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Article

# Formulation and evaluation of transdermal drug-delivery system of isosorbide dinitrate

**Xiaoping Zhan[1], Zhenmin Mao[1,*], Sijing Chen[1], Shaoxiong Chen[2], Liqun Wang[2]**

*[1]School of Pharmacy, Shanghai Jiaotong University, Shanghai, P R China, [2]Strategic Alliance of Biomedical Technology, Shanghai, P R China*

The purpose of this study was to develop a reservoir-type transdermal delivery system for isosorbide dinitrate (ISDN). The developed patch consisted of five layers from bottom to top, namely, a temporary liner, an adhesive layer, a rate-controlling membrane, a reservoir and a backing. The effects of chemical penetration enhancers, reservoir materials and rate-controlling membranes on the release behaviour of ISDN from the transdermal patch were studied, and the *in vitro* release of ISDN from the developed patch was studied and compared with the commercially available ISDN patch. The results showed that there was no significant difference in permeation rates between the developed reservoir-type patch and the commercially available ISDN patch ($p > 0.05$). Moreover, the cumulative release ratio of the commercially available ISDN patch in 48 h was up to 89.8%, whereas the developed patch was only 34.9%, which meant the sustained release time of the developed patch was much longer than the commercially available ISDN patch, and would promote the satisfaction of the patient.

**Uniterms:** Isosorbide dinitrate/transdermal drug-delivery system. Transdermal patches/*in vitro* release. Transdermal patches/sustained release. Controlling membrane/drugs release rate. Skin penetration/drugs release.

O objetivo do presente estudo foi desenvolver um sistema de liberação transdérmico do tipo reservatório para o dinitrato de isossorbida (ISDN, abrevitura em Inglês). A formulação transdérmica desenvolvida constou de cinco camadas de baixo para cima, ou seja, um revestimento temporário, uma camada adesiva, uma membrana controladora da taxa de liberação, um reservatório e um reforço. Estudaram-se os efeitos dos potenciadores de penetração química, materiais do reservatório e membranas de controle da taxa de liberação no comportamento da formulação transdérmica de dinitrato de isossorbida. A liberação *in vitro* da formulação transdérmica de dinitrato de isossorbida desenvolvida foi estudada em comparação com a formulação de dinitrato de isossorbida disponível comercialmente. Os resultados mostraram que não existem diferenças significativa nas taxas de permeação entre o tipo de reservatório desenvolvido e o de dinitrato de isossorbida desenvolvido comercialmente ($p$>0,05). Ademais, a taxa de liberação cumulativa da formulação de dinitrato de isossorbida disponível comercialmente em 48 horas foi de até 89,8% e a da formulação desenvolvida, de apenas de 34,9%, o que provou que a liberação sustentada da formulação desenvolvida foi muito maior do que a de dinitrato de isossorbida desenvolvida comercialmente, o que promoveria a satisfação do paciente.

**Unitermos:** Dinitrato de isossorbida/Sistema de liberação transdérmico. Formulações transdérmicas/liberação *in vitro*. Formulações transdérmicas/liberação sustentada. Membrana de controle/taxa de liberação de fármacos. Penetração cutânea/liberação de fármacos.

***Correspondence:** Zhenmin Mao. School of Pharmacy. Shanghai Jiaotong University. Shanghai 200240, P. R. China. E-mail: zmmao@sjtu.edu.cn

## INTRODUCTION

Transdermal-patch technology has advanced tremendously since the first scopolamine patch was introduced into the market in 1979. It can be attributed to today's advanced patch-making technology, through which nearly a billion patches are manufactured every year (Prausnitz, Langer, 2008). These transdermal patches are classified into three types: drug in adhesive (the drug is directly dispersed into the adhesive polymer), reservoir (consists of a drug reservoir between a backing membrane and rate-controlling membrane, with a skin-contacting adhesive layer) and matrix (consists of a drug reservoir in the centre with a peripheral adhesive ring around the edges) (Tan, Pfister, 1999; Subedi *et al.*, 2010).

Isosorbide dinitrate (ISDN) is commonly used for the therapy of stable angina pectoris and is traditionally administrated via oral or sublingual routes. However, loss of consciousness appears in patients when angina pectoris breaks out, and thus it is difficult for patients to take the medicine by themselves. Additionally, administrated orally ISDN has low bioavailability, owing to its high first-pass metabolism in the gastrointestinal tract and liver. Moreover, the critical point of anti-anginal therapy depends, to a certain extent, on the ability of the drug to produce an immediate effect (Johnson, Gladigau, Schnelle, 1981; Fung, 1985; Taylor *et al.*, 1985). Thus, transdermal delivery may be an appropriate administration route for ISDN.

