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Double-layer weekly sustained release transdermal patch containing gestodene and ethinylestradiol

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ABSTRACT

The combination therapy of gestodene (GEST) and ethinylestradiol (EE) has shown advanced contraception effect and lower side effect. The present study was designed to develop a weekly sustained release matrix type transdermal patch containing GEST and EE using blends of different polymeric combinations. The multiple-layer technique was adopted in order to maintain a steady permeation flux for 7 days. The effects of polymer types, polymer ratios, permeation enhancers, drug loadings and drug ratios in different layers on the skin permeations of the drugs were evaluated using excised mice skin. Polariscope examination was carried out to observe the drug distribution behavior. The formulation with the mixture of polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP) (7:1) was found to provide the regular release and propylene glycol (PG) could enhance the permeation fluxes of drugs. Double-layer transdermal drug delivery system (TDDS) could sustain the steady permeation flux of drugs for 7 days when the ratio of drug in drug release layer and drug reservoir layer was 1:4 with the identical total drug amount. The *in vitro* transdermal permeation fluxes were 0.377 μg/cm^2/h and 0.092 μg/cm^2/h, for GEST and EE respectively. The uniformity of dosage units test showed that the distribution of drugs in the matrix was homogeneous, which was further demonstrated by the polariscope result. The developed transdermal delivery system containing GEST and EE could be a promising non-oral contraceptive method.

1. Introduction

During the past decades, women had been looking forward to alternatives to the oral contraceptives (OCs) and there were many studies with focus on the safety of OCs. OCs have been shown to be associated with higher risk of cardiovascular diseases such as stroke, migraine, breast cancer, cervical cancer and hepatic injury or sudden hemorrhage resulted by forgetting intake (Dominique et al., 2000; Ferreira et al., 2001; Pietrzak et al., 2007). The bioavailability of OCs also is low due to the hepatic first-pass effect (Back et al., 1981, 1990). Furthermore, one of the significant drawbacks of OCs is the inconvenience of daily intake resulting in the low compliance and the 'surprise' in case of a forgotten pill (Rosenberg et al., 1998; Bajos et al., 2003). Therefore, non-oral delivery methods of steroids have been developed including the intrauterine contraceptive device, implants and injections which could offer a long-period effect with good compliance and tolerance (Sunanda, 2003; Régine, 2005). However, all these methods demanded professional staff to conduct, which restricted the place and the time of usage.

TDDS has been developed for contraception and hormonal replacement therapy (HRT) (Friend, 1990; Régine, 1995). It shows the following advantages superior to those methods mentioned: it has satisfactory contraception effect with lower dose of steroids by avoiding the hepatic first-pass effect (Terry et al., 2006); it can sustain a more steady plasma level to decrease side effects, particularly those associated with plasma levels fluctuation; moreover, it realizes long-period effect, good compliance by reducing the frequent dosing and the unexpected pregnancy rate (Wolfgang et al., 2005). A number of TDDS containing at least one hormone, progestin or estrogen, has been developed to achieve effective plasma concentrations. EVRA® requiring less frequent dosing has been developed and commercialized as the combined contraceptives patch (Archer et al., 2002a,b).

GEST is one of the third generation progestogens. Clinically, it is frequently used in OCs combined with EE. Both of them are so potent, the combination of 75 μg GEST and 30 μg EE has been recommended and the OCs products like Minulet® have been commercialized. It possesses most of the ideal physicochemical and biological properties, e.g. a biological half-life time ranging between 6 and 14 h, a small dosage ranging from 0.03 to 2 mg daily, a favorable oil–water partition coefficient ranging from 1 to 4 (Ulrike et al., 1995; Ines et al., 2007).

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The 7-day patch is so convenient that three patches could meet the need of one menstrual cycle. However, due to the high concentration gradient caused by 7-day dose, single-layer patch might have a burst release in the early days while the release rate decrease in the later days, which could not maintain the steady release for 7 days (Zhang and Dong, 1994).

The objective of this study was to develop a weekly sustained release matrix type TDDS containing GEST and EE employing different polymeric combinations. In order to obtain a 7-day sustained release of drug, the multi-layer technique was considered. The effects of polymer types, polymer ratios, permeation enhancers, drug loadings and drug ratios in different layers on the skin permeations of the drugs were evaluated. The results of *in vitro* release and permeation experiments indicated that double-layer patch obtained could sustain the steady release and permeation for 7 days.

