According to ASTM D3121, tack tests were carried out on adhesive tapes using a Chemie Instruments Probe-Tack PT-500 (Fairfield, Ohio, USA) on at least five samples from each formulation [14, 38].

Peel Strength Test at 180°: According to ASTM D3330, peel tests were carried out on adhesive coated tapes each with 25 mm width. After preparation of drug in adhesive patches consisting of the acrylic PSA tape/ stainless steel joints, the samples were stored at room temperature for 20 minutes. Using a Chemie Instruments' AR-1000 adhesive/release tester (Fairfield, CT, USA), peel force was measured in 180º direction at a peel rate of 30.50 cm/ min at room temperature. Peel tests were carried out on at least three samples from each formulation using steel joints [34, 38].

Statistical analysis: Statistical analysis was performed using MiniTab software (Release 11.12, Minitab Inc., State College, PA, USA). Data were reported as mean ± standard deviation at a significance level of $p<0.05$. Using the T procedure, outliers were rejected during data processing [39]. Differences among the groups were analyzed using one-way variance analyses and were considered statistically significant when the p value was less than 0.05.

RESULTS

In vitro skin permeation

Effect of permeation enhancer on in vitro skin permeation: In this study, drug concentration was fixed at 7.5% within formulations 1-4 where various levels of EG were incorporated. The observed increase in the total drug flux was resulted from increasing chemical enhancer concentration. The permeation profile of CF and VB_5 are shown in Figures 1 and 2. The steady-state flux is presented in Table 3.

The permeation rates of CF and VB_5 through rat skin were divided into two sections. In the first section (sec1), drugs' skin permeation examinations were carried out in a high rate. This section was continued for 3 h for formulation 1, whilst it was continued for to 4.5 h for formulations 2 to 4. The drugs skin permeation examinations were carried out through more gentle slopes in the second section (sec2) compared to that of the first section. Lag time value which was only computable for VB_5 on the first formulation, was found to be 1.1 ± 0.1 h. Permeation rates of CF and VB_5 were found to be in the range of 15.7- 31.1

and 0.28- 3.14 μg/cm^{2}h in sec1 and 1.76- 7.18 and 0.05- 0.18 μg/cm^{2}h in sec2, respectively.

Effect of loading dose on in vitro skin permeation: As shown in Figures 3 and 4, a drug loading of 20.0% gave the highest cumulative amount of total CF and VB_5, while loading dosages of 15.5% and 14% resulted in the next two high values. The permeation rates of CF and VB_5 through rat skin were divided into two sections in different drug loadings, too. Sec1 was performed for 4.5 h for the formulations containing 15.5 and 20.0% loading doses. The steady state flux is presented in Table 3. Correspondingly, total flux of CF was found to in the range of 15.7-18.6 and 1.76-2.82 μg/cm^{2}h in sec1 and sec2, respectively, while VB_5 skin flux was in the range of 0.28-0.49 μg/cm^{2}h in sec1 and around 0.05μg/cm^{2}h in sec2. Lag times were not computable for formulations 5 and 6.

Drug release

Effect of permeation enhancer on drug release: *In vitro* drug release of CF and VB_5 from TDDSs was studied and the results are summarized in Table 3. For this purpose, the cumulative released drug during 24 h was obtained, so that the percent of released drug is calculated using Eq. 2.

Released Drug (%) = Q_t / C_0 *100

where Q_t and C_0 are the cumulative amounts of drug released at time t per unit of exposed area and the drug loading, respectively.

According to Table 3, the influence of different levels of chemical enhancer on the amount of released drug is similar to their effect on skin permeation rate. It is found that the formulation with the highest percentage of chemical enhancer is associated with the highest values of released drug. A dispersed drug in an adhesive vehicle is also described by Higuchi's model:

$Q_t = (2DC_pC_ot)^{0.5}$

where Q_t is the cumulative amount of drug released at time t per unit of exposed area, D is the constant diffusion coefficient, C_o and C_p are the drug loading and drug solubility in the polymer matrix, respectively [23]. The slopes of Higuchi's equation have been shown in Table 3.