Among the ISDN transdermal patches designed and reported previously, the drug-in-adhesive patches are the simplest and the most commonly used design. Zhao *et al.* (2007) developed single-layer drug-in-adhesive transdermal patches, in which the adhesive layer not only serves as an adhesion layer to the skin, but also is responsible for the release of the drug. The *in vitro* release results show that the release kinetics of ISDN is a first-order process, suggesting that the outwards moving of ISDN from the adhesive is associated with a passive diffusion process.

Drug release from a drug-in-adhesive patch depends directly upon the drug concentration in the patch and follows first-order kinetics. However, reservoir-type transdermal drug delivery could be observed the zero-order kinetics. The rate controlling membrane, as a most important component in the reservoir-type transdermal patch, was responsible for controlling drug delivery. The rate-controlling membranes reported in previous publications included ethyl cellulose (Lewis, Pandey, Udupa, 2006), collagen and chitosan (Thacharodi, Rao, 1996), ethylene-vinyl acetate (EVA) (Shen *et al.*, 2013) and acrylate polymers (Zhan *et al.*, 2007a, b, c). Previous work in our lab has proven that acrylate polymers, as a new type of rate-controlling membranes, could control clonidine HCl solution release with zero order (Zhan *et al.*, 2007a, b, c). But, such film-like acrylate polymers have not been applied in the production of patches to date.

The aim of this study was to develop a reservoir-type transdermal patch of ISDN with acrylate polymer as the rate-controlling membrane, which could keep drug release at a constant rate for at least 48 h. The effects of chemical penetration enhancers, reservoir materials and rate-controlling membranes on the release behaviour of ISDN permeation across the transdermal patch were studied. Consequently, the *in vitro* release of ISDN from such a patch was studied in comparison to commercially available ISDN patches.

## MATERIAL AND METHODS

### Material

2-Hydroxy-3-phenoxypropylacrylate (marked A), 4-hydroxybutyl acrylate (marked B), diethyl maleate (marked C1), dibutyl maleate (marked C2) and 2-methyl-2-nitropropyl methacrylate (marked C3) were purchased from Aldrich (USA). Benzoyl peroxide, ethyl cellulose (EC), polyethylene glycol 400 (PEG400), methylene chloride, ethanol absolute, oleic acid, urea, propylene glycol and polyvinyl alcohol 17-88 (PVA17-88) were purchased from National Medicine Corporation (CHN). Isosorbide dinitrate (ISDN) was purchased from Shanghai Yuanji Chemical Co., Ltd. Polyvinyl alcohol 05-88 (PVA05-88) was purchased from Shanghai Jin Wei Trading Co., Ltd. Polyvinyl pyrrolidone K90 (PVP K90) was presented by Boai New Kaiyuan Pharmaceutical Co., Ltd. Methanol was of HPLC grade. Frandol® tape was purchased from Yamanuchi Pharm (JP) (40 mg in 40 $cm^2$). All other chemicals were of reagent grade and used as received.

### Fabrication of a reservoir-type transdermal patch of ISDN

The structure of the transdermal patch consisted of five layers, namely, a temporary liner, an adhesive layer, a rate-controlling membrane, a reservoir and a backing (Figure 1). First, the adhesive solution was coated onto the temporary liner (3M, Scotchpak™ 1022) and was allowed to dry completely. Then, a polyacrylate membrane, as a rate-controlling membrane, was pressed over the adhesive layer. Then, the reservoir layer was pressed onto the rate-

controlling membrane. Last, the polyester film laminate (3M, Scotchpak™ 9732), as a backing layer, covered the reservoir. The temporary liner and the backing layer were then heat-sealed and cut to the appropriate sizes. The patch was stored in a sealed aluminium pouch to minimise the loss of solvent.

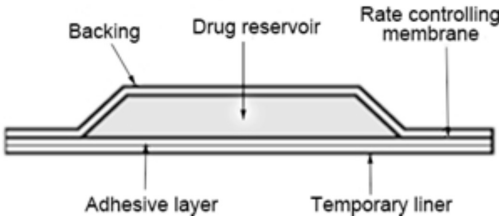


**FIGURE 1-** The construction of the ISDN transdermal patch.

## Preparation of the pressure-sensitive adhesives (PSAs)

PSAs with ISDN (0.96 g PVP K90, 0.18 g PEG400, 0.06 g gelatin and 100 mL deionised water) were put into a three-necked boiling flask and stirred at 80 ºC until the solid reagents dissolved completely; this was then refluxed for 2 h 120 ºC to decrease the strength and viscosity of the gelation solution. The solution was cooled before adding 0.06 g ISDN and stirring until the ISDN was homogeneously dispersed. The mixture was cast onto the temporary liner in 200 $cm^2$ areas and dried at 60 ºC; 0.3 mg of ISDN per $cm^2$ was obtained in the PSAs.