2. Materials and methods

2.1. Materials

GEST and EE were obtained from ZiZhu pharmaceutical Co. Ltd. (Beijing, China) and XianJu pharmaceutical Co. Ltd (Zhejiang, China), respectively. Eudragit® RL 100, Eudragit® RS 100 and Eudragit® E100 were kindly provided by Röehm Pharma Polymers (Darmstadt, Germany). PVA of molecular weights of 17,600 and 78,800 (degree of hydrolysis 88%) were obtained from GuanHua trade Co. Ltd. (Nanjing, China). PVP K30 was a gift from ISP. The following reagents were used as purchased without further purification: Polyethylene Glycol400 (XiLong Chemical Plant, China), Laurocapram (Azone, GongZhou Chemical Plant, China), Oleic acid (OA, LingFeng Chemicals, China), Isopropyl myristate (IPM, DaZhong Chemical Plant, China), PG (ShuangLin Chemical Plant, China). All other chemicals were of HPLC grade and purchased from WanQing Co. Ltd. (Nanjing, China).

2.2. Patch preparation

2.2.1. Single-layer patch preparation

TDDS composed of different polymers containing GEST and EE were prepared by salivating technique. Firstly, drugs were dissolved in ethanol aqueous solution. Base materials were added into the solution and swelled in ambient temperature. Permeation enhancers and plasticizer were added to the solution, then agitated and homogenized. The mixture solution was applied to the surface of a flexible backing membrane, placed at static state and dried at 60 °C for 1–4 h. After being cooled, the single-layer patch with a thickness of 0.4 mm was obtained.

2.2.2. Multi-layers patch preparation

The formulation solution of the drug reservoir layer was poured on a backing membrane, dried for 3 h. Then, the formulation solution of the drug release layer was directly poured out onto the formed film, dried and cooled. Patches with drug ratios in different layers (in Table 3) were prepared. The thickness of each layer was 0.4 mm. Finally, the patches were cut, covered by a protective membrane layer, packed by aluminium–plastic membrane, and kept in a drier until used.

2.3. In vitro transdermal permeation experiment

The *in vitro* transdermal permeation behavior of drugs from TDDS across depilated mouse abdominal skin was investigated by using a modified Franz diffusion cell. Mice were sacrificed by cervical dislocation. Hairs on the abdominal area were removed using a razor for children (Phillips). Abdominal skin was excised and the fatty tissue and blood vessel attached to the dermis were removed carefully. If not used immediately, the skin was kept in refrigerator (0–4 °C) and used within 3 days. Skin was clamped between the donor cell and receptor cell with the stratum corneum facing upward into the donor cell. The effective area was 2.92 cm^2 and the receptor volume was 17 ml. Sodium chloride containing 20% (v/v) of polyethylene glycol 400 was used as receptor medium. 0.01% (w/v) gentamicin was added into the receptor medium as preservative. The study was performed at 32 ± 0.5 °C with a stirring rate of 250 rpm to prevent any boundary layer effects. The prepared patch (2.92 cm^2) was applied on the stratum corneum. Because the patch was adhesive only in the presence of water, the exposed skin area was wetted with 50 μl water. Sampling time-points were set at 2.0, 6.0, 12.0, 24.0, 48.0, 72.0, 96.0, 120.0, 144.0 and 168.0 h. 5 ml solution was taken from the receptor cell and the same volume fresh receptor medium was supplemented after each point. Sink conditions were maintained at any time. The samples were filtered through 0.45 μm filter films and analyzed for the determination of drugs permeated.

According to the concentration of each sample, the cumulative amount of the drug permeated per unit area at time point '*n*' (Q, μg/cm^2) was calculated by the following formula.

$$Q = \frac{C_n \times V + \sum_{i=1}^{n-1} C_i \times 5}{S} \quad (1)$$

where S is the effective area, V is the volume of receptor cell, C_n is the drug concentration at time point '*n*', and C_i is the drug concentration at time point '*i*'.