Effect of loading dose on drug release: The influence of loading dose on *in vitro* drug release of CF and VB_5 from TDDSs within 24 h is presented in Table 3. CF release percentage raised with increasing drug loading dose; whilst, VB_5 release percentage decreased in formulation 6 as a result of a rise up in the loading dose. However, cumulative VB_5 skin permeation at a loading dose of 20.0% was more than those in other formulation.

Adhesion performance: To characterize adhesion performance of the PSA formulations, tack and peel strengths of adhesion were determined and shown in Figure 5.

DISCUSSION

It was observed that for both drugs, their release behaviors follow Higuchi's kinetic model. The related regression correlation coefficients (R^2) are summarized in Table 3. According to Higuchi's equation, the slope of (Q_t–$t^{0.5}$) is equal to $(2DC_pC_o)^{0.5}$ which leads to different values of slope for various formulations. As the drug compositions is the same in formulations 1-4, the C_o value would be the same; therefore, the different slopes can be explained by the changes in DC_p. By introducing a chemical enhancer into the TDDSs, both D and C_p rose up [14]; thus, the skin permeation rate increased.

Taking the slope of (Q_t–$t^{0.5}$) into consideration, it was found that for CF, the release behavior follows Higuchi's kinetic model; however, the VB_5 release as well follows this model as it does zero order equation in different time intervals. The related regression correlation coefficients (R^2) are summarized in table 3. Based on these results, EG acts as a chemical enhancer on the adhesive; thus, it was observed that the tack and peel strengths were decreased at higher EG concentrations. Similarly, increasing drug concentrations led tack and peel strength values to decline. This is in agreement with [2, 5, 34].

CONCLUSION

The present work was focused on improvement of skin permeation rate with different levels of chemical enhancer and drug loading doses. Comparing this study to similar studies, one may see that as of writing this research, there were no academic reports on VB_5 skin permeation, while the present research is an attempt to achieve VB_5 release rate of the available commercial patches of this novel transdermal devices and also to evaluate them in terms of both *in vitro* skin permeation and adhesion properties. It was found that the cumulative rate of skin permeation and drug release were increased using a high percentage of chemical enhancer and drug loading dose in the formulations. The formulation with 85 *%w/w* of EG (considering dry adhesive weight) demonstrated suitable drug skin release and adhesion properties.

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DECLARATION OF INTEREST

The authors have declared no conflict of interest.

COMPLIANCE WITH ETHICS OF REQUIREMENT

This article does not contain any studies with human or animal subjects.

Table 1: MATERIAL COMPOSITIONS OF TRANSDERMAL PATCHES

Ingredients	Formulations					
	F_1	F_2	F_3	F_4	F_5	F_6
Adhesive	52.9	51.4	50.0	48.7	52.3	50.2
CF	6.4	6.4	6.4	6.4	6.8	8.2
VB_5	1.1	1.1	1.1	1.1	1.3	2.0
EG	39.6	41.1	42.5	43.8	39.6	39.6

Table 2 DEMONSTRATION OF SYSTEM SUITABILITY OF THE HPLC ASSAY FOR CF AND VB_5

Characteristics	CF	VB_5
System Suitability Test (SST)		
Theoretical plates	7562	6521
Precision (Repeatability and Reproducibility) (%RSD)	2.3	4.2
Repeatability of Retention time (%RSD)	1.2	0.5
Repeatability of Peak area (%RSD)	1.2	2.2
Accuracy (%RSD)	4.1	5.0
Limit of Detection (LOD) (µg/ml)	0.1	0.1
Limit of Quantitation (LOQ) (µg/ml)	0.5	0.5

Table 3: STEADY STATE FLUX (J_{ss}) , SLOPE OF HIGUCHI EQUATION ($Q/ t^{0.5}$) AND CUMULATIVE PERCENT PERMEATION DATA OF CF AND VB_5 FROM TDDSs, ACROSS THE RAT SKIN AFTER 24 h (n=3, ±SD).