The preparation process of PSA without ISDN was the same procedure, except no ISDN was added.

## Preparation of the rate-controlling membranes

We used a previously reported method that had already been used in our lab to prepare three rate-controlling membranes (Figure 2), that is, membrane M1 made of monomers A, B and C1 (Zhan *et al.*, 2007a), membrane M2 made of monomers A, B and C2 (Zhan *et al.*, 2007b), and membrane M3 made of monomers A, B and C3 (Zhan *et al.*, 2007c). The ratio of the monomers in each membrane was 4:4:2 (w/w/w). We used 3% (w/w) benzoyl peroxide as the photo-initiator reagent. The mixture of monomers with the photo-initiator was treated under UV light in UV-curing equipment (Beijing Aishibo Machinery Electronic Equipment Centre, China). The thickness of all resultant membranes was 14μm.

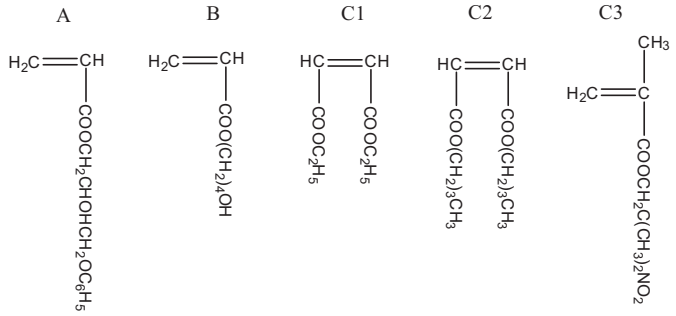


**FIGURE 2 -** The structure of acrylate monomers.

## Preparation of the drug reservoir

ISDN in EC reservoir: 5.1 g of EC were completely dissolved in methylene chloride, and then 0.54 g of ISDN was added and dissolved. The mixed solution was casted onto a glass plate with 200 $cm^2$ areas; the plate was then put in an air-conditioned chamber at room temperature for 24 h under 60–70% relative humidity in order to evaporate the methylene chloride, and a 2.7 mg ISDN layer (per $cm^2$ EC) was formed. The drug reservoir layer was separated carefully from the glass plate, and then pressed tightly on the rate-controlling membrane.

ISDN in PVA reservoir: 5.1 g of a PVA mixture (PVA0588/ PVA1788=1:2.5 mol/mol) was dissolved in water/ethanol (1:1 v/v) solution, and then 0.54 g of ISDN was added and dissolved. The mixed solution was casted onto a glass plate with 200 $cm^2$ areas, and then the plate was put in an oven (Hanzhou Huier Equipment Co., Ltd., China) at 65 °C for 24 h in order to evaporate $H_2O$ and ethanol, and a 2.7 mg ISDN layer (per $cm^2$ PVA) was formed. The drug reservoir layer was carefully separated from the glass plate and tightly attached to the rate-controlling membrane.

## Preparation of the drug reservoir containing penetration enhancer

The preparation of the drug reservoir with a penetration enhancer followed the same procedure as the preparation of the PVA reservoir layer. When ISDN was dissolved in PVA solution, 15% (w/w) oleic acid, 15% (w/w) 1,2-propylene glycol and 5% (w/w) urea were used as penetration enhancers, which were added into the PVA reservoir layer, stirred and homogeneously dispersed in the reservoir.

## Characterisation of optimised formulation

### *Thickness*

The patch thickness was measured using a digital meter (Shanghai Measuring and Cutting Tools Factory, China) at five different places. The average and standard

deviation of five readings were calculated for three batches of the optimised formulation with an area of 1 $cm^2$.

*Weight*

Three different patches from three batches, each with an area of 1 $cm^2$, were weighed individually, and the average weight and standard deviation was calculated.

*Drug content*

Three individual patches from three batches, each with an area of 1 $cm^2$, were cut into small pieces and dissolved in 100 mL of methanol/water solution (54:46 v/v). The solution was filtered, diluted suitably and measured by HPLC.

*Skin for permeation studies*

Hairless rat skin was used to evaluate the effects of penetration enhancers on the permeation and to evaluate the permeation of ISDN release from the optimised developed patch. The skin was isolated from hairless rats (male, 8-10 weeks old and 350-400 g in weight) that were obtained from Slac Lab. Animal (Shanghai, China). All studies were performed as per the protocol approved by the Institutional Animal Care and Use Committee (IACUC) at Shanghai Jiaotong University. Hairless rats were euthanised by carbon dioxide asphyxiation prior to the permeation experiment and its abdominal skin was carefully excised using scissors and forceps. The underlying subcutaneous fat was removed from the excised skin and the thus-obtained abdominal skin (ca. 1 mm thick) was used for permeation experiments.