The cumulative amount per unit area (Q, μg/cm^2) of drug permeated through the skin was plotted as a function of time (t, h). The slope of the linear portion of the plot was represented as the permeation flux (J_s, μg/cm^2/h). For the experiments with chemical enhancers, an enhancement ratio (ER) was calculated by the following formula.

$$\mathrm{ER} = \frac{J_{s1}}{J_{s2}} \quad (2)$$

where J_{s1} is the permeation flux in the presence of chemical enhancers and J_{s2} is the permeation flux in the absence of chemical enhancers. The higher value of ER indicates the better enhancement effect.

All the transdermal permeation experiments were repeated five times and their mean values with standard deviations were calculated.

2.4. In vitro release experiment

The *in vitro* release kinetics of GEST and EE from transdermal patch were performed by using an apparatus according to the Chinese Pharmacopoeia (2005, Part II, Appendix XD method III). 900 ml sodium chloride containing 20% (v/v) of polyethylene glycol 400 was used as dissolution medium. A patch sample of 10 cm^2 was fixed in the stainless steel disk-assembly with the release surface facing up. The study was performed at 32 ± 0.5 °C with a paddle stirring rate of 75 rpm. Sampling time-points were set at 2.0, 6.0, 12.0, 24.0, 48.0, 72.0, 96.0, 120.0, 144.0 and 168.0 h. 5 ml solution was taken from the vessel and the same volume fresh dissolution medium was supplemented after each point. Sink conditions were maintained at any time. The samples were filtered through 0.45 μm filter films and analyzed.

2.5. Evaluation of characteristics of the optimized patch

2.5.1. Uniformity of dosage units test

An accurately weighed portion of the patch (1 cm × 1 cm) was cut into small pieces, and transferred to a 10 ml brown volumetric flask, dissolved in 3 ml water. The sample was heated in 50 °C water

bath for 20 min, subsequently sonicated for 5 min and then cooled. The flask was adjusted with methanol and mixed well. The solution was filtered through 0.45 μm filter films and then analyzed.

2.5.2. Polariscope examination

The test for observing drugs crystals in patch was performed with a Polariscope (Leica DM Lp, Germany) examination. Film of patch was put on an object slide and observed by a linear polarization equipment to distinguish drugs crystals from the patch matrix.

2.5.3. Adhesion test

The adhesive strength of the final patch was evaluated with tack test, shear adhesion test, and peel adhesion test according to the Chinese Pharmacopoeia (2005, Part II, Appendix XJ). The tack strength was observed by the biggest ball number that can be stick to patch (CZY-G, Labthink, China). The prepared patch was cut into strips 1.5 cm × 2.0 cm and conditioned for 2 h at 23 ± 2 °C and 50 ± 5% RH. CZY-6S (Labthink, China) was used to determine the shear strength. One side of the strip was attached on the panel and the other side was hung on a weight of 500 g. The peel adhesion force was evaluated with BLD-S (Labthink, China). The samples were applied to the stainless steel plate, rolled and pulled from the substrate at a 180° angle at a rate of 300 mm/min.

2.5.4. Skin irritation study

The dorsal part of rabbit was carefully shaved, and a 9-cm^2 patch was applied on the shaved skin for 7 days. After the patch was removed, conditions of the dorsal skin were observed and classified into five grades (points 0–4) on the basis of the severity of skin injury.

2.6. HPLC determination of GEST and EE

The amounts of GEST and EE in the samples were quantified by high pressure liquid chromatography (HPLC, Shimadzu LC-10A, Kyoto, Japan) equipped with an ultraviolet (UV) detection and a fluorescence detection, a Shim-pack VP-ODS (150 mm × 4.6 mm) column. Mobile phase was methanol/water (70:30, v:v), flow rate was kept at 1 ml/min, and the column temperature was 28 °C. Sample injection volume was 20 μl. Drugs and excipients were validated and no interference with each other. The conditions of the analysis for *in vitro* transdermal permeation and release experiments were the UV detection operated at 246 nm (GEST) and the fluorescence detection using a wavelength of 285 nm for excitation and 310 nm for emission (EE). The linearity of the method was studied in the concentration range of 0.8–12.8 μg/ml (R^2 = 0.9998, GEST) and 0.2–3.2 μg/ml (R^2 = 0.9994, EE). In addition, the UV detection operated at 240 nm and 281 nm in the uniformity of dosage units test for GEST and EE, respectively. The linearity was obtained in the concentration range of 5.0–40.0 μg/ml (R^2 = 0.9998, GEST) and 1.0–15.0 μg/ml (R^2 = 0.9999, EE). The coefficients of variation of the intra-day and inter-day precision for GEST and EE were less than 2%. The recovery rates for both drugs were in the range of 98–102%, and the relative standard deviations (RSD) were below 2%.