Analyte	J_{ss} (µg/cm^{2}h)				$Q/t^{0.5}$ (µg/h$^{0.5}$)				Q_{24} (%)
	Sec1	R^2	Sec2	R^2	Sec1	R^2	Sec2	R^2	
F_1									
CF	15.7±0.2	0.96	1.76±0.2	0.96	78.1±4.3	0.99	27.6±2.6	0.98	14.3±2.5
VB_5	0.28±0.05	0.99*	0.05±0.01	0.96	1.70±0.4	0.99	0.71±0.3	0.99	1.4±0.2
F_2									
CF	20.8±0.5	0.99	6.42±0.3	0.99*	114±9.0	0.99	-	-	39.8±0.5
VB_5	2.33±0.1	0.93	0.11±0.04	0.99	13.7±1.0	0.99	1.8±0.2	0.99	12.7±0.1
F_3									
CF	25.3±0.6	0.98	6.82±0.3	0.99	127±8.0	0.99	-	-	45.9±1.3
VB_5	2.88±0.1	0.92	0.14±0.03	0.99	17.0±0.8	0.99	2.21±0.4	0.99*	16.5±0.2
F_4									
CF	31.1±0.5	0.97	7.18±0.2	0.99*	143±8.3	0.98	-	-	53.0±0.9
VB_5	3.14±0.05	0.91	0.18±0.03	0.99*	18.6±1.1	0.98	2.86±0.2	0.99*	18.8±0.1
F_5									
CF	16.0±3.5	0.93	2.15±0.4	0.99	94.3±3.4	0.99	34.6±7.5	0.99*	17.3±4.5
VB_5	0.45±0.06	0.99	0.05±0.02	0.99*	2.21±0.2	0.99	0.71±0.2	0.99*	1.7±0.4
F_6									
CF	18.6±3.7	0.93	2.82±0.2	0.99	110±6.2	0.99	45.4±8.2	0.99*	17.9±3.3
VB_5	0.49±0.1	0.99	0.05±0.01	0.99	2.41±0.4	0.99	0.84±0.1	0.99*	1.4±0.3

For all formulations $P < 0.05$. * The third digit after the decimal point is more than 5.

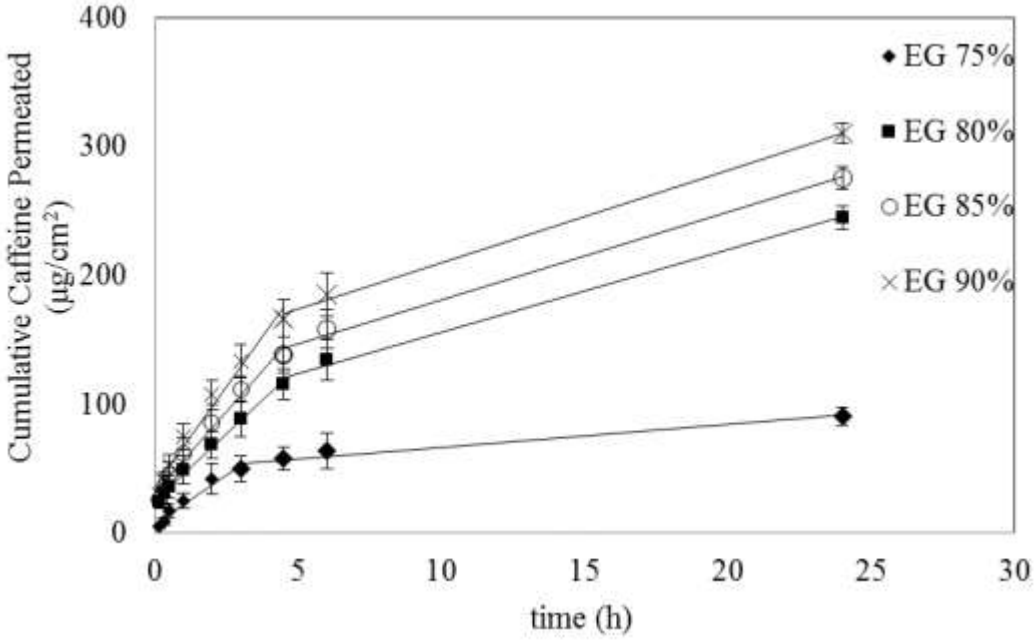

Fig. 1: Influence of different concentrations of EG on skin permeation profile of CF