*Permeation studies*

Permeation experiments were carried out to evaluate the rate-controlling membrane, the drug reservoirs, the penetration enhancers and the integral developed patch. The tested component was cut into appropriate sizes and mounted on a modified Franz diffusion assembly (Ng e*t al.*, 2010) produced at Huanghai Medicine & Drug Testing Instruments (China). When the tested component was the integral developed patch, the temporary line should be removed, and the dermis side of the skin faced the receptor compartment. The effective diffusion area was 0.785 $cm^2$. The receptor compartment was filled with 25.0 ± 0.1 mL of PEG400 and normal saline in the volume ratio of 4:6, and the sink conditions were maintained during the permeation experiment. The receptor medium was maintained at 37.0 ± 0.1 ºC and stirred constantly at 200 ± 2 rpm. At pre-determined time intervals 0.5, 1.5, 3, 4.5, 6.5, 8.5, 10.5, 12 and 24 h, 200 µL of the receptor solution was taken out and replaced with an equal volume of fresh receptor medium. The samples were analysed by HPLC.

## HPLC analysis of ISDN

The HPLC system (Waters, USA) consisted of a 1525 binary pump, a 717 plus auto-sampler and a 2487 dual-wavelength UV absorbance detector. Data acquisition and processing were dealt with by Waters Empower professional software. The mobile phase was a mixture of methanol and water in the volume ratio of 54:46. The mobile phase was filtered through a 0.45 mm porosity filter and degassed. The liquid chromatograph was equipped with a 80 Å, 5 µm, 4.6 mm×250 mm C18 column (Agilent Zorbax Extend) with a flow rate of 1 mL/min. The volume of each injected sample was 20 µL, the wavelength of the UV detector was set at 220nm, and the run time was 15 min (USP 32, 2009).

## Validation studies of the HPLC method

*Linearity and range*

A stock solution of ISDN (1 mg/mL) was prepared in the mobile phase. Seven standard solutions (1 to 100 µg/mL) were diluted from this stock solution using the mobile phase for the assessment of linearity. The peak areas for ISDN ($n$=5) versus concentrations were plotted and fitted to be linear over the entire concentration range.

*Accuracy*

The accuracy was determined by recovery studies. The samples were analysed at three concentrations of 5, 25 and 100 µg/mL by the proposed method. The experiments were conducted in triplicate.

*Precision*

The intra-day variability was checked at three time points on the same day, and the inter-day variability was checked on three consecutive days. The samples were analysed at three concentrations of 5, 25 and 100 µg/mL by the proposed method. The results were expressed as the percent relative standard deviation (% RSD) of concentration.

*Specificity*

A set of sample solutions were prepared to ascertain the specificity of the method. The blank solution was the mobile phase, the standard solution contained 50 µg/mL ISDN dissolved in the mobile phase, the drug substance solution was 50 µg/mL ISDN dissolved in the receptor medium (PEG400: normal saline = 4: 6 v/v), placebo

1 solution contained PSAs (50 μg/mL PVP K90 and 50 μg/mL gelation dissolved in the receptor medium and then filtered), placebo 2 solution contained penetration enhancers (50 μg/mL oleic acid, 50 μg/mL 1,2-propylene glycol and 50 μg/mL urea dissolved in the receptor medium and then filtered) and placebo 3 was for the drug reservoir (50 μg/mL EC, 50 μg/mL PVA0588 and 50 μg/mL PVA1788 dissolved in the receptor medium and then filtered).

*Detection and quantitation limits*

The LOD (limit of detection) and LOQ (limit of quantitation) were defined as the concentrations that yielded a measured peak with a S/N (signal-to-noise ratio) of 3 and 10, respectively.

### Test of sink conditions

Assuring the sink conditions in the *in vitro* release experiment is very important. According to the requirements of the sink conditions, the volume of the receptor medium was generally greater than 5-10 times that of the saturation point of ISDN in this medium. Thus, the saturated solubility of ISDN in the receptor medium needed to be quantified. ISDN was added to the flask with 25.0 mL of the receptor medium and sonicated for 24 h at 20 °C until no more solid dissolved. The saturated solution was filtered and then measured by HPLC.

### Data analysis

The cumulative amount of ISDN was calculated as follows:

$$Q = \frac{C_n V + \sum_{i=1}^{i=n-1} C_i V_i}{A} \qquad \text{(Eq. 1)}$$

where $Q$ is the cumulative amount of the drug (μg/cm$^2$), $V$ is the receptor solution volume (mL), $V_i$ is the sample volume (mL), $C_n$ and $C_i$ are the drug concentrations in the receptor cells and the concentration of the extraction samples (μg/mL), respectively, and $A$ is the transporting area (cm$^2$).