2.7. Statistical analysis

Data reported were the arithmetic mean values ± standard deviations (Mean ± S.D.). Statistically significant differences were determined using two-tailed Student's *t*-test with $p < 0.05$ or $p < 0.01$ as a level of significance.

3. Results and discussion

3.1. Effects of polymer types on skin permeations of GEST and EE

Acrylic acid and hydrophilic polymers were usually used as base materials. Eudragit®E100, Eudragit®RS100, Eudragit®RL100, mixtures of PVA and PVP were studied in our work. Two different molecular weights of PVA were used, namely 17,600 (PVA17) and 78,800 (PVA78). Compared the flexibility and permeation kinetic patterns (R^2), the best suitable material was picked out. The results were listed in Table 1.

Compared with the Eudragit®RS100 and Eudragit®RL100, patches prepared with the mixtures of PVA and PVP and Eudragit®E100 were more uniform and transparency. The permeation fluxes of drugs in the matrices of PVA and PVP were much larger than those in the matrix of Eudragit®E100 and the differences were significant in statistics ($p < 0.05$). The drugs permeation in terms of permeated amount or kinetics would be increased with the molecular weight of PVA ranging from 17,600 to 78,800. Due to the fact that the formulation prepared with the mixture of PVA78 and PVP showed the largest drug delivery permeation flux, it was considered to be the formulation with highest potential and was therefore selected for all the following investigations.

The base materials would not only influence the properties of the formed patch such as flexibility, adhesion and the appearance of the patch such as transparency, smoothness, gloss, but also affect the drug permeation flux (Zurdo et al., 2007). According to their chemical properties, polymers and drugs can interact in different ways like hydrogen bonding or ionic forces and so on (Jenquin and McGinity, 1994). With the evaporation of the solvent in the formulation, the drug concentration will increase, which could facilitate the permeation of drugs from the patch to skin and increase the drug permeation flux (Davis and Hadgraft, 1991; Moser et al., 2001). However, when the drug concentration in the patch exceeds the saturation level, crystallization might occur which has a negative impact on the drug permeation flux. PVP might influence the physical state of the drug in the matrix, and then affect the drug permeation flux by acting as a crystallization inhibitor.

3.2. Effects of polymer ratios on skin permeations of GEST and EE

The hydrophilic polymeric matrix PVA has many characteristics such as low toxicity and low irritation to skin, good flexibility and water-solubility. PVP plays the roles of adhesive, crystallization inhibitor and porosity-making agent. Various ratios of PVA and PVP could have different influences on the permeation fluxes.

As shown in Table 2, when the ratio of PVA and PVP changed from 1:1 to 9:1, the permeation fluxes of GEST and EE correspondingly

Table 1
Skin permeation fluxes (J_s) of GEST and EE through mice skin from the patches made with different polymers.

Polymer	Flexibility	Permeation kinetic pattern	J_s of GEST (μg/cm^2/h)	R^2	J_s of EE (μg/cm^2/h)	R^2
Eudragit®E100	Better	Zero-order equation	0.1882 ± 0.021[a]	0.9974	0.1117 ± 0.031	0.9835
PVA17 + PVP	Good	Zero-order equation	0.3053 ± 0.106*	0.9387	0.1664 ± 0.089*	0.9517
PVA78 + PVP	Better	Zero-order equation	0.4864 ± 0.042**	0.9688	0.2905 ± 0.013**	0.9708

[a] Mean ± S.D. (n = 5).
* Significantly different from the Eudragit®E100 ($p < 0.05$).
** Significantly different from the Eudragit®E100 ($p < 0.01$).

Table 2
Skin permeation fluxes (J_s) of GEST and EE through mice skin from the patches made with different ratios of polymers.