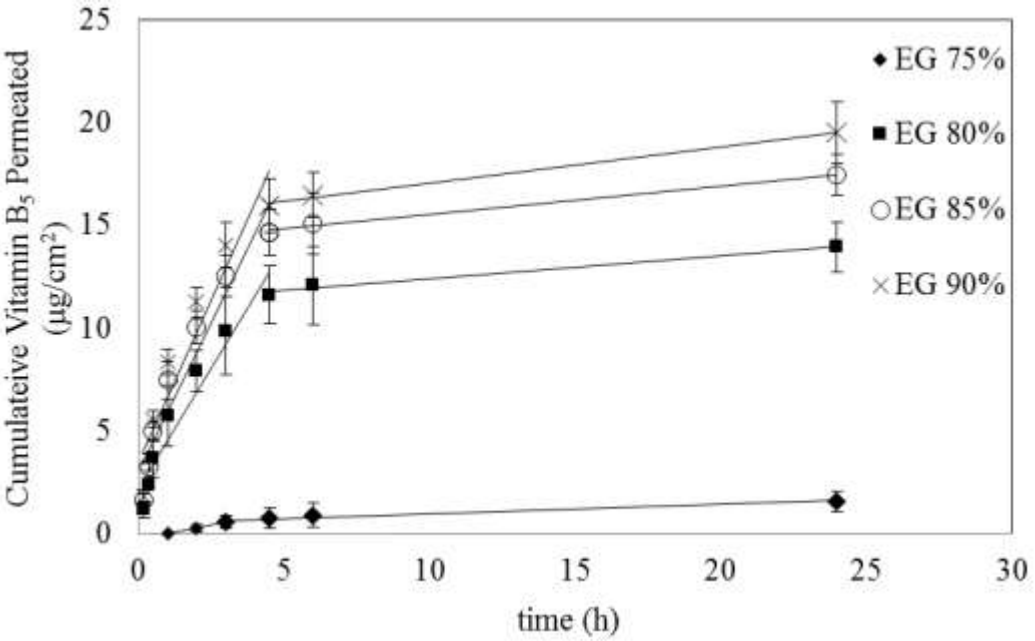

Fig. 2: Influence of different concentrations of EG on skin permeation profile of VB_5

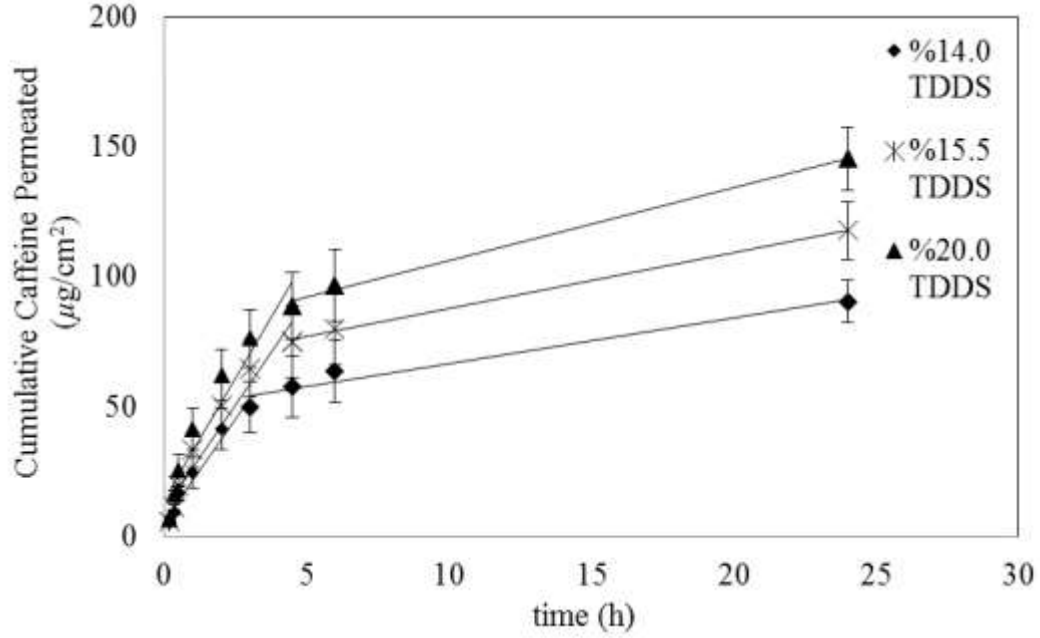

Fig. 3: Influence of different drug loading doses on skin permeation profile of CF