The cumulative drug amount ($Q_t$, μg/cm$^2$) was plotted versus time ($T$, h). The slope of the linear portion of the plot was presented as the permeation rate ($J_{ss}$, μg/cm/h). The intercept on *the x*-axis was taken as the lag time ($T_L$, h). All of the release experiments were repeated three times from independent batches, and mean values of the permeation rates with standard deviations were calculated. Student's *t*-test and analysis of variance (ANOVA) were used to statistically determine significant differences. The *p* value used in this study was 0.05.

## RESULTS AND DISCUSSION

### Method validation of HPLC

*Linearity and range*

The calibration curve for ISDN obtained from the developed HPLC method was displayed in Figure 3. As shown, the peak areas of the drugs were obtained to be strictly linear in the concentration range of 1 to 100 μg/mL; the correlation coefficient value was 0.9999.

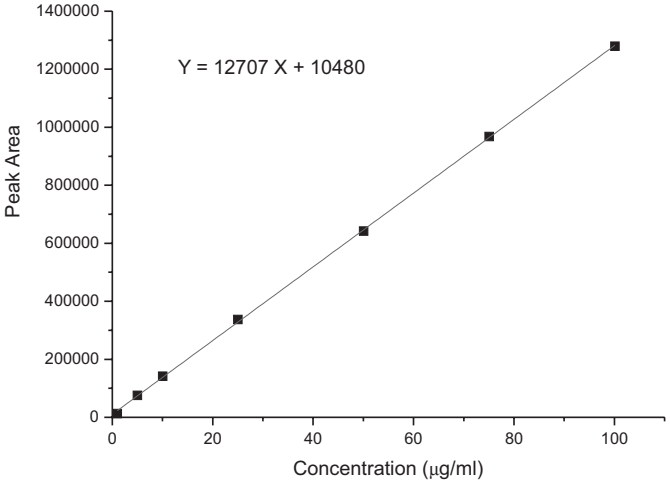


**FIGURE 3** – Calibration curve for ISDN from the HPLC method.

*Accuracy*

The percentage recoveries of the three concentrations from low to high were found to be 102.07 ± 0.63, 102.75 ± 0.21 and 99.78 ± 0.32%, respectively, which confirms that the method was accurate.

*Precision*

The % RSD of the developed intra-day HPLC method was 0.44 ± 0.28, and the % RSD of inter-day method was 0.52 ± 0.22, which suggests the excellent precision of the developed HPLC method.

*Detection and quantitation limits*

The LOD and LOQ values were found to be 100 and 350 ng/mL, respectively.

*Specificity*

The method specificity was assessed by comparing the chromatograms obtained from the sample solutions

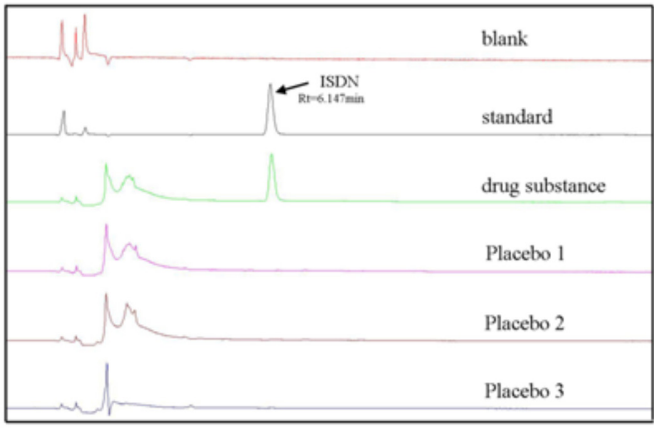


**FIGURE 4** – Chromatograph from HPLC obtained from different sample solutions.

(Figure 4); the retention time of ISDN observed from the HPLC was 6.147 min.

## Evaluation of sink conditions

The solubility of ISDN in the PEG400/normal saline solution (4:6 v/v) at 20 °C was 1.85 ± 0.03 mg/mL ($n$=3). The solubility of ISDN in water at 20 °C was 1.45 ± 0.01 mg/mL ($n$=3). The solubility of ISDN in PEG400/normal saline solution increased slightly compared to its solubility in water. The highest drug content in our research was 5 mg (the concentration in donor compartment: 5 mg/mL, volume: 1 mL), and the receptor media volume was 25 mL; the concentration was 0.2 mg/mL if 5 mg of ISDN was completely dissolved in 25 mL of media. Thus, the receptor media volume was nine times greater than the saturation point and the sink conditions were achieved during the permeation experiment. In fact, other researchers also used water as the receptor media for the purpose of simulating a human physiological environment (Zhao *et al.*, 2007).