PVA:PVP	J_s of GEST (μg/cm²/h)	R^2	J_s of EE (μg/cm²/h)	R^2
1:1	0.1406 ± 0.010[a]	0.9495	0.0881 ± 0.016	0.9898
3:1	0.2505 ± 0.093	0.9805	0.1319 ± 0.026	0.9852
5:1	0.3569 ± 0.045	0.9864	0.1968 ± 0.018	0.9827
7:1	0.3866 ± 0.084	0.9716	0.2313 ± 0.021	0.9757
9:1	0.4073 ± 0.106	0.9623	0.2492 ± 0.010	0.9588
11:1	0.2954 ± 0.012	0.9784	0.1641 ± 0.012*	0.9782

[a] Mean ± S.D. (n = 5).
* Significantly different from the ratio (9:1) (p < 0.05).

increased. However, with increasing to 11:1, the permeation fluxes of both drugs decreased, significantly for EE. Overall these results suggested that the ratio of PVA and PVP was one of the important determinants for the drugs release. Comprehensively considered other factors, the ratio of 7:1 was used in our study.

3.3. Effects of permeation enhancers on skin permeations of GEST and EE

In order to select a suitable permeation enhancer, several formulations with the pre-selected enhancers at a fixed concentration 3% (w/w) were tested. Results were listed in Fig. 1. PG had the highest permeation enhancing effect for both drugs. IPM, OA and Azone also showed high enhancing effects. The difference of the permeation fluxes between the formulations in the presence and absence of permeation enhancers was significant (p < 0.01). The permeation enhancement induced by OA may result from the mechanism involving stratum corneum lipid fluidization and phase separation (Aarti et al., 1995). However, OA is a possible source for skin irritation and therefore should be used with caution (Boelsma et al., 1996). Due to lipophilicity of IPM and Azone, the system could not be homogeneous and oil spots could be seen on the surface of patches.

As the drugs were completely dissolved in the formed patches (no drug crystals were observed in the polariscope examination), the permeation enhancer might act as co-solvents to improve the partition of lipophilic drugs into the skin by dissolving them in the aqueous regions in the intercellular structures of the stratum corneum (Barry, 1991). Permeation enhancers may enhance the delivering efficiency of the formulation and reduce the drug content needed in the formulation in order to cut down the cost. In addition, the permeation enhancer should have a good local tolerability to the skin. PG could meet these requirements. It was reported that PG had an apparent enhancing effect on lipiophilic drugs as sole enhancer which was also demonstrated in our work (Goodman and Barry, 1989; Babu and Pandit, 2005).

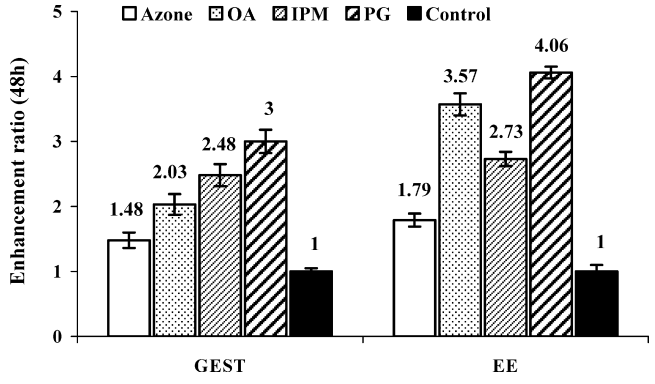

Fig. 1. Enhancement ratios for GEST and EE with different permeation enhancers based on the permeation fluxes after 48 h. Mean ± S.D. (n = 5).