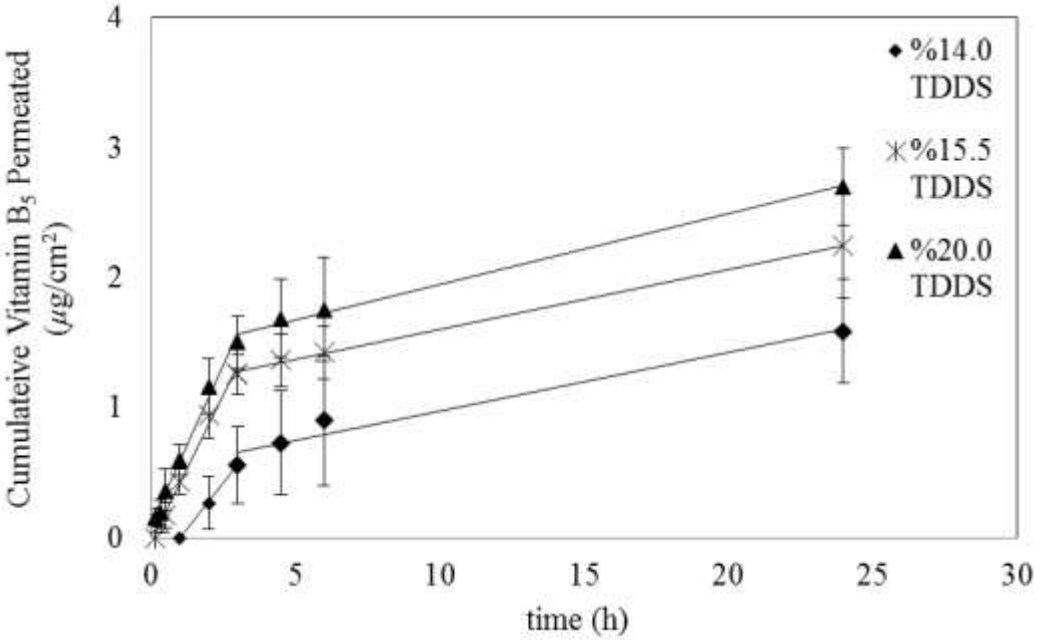

Fig. 4: Influence of different drug loading doses on skin permeation profile of VB_5