## Effect of the rate-controlling membrane on the permeation

To screen a suitable rate-controlling membrane, different concentrations of ISDN in 1,2-propylene glycol solution (1.0, 2.0, 3.0, and 5.0 mg/mL) were used as donor solutions in the modified Franz cells with a volume of 1 mL. The release behaviours of ISDN across different rate-controlling membranes were studied over 24 h. It was found that the rate-controlling membrane marked M1 showed the highest value of permeation rates under the same drug-donor concentration, as shown in Figure 5. This result could be explained by the pore sizes of membrane M1, which was fabricated randomly by polymer chains. As polyacrylates are non-degradable polymers, they are controlled drug molecules across the pores in the membrane instead of degradation, erosion or dissolution of the polymer. The shorter side chain of monomer C1 would occupy the pore's inner space less compared with C2 and C3, as a result of the drug molecules release across the membrane M1 more easily. Membrane M1 also showed higher permeation rates compared to M2 and M3 when the tested drug was clonidine HCl (Zhan *et al.*, 2007a,b,c).

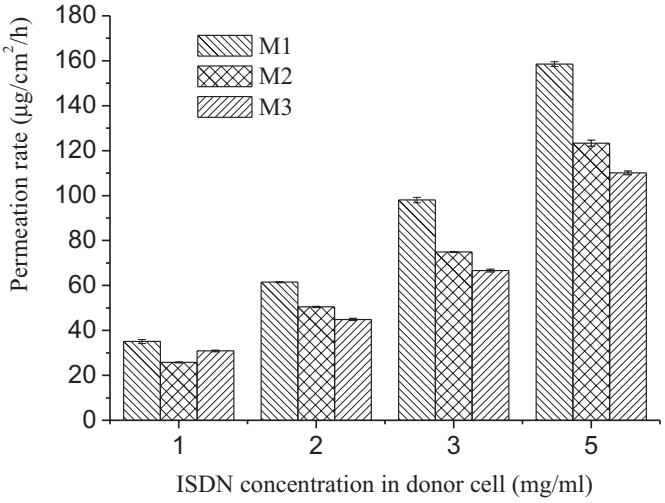


**FIGURE 5** - The permeation of ISDN through different rate-controlling membranes.

## Effect of the drug reservoir on the permeation

Two types of drug reservoir, EC and PVA, were designed. The permeation of ISDN from the drug reservoir across rate-controlling membrane M1 was studied. Table I

**TABLE I** - The permeation of ISDN releasing from the drug reservoir across M1

| Formulation | Reservoir | $J_{ss}$ (μg/cm²/h) | Cumulative amount of release at 24 h (μg) | Correlation factor ($r^2$) | $T_L$ (h) | Cumulative ratio of release at 24 h (%) |
|---|---|---|---|---|---|---|
| F1 | EC | 1.7 ± 0.1 | 30.7 ± 1.1 | 0.9967 | 8.1 | 1.3 ± 0.03 |
| F2 | PVA | 43.2 ± 1.8 | 957.2 ± 54.3 | 0.9817 | - | 40.1 ± 0.8 |

shows that PVA had a better permeation compared with EC, which increased the permeation rate 25.4 fold, a 31.1-fold cumulative release after 24 h, and a 30.8-fold cumulative ratio of release at 24 h. Moreover, ISDN releasing from PVA were not observed in the lag time, but EC had serious lag time of 8.1 h. So, the PVA was chosen to perform latter experiment.

## Effect of the penetration enhancers on the permeation

To screen the penetration enhancers, three penetrations covering a broad range of lipophilicity values (log*P* values of penetrations amounted to –2.11, –0.92 and 7.7 for urea, propylene glycol and oleic acid, respectively) (Table II) (Yangali-Quintanilla *et al.*, 2009) were chosen to investigate the impact of the partitioning characteristic of penetrations on the permeation. The weight ratio of the penetration enhancers were reported in a previously published paper (Halina; Krzysztof; Stanislaw, 2000; Chen *et al.*, 1992). The release behavior of ISDN in the PVA reservoir with the penetration enhancer transporting across the M1 was evaluated (Table III). It was found that there was no significant promotion on the permeation rates among the groups of drug reservoirs with or without penetration enhancers ($p > 0.05$) (Table IV).