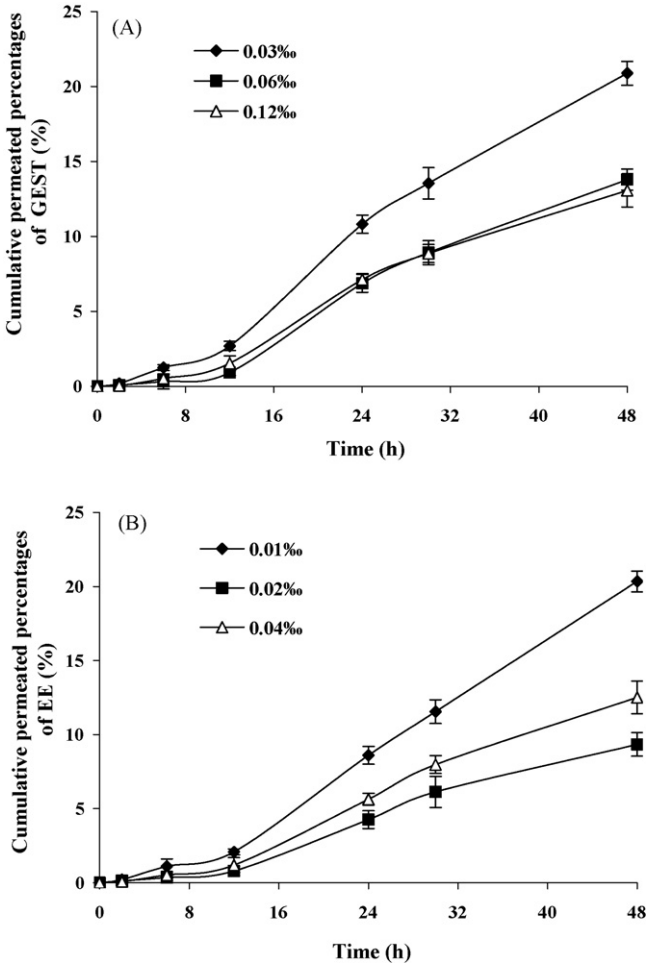

Fig. 2. Effect of drug loading in the patches made with PVA and PVP on the cumulative permeated percentages of GEST and EE. Keys: (A) cumulative permeated percentages of GEST *vs.* time; (B) cumulative permeated percentages of EE *vs.* time. Mean ± S.D. (n = 5).

3.4. Effects of drug loadings on skin permeations of GEST and EE

The formulations with different drug loadings, using PG as permeation enhancer and the mixture of PVA78 and PVP as base material, were studied to investigate the influence of drug loading on the skin permeations of GEST and EE. The results can be observed in Fig. 2. When the loading of GEST increased from 0.03‰ to 0.12‰, the permeated amount correspondingly increased while the permeated percentage decreased. The same tendency was observed for EE when its loading increased from 0.01‰ to 0.02‰. However, the permeated percentages of GEST did not vary obviously between the loading of 0.06‰ and 0.12‰ while the permeated percentage of EE increased with the drug loading changed from 0.02‰ to 0.04‰.

The drug loading was not proportional to the permeated percentage within the test range. The reason may be that the swelling and corrosion of PVA and PVP could produce limited pores which were beneficial for the diffusion of drugs. The high permeated percentage at low drug loadings was due to that most of drug molecules could freely pass through these pores. With the drug loading increased, the permeated percentage reduced, which may be caused by that majority pores were occupied or the functional groups of drug molecules interacted with those of the polymers (Jenquin and McGinity, 1994; Raghavan et al., 2001; Mukherjee et al., 2005). This tendency would not last forever because a critical point of drug loading might exist (Zhan et al., 2006). When the drug loading exceed the critical point, the effect of high osmotic pressure

Table 3
Skin permeation fluxes (J_s) of GEST and EE through mice skin from the patches with different drug ratios.

Type	Drug ratios in different layers	J_s of GEST (μg/cm²/h)	R^2	J_s of EE (μg/cm²/h)	R^2
S[b]	1:0	0.3138 ± 0.121[a]	0.9698	0.0732 ± 0.005	0.8924
D_1[c]	1:2	0.2843 ± 0.093	0.9482	0.0665 ± 0.011	0.8892
D_2	1:3	0.2869 ± 0.102	0.9878	0.0830 ± 0.012	0.9826
D_3	1:4	0.3774 ± 0.114	0.9935	0.0921 ± 0.013*	0.9834
D_4	1:5	0.3586 ± 0.097	0.9898	0.0760 ± 0.010	0.9815
T[d]	1:2:4	0.3931 ± 0.081	0.9942	0.1071 ± 0.008*	0.9882

[a] Mean ± S.D. (n = 5).
[b] Single-layer patch.
[c] Double-layer patch.
[d] Triple-layer patch.
* Significantly different from single-layer patch (p < 0.05).

might surpass that of the interaction between drug molecules and polymers, which leaded to the increase of permeation flux and permeated percentage. The similar results have been reported by Zhan et al. (2006) and Padula et al. (2007).