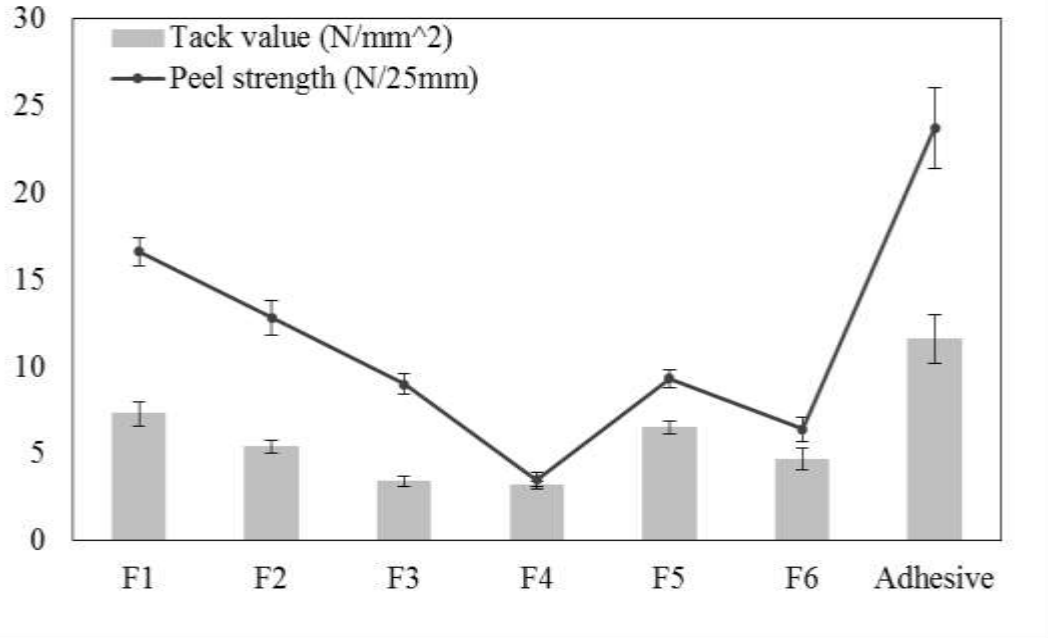

Fig. 5: Tack and Peel values for all formulations

REFERENCES

1- Shirsand SB et al. Design and evaluation of matrix transdermal patches of meloxicam. RGUHS J Pharm Sci 2012; 2(4): 58-65.
2- Khade P et al. Formulation and evaluation of microemulsion system for transdermal delivery of nimodipine. World J Pharm Sci 2014; 2(9): 988-96.
3- Natarajan R, Narasimmier Rajendran N. Cefixime trihydrate loaded chitosan-alginate transdermal patches. World J Pharm Sci 2014; 2(9): 997-1008.
4- Ng S et al. Validation of a static franz diffusion cell system for *in vitro* permeation studies. AAPS PharmSciTech 2011; 11(3): 1432-41.
5- Michaelis M et al. Plasticization and antiplasticization of an acrylic pressure sensitive adhesive by ibuprofen and their effect on the adhesion properties. Eur J Pharm Biopharm 2014; 86: 234-43.
6- Michaelis M, Leopold CS. Mixture design approach for early stage formulation development of a transdermal delivery system. Drug Dev Ind Pharm 2014; 13: 1-9.
7- Wang C et al. A drug in adhesive matrix based on thermo-plastic elastomer: evaluation of percutaneous absorption, adhesion and skin irritation. AAPS PharmSciTech 2012; 13(4): 1179-89.
8- Gannu R et al. *In vitro* permeation of carvedilol through porcine skin: effect of vehicles and penetration enhancers. PDA J Pharm Sci Technol 2008; 62(4): 256-63.
9- Subedi RK et al. Recent advances in transdermal drug delivery. Arch Pharmacal Res 2010; 33(3): 339-51.
10- Maurya A et al. Pre-treatment with chemical penetration enhancers in dermal/transdermal drug delivery. J Drug Delivery Sci Technol 2014; 24(3): 251-54.
11- Pathan IB, Setty CM. Chemical penetration enhancers for transdermal drug delivery systems. Trop J Pharm Res 2009; 8(2): 173-79.
12- Williams AC, Barry BW. Penetration enhancers. Adv Drug Delivery Rev 2012; 64: 128-37.
13- Chen Y et al. Novel chemical permeation enhancers for transdermal drug delivery. Asian J Pharm Sci 2014; 9: 51-64.
14- Taghizadeh SM et al. A statistical experimental design approach to evaluate the influence of various penetration enhancers on transdermal drug delivery of buprenorphine. J Adv Res 2015; 6: 155-62.
15- Quinones OG et al. *In vitro* and *in vivo* influence of penetration enhancers in the topical application of celecoxib. Drug Dev Ind Pharm 2014; 40: 1180-89.
16- Yang H et al. Polyethylene glycol-polyamidoamine dendritic micelle as solubility enhancer and the effect of the length of polyethylene glycol arms on the solubility of pyrene in water. J Colloid Interface Sci 2004; 273: 148-54.
17- Gungor S et al. Plasticizers in transdermal drug delivery systems. Recent Advances in Plasticizers 2012; 5: 91-112.
18- Junyaprasert VB et al. Influence of chemical penetration enhancers on skin permeability of ellagic acid-loaded niosomes. Asian J Pharm Sci 2013; 8: 110-17.
19- Mohammed D et al. Influence of skin penetration enhancers on skin barrier function and skin protease activity. Eur J Pharm Sci 2014; 51: 118-22.
20- Swain K et al. Drug in adhesive type transdermal matrix systems of ondansetron hydrochloride: optimization of permeation pattern using response surface methodology. J Drug Targeting 2010; 18(2): 106-14.
21- Ansari K et al. Recent advancement in transdermal drug delivery system. Int J Pharm Pharm Sci 2011; 3(5): 52-9.
22- Pattnaik S et al. Influence of polymeric system and loading dose on drug release from Alfuzosin hydrochloride transdermal films. Lat Am J Pharm 2009; 28(1): 62-9.
23- Taghizadeh SM et al. Preparation and *in vitro* evaluation of a new fentanyl patch based on functional and non-functional pressure sensitive adhesives. AAPS PharmSciTech 2010; 11(1): 278-84.
24- Shakeel F, Ramadan W. Transdermal delivery of anticancer drug caffeine from water-in-oil nanoemulsions. Colloids Surf, B 2010; 75; 356-62.