**TABLE II** - Physicochemical properties of penetration enhancers

| Penetration enhancers | Molar mass (g/mol) | log*P** |
|---|---|---|
| Oleic acid | 282.46 | 7.7 |
| Propylene glycol | 76.05 | –0.92 |
| Urea | 60.06 | –2.11 |

* Experimental data

Although the penetration enhancers had no effects when the drug transported across the rate-controlling membrane, M1, it was well known that the penetration enhancers had serious effects on the skin in general. To further illustrate how the penetration enhancers affected the permeation rates, the release behaviours of ISDN in the formulation groups F2, F3, F4 and F5 transporting across the rats' *ex-vivo* skin were studied. It was found that formulation F5, of which urea was the penetration enhancer, had a higher permeation rate, cumulative amount of release and cumulative ratio of release compared to oleic acid and propylene glycol ($p<0.05$) (Figure 6). The fastest rate of permeation in the case of urea was explained by it having the lowest molecular weight when compared to the more lipophilic penetrations (Table II), as well as by its permeation-enhancing potential for the hydrophilic drugs because the value of log*P* of ISDN was 0.95, as calculated using Advanced Chemistry Development (ACD/Labs) Software V 11.02 (Ochalek *et al.*, 2012).

Actually, the excised animal skin could not represent the real permeation of drugs transporting through human skin. But, the animal skin is frequently used as a replacement for human skin, because the human skin is difficult to acquire under ethical principles. Schmook *et al* (2001) compared the penetration properties of human skin with animal (pig and rat) skin for four topical dermatological drugs (salicylic acid, hydrocortisone, clotrimazole and terbinafine) with widely varying polarity. The results revealed that the order of permeation rates was rat > pig > human (terbinafine), rat > pig ~ human (clotrimazole), rat>human>pig (hydrocortisone), rat>human>pig (salicylic acid). Thus, we could surmise the permeation rates of the drug transporting through the human skin would be less than the values of rat skin.

## Effect of the pressure sensitive adhesive on the permeation

When PVA was used as the drug reservoir, urea was used as the penetration enhancer and M1 was used as the rate-controlling membrane, the *in vitro* release behaviour of the PSA through the rats' *ex-vivo* skin was studied. Comparing the values of the permeation rates of formulation groups F5 and F6, it was found that the value of $J_{ss}$ showed a significant decrease when the PSA was

**TABLE III** - Permeation of ISDN in PVA reservoir with different penetration enhancers transporting through the M1

| Formulation | Penetration enhancer | $J_{ss}$ (μg/cm²/h) | Cumulative amount of release at 24 h (μg) | Correlation factor ($r^2$) | Cumulative ratio of release at 24 h (%) |
|---|---|---|---|---|---|
| F3 | 15% Oleic acid | 44.5 ± 0.7 | 1040.4 ± 53.9 | 0.9942 | 39.4 ± 2.2 |
| F4 | 15% 1,2-Propylene glycol | 44.3 ± 0.5 | 1000.1 ± 19.0 | 0.9917 | 40.4 ± 0.6 |
| F5 | 5% Urea | 44.8 ± 0.8 | 1017.9 ± 54.1 | 0.9889 | 41.1 ± 1.6 |

**TABLE IV -** Comparison of the *p* values from the permeation rates in four formulation groups

| Comparison formulation groups | | *p* value |
|---|---|---|
| F2 | F3 | 0.33 |
| | F4 | 0.38 |
| | F5 | 0.24 |
| F3 | F4 | 0.75 |
| | F5 | 0.62 |
| F4 | F5 | 0.41 |

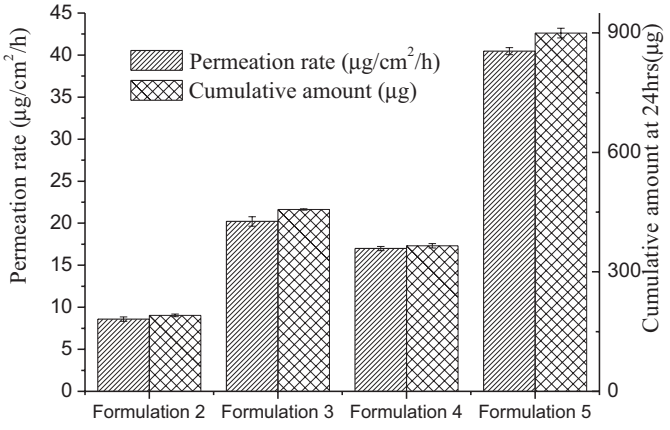


**FIGURE 6 -** *In vitro* release of ISDN in four formulations with different penetration enhancers across through the rats' *ex-vivo* skin.

used in the formulation ($p<0.05$). To increase the value of $J_{ss}$, 5% (w/w) ISDN was added into the PSA, as shown in formulation F7, and promotion effects of permeation were observed in formulation F7 compared with formulation F6 ($p<0.05$) (Table V).