3.5. Effects of drug ratios in different layers on skin permeations of GEST and EE

Various formulations were estimated to screen a suitable drug ratio allocated in different layers by *in vitro* permeation experiments. The multiple-layer technique was reported in several references (Chien et al., 1988; Chen et al., 1995). The permeation fluxes of GEST and EE in single-layer, double-layer and triple-layer patches with different drug ratios, but identical total drugs amounts, were investigated. The results were illustrated in Table 3 and Fig. 3.

Results in Table 3 indicated that the rank orders in the permeation fluxes of GEST and EE were T > D_3 > D_4 > S > D_2 > D_1 and T > D_3 > D_2 > D_4 > S > D_1, respectively. The permeation fluxes of EE in T and D_3 patches were significantly larger than that in S patches in statistics (p < 0.05). As Fig. 3 shown, single-layer patch cannot sustain the steady release for 7 days, the daily permeated amounts of GEST and EE after the second day were much less than those in the first day. Maybe with the corrosion of PVA and PVP, the reticulate structure was a failure, which resulted in the unsteady release. In addition, it also can be seen that the daily permeated amounts of drugs from T and D_3 patches were much steadier than those from S patches in one week. Maybe the drugs allocated in drug reservoir layer could compensate for the drug depleted from the drug release layer, which maintained that drugs were released from patch at a steady rate. The comparison of permeation fluxes between double-layer and triple-layer patch indicated that there was no significant difference in statistics (p > 0.05), so double-layer technique was adopted and D_3 patch was the final choice.

The final patch was a laminated composite (see Fig. 4) that including: (1) a backing membrane layer that was substantially impermeable for the drug and defined the face surface of the composite, (2) the drug reservoir layer that maintain a steady release rate, (3) the drug release layer that provided the initial therapeutic dose, (4) a protective membrane layer that maintained the product appearance before administration. Double-layer patch meant that the patch body was composed of two drug layers besides the backing and protective membrane layers.

3.6. In vitro release and transdermal permeation kinetics

The *in vitro* released and permeated percentages of GEST and EE *vs.* time profiles from the final formulation were illustrated in Fig. 5. Results showed that the cumulative released percentages of GEST and EE were up to 90.7% and 92.2%, respectively. The kinetics of drug release from matrix system was more closely described by One-order model or Higuchi model. The equations of released percentages (y_1, %) *vs.* time(x, h) were $\ln(1 - y_1) = -0.006x + 0.0666$ (R^2 = 0.9839) for GEST and $y_1 = 7.3113x^{1/2} + 2.8517$ (R^2 = 0.9907) for EE, respectively.