25- Sintov AC, Greenberg I. Comparative percutaneous permeation study using caffeine-loaded microemulsion showing low reliability of the frozen/thawed skin models. Int J Pharm 2014; 471: 516-24.
26- Heard CM et al. *In vitro* transdermal delivery of caffeine, theobromine, theophylline and catechin from extract of Guarana, Paullinia Cupana. Int J Pharm 2006; 317: 26-31.
27- Bonina FP et al. Vehicle effects on *in vitro* skin permeation of and stratum corneum affinity for model drugs caffeine and testosterone. Int J Pharm 1993; 100: 41-7.
28- Touitou E et al. Modulation of caffeine skin delivery by carrier design: liposomes versus permeation enhancers. Int J Pharm 1994; 103: 131-36.
29- Monti D et al. Comparison of the effect of ultrasound and of chemical enhancers on transdermal permeation of caffeine and morphine through hairless mouse skin *in vitro*. Int J Pharm 2001; 229: 131-37.
30- Srivastava M et al. Experimental Raman and IR spectral and theoretical studies of vibrational spectrum and molecular structure of Pantothenic acid (vitamin B_5). Spectrochim Acta, Part A 2014; 129: 131-42.
31- Aioi A et al. Effect of vitamin E and squalene on skin irritation of a transdermal absorption enhancer, lauroylsarcosine. Int J Pharm 1993; 93: 1-6.
32- Taepaiboon P et al. Vitamin-loaded electrospun cellulose acetate nanofiber mats as transdermal and dermal therapeutic agents of vitamin A acid and vitamin E. Eur J Pharm Biopharm 2007; 67: 387-97.
33- Madhaiyan K et al. Vitamin B_{12} loaded polycaprolactone nanofibers: A novel transdermal route for the water soluble energy supplement delivery. Int J Pharm 2013; 444: 70-6.
34- Khire A, Vavia P. Effect of permeation enhancers on dynamic mechanical properties of acrylate pressure sensitive adhesives. Int J Pharm 2013; 458: 141-47.
35- Taghizadeh SM, Bajgholi S. A new Liposomal-drug-in-adhesive patch for transdermal delivery of Sodium Diclofenac. J Biomater Nanobiotechnol 2011; 2: 576-81.
36- Mamatha T et al. Development of matrix type transdermal patches of lercanidipine hydrochloride: physicochemical and in-vitro characterization. Daru J Pharm Sci 2010; 18(1): 9-16.
37- Lakshmi PK et al. Formulation and evaluation of membrane-controlled transdermal drug delivery of tolterodine tartarate. Asian J Pharm Clin Res 2014; 7(2): 111-15.
38- Taghizadeh SM, Ghasemi D. Synthesis and optimization of a four-component acrylic-based copolymer as pressure sensitive adhesive. Iran Polym J 2010; 19(5): 343-52.
39- Imani M et al. Effect of adhesive layer thickness and drug loading on estradiol crystallization in a transdermal drug delivery system. AAPS PharmSciTech 2010; 11(3): 1268-75.