## Pharmaceutical equivalent evaluation

Commercially available Frandol® Tape was used a controlling group. Comparing *in vitro* releasing behavior of formulation group F7 and Frandol® Tape, it was found that both groups could control drug release at a sustained rate for up to 48 h. There were no significant differences in the permeation rates between formulation F7 and the controlling group ($p>0.05$). The cumulative release ratio at 48 h of the developed patch was only 34.9%, but up to 89.8% in the Frandol® Tape (Figure 7), which meant that the developed patch would release at a constant rate for a longer time than the controlling group. The reason for the significant difference in cumulative release ratio of the two patches was that the amount of ISDN in the developed patch was up to 3 mg/cm$^2$ but in the Frandol® Tape it was only 1 mg/cm$^2$. In comparison with Frandol® Tape, which was a single-layer adhesive-type patch, the developed reservoir-type patch showed advantages, whereby the permeation rates were controlled by the rate-controlling membrane and the drug-release time was decided by the amount of drugs in the reservoir layer. Thus, it was easy to tune the release rate and release time to achieve the prediction.

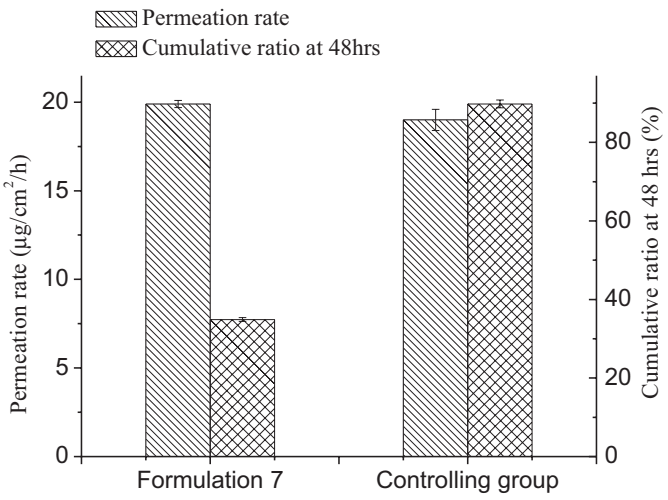


**FIGURE 7** – Comparison release of the developed patch with the controlling group.

## Physical evaluation of optimised patches

The optimised formulation, F7, with an area of 1 cm$^2$

**TABLE V -** *In vitro* release of formulations with different pressure sensitive adhesives

| Formulation | PSA | $J_{ss}$ (μg/cm$^2$/h) | Cumulative amount of release at 48 h (μg) | Correlation factor ($r^2$) | Cumulative ratio of release at 48 h (%) |
|---|---|---|---|---|---|
| F5 | - | 24.4 ± 0.1 | 1045.7 ± 19.5 | 0.9974 | 42.8 ± 1.1 |
| F6 | PSA without ISDN | 16.7 ± 0.7 | 713.5 ± 35.4 | 0.9937 | 28.9 ± 1.2 |
| F7 | PSA with ISDN | 19.9 ± 0.2 | 861.4 ± 3.3 | 0.9972 | 34.9 ± 0.5 |
| Controlling group | | 19.0 ± 0.6 | 852.9 ± 9.6 | 0.9945 | 89.8 ± 1.0 |

was tested for various physical parameters. The thickness of formulation F7 was found to be 256.4±11.8 μm. The weight of formulation F7 was 43.7±1.3 mg. The drug content in formulation F7 was 2954.7±20.3 mg. Compared to the calculated drug content of 3 mg/cm$^2$, the percent loss of drug in the preparation process was below 2%. The results indicated that the reservoir-type transdermal patch of ISDN developed in this study possessed uniform thickness, weight and drug content.

## CONCLUSION

A reservoir-type transdermal patch of ISDN was prepared in this paper. The developed patch was fabricated by a temporary liner, an adhesive layer, a rate-controlling membrane, a reservoir and a backing. In the developed patch, the optimised drug reservoir included 75% PVA, 10% ISDN and 5% urea; the optimised rate-controlling membrane was synthesised by 2-hydroxy-3-phenoxypropylacrylate, 4-hydroxybutyl acrylate and diethyl maleate; the optimised PSA was 5% ISDN dispersed in the mixture of PVP K90, PEG400 and gelatine. Compared with the commercial patch, the developed patch presented a longer release time at a sustained release rate and would promote patient satisfaction. Such a reservoir-type transdermal patch had more advantages over the adhesive-type patch, including sustained release rate, owing to the rate-controlling membrane, and a higher loading-drug amount, owing to the separated drug reservoir layer. Thus, it was easy to tune release rate and release time to achieve the prediction.

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