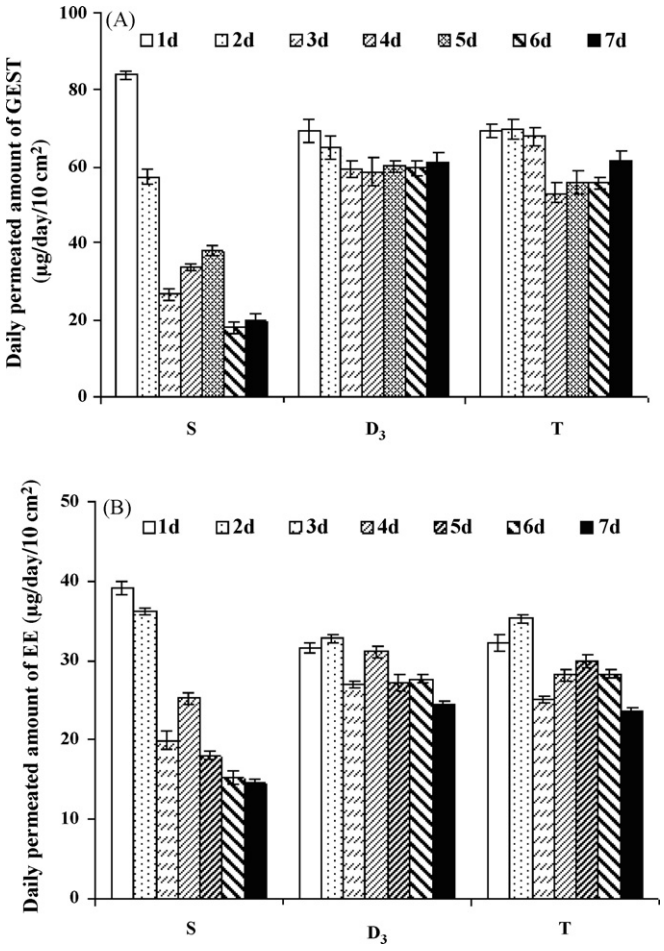

Fig. 3. Daily permeated amounts of GEST and EE through mice skin from different patches in one week. Keys: (A) daily permeated amount of GEST in one week; (B) daily permeated amount of EE in one week. Mean ± S.D. (n = 5).

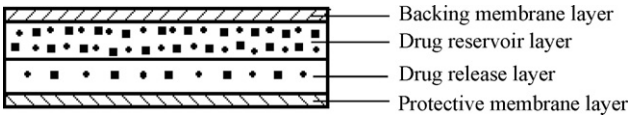

Fig. 4. Cross-sectional scheme of the double-layer patch.

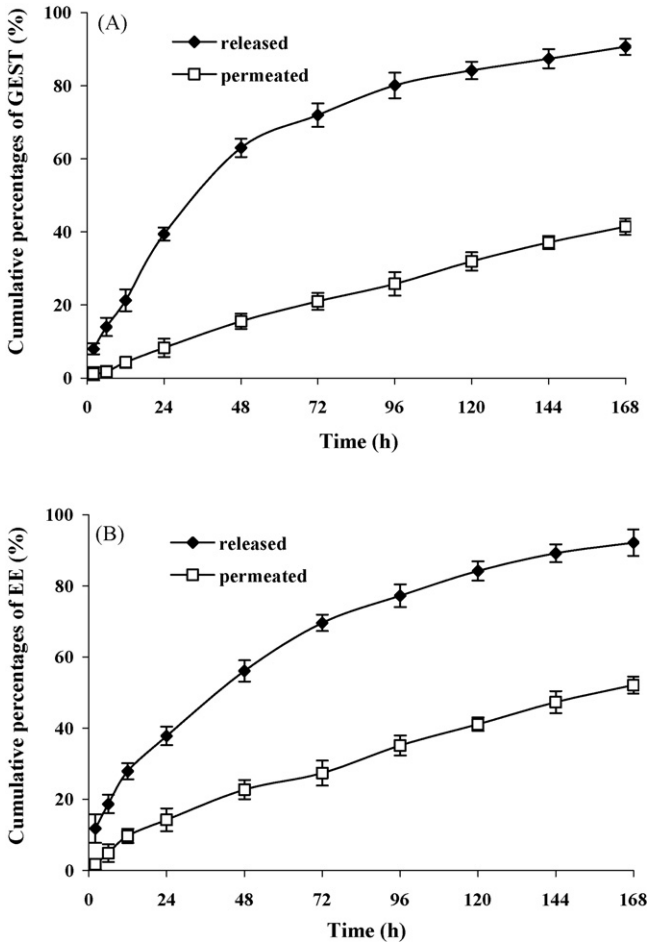

Fig. 5. *In vitro* released and permeation profiles of the transdermal patches containing GEST and EE. Keys: (A) cumulative percentages of GEST *vs.* time; (B) cumulative percentages of EE *vs.* time. Mean ± S.D. ($n = 5$).

The permeation kinetics of GEST and EE exhibited a zero-order process. The equations of permeated percentages (y_2, %) *vs.* time(x, h) were $y_2 = 0.2459x + 1.8198$ ($R^2 = 0.9933$) for GEST and $y_2 = 0.2941x + 5.3088$ ($R^2 = 0.9863$) for EE, respectively. The results above suggested that the skin was the rate-limiting step when the patches were administrated.

As Fig. 6 shown, the daily permeated amount of GEST exceeded 75 μg while the value of EE surpassed 30 μg, which indicated that drugs can be released steadily from the double-layer patch consisted of PVA and PVP for 7 days, meeting the requirements of dosage form design.

Due to the difference of skin construction between mice and human, the results we obtained can be only regarded as references (Biana and Elka, 2007). Thus, experiments on human skin or in vivo evaluation are still necessary to get further information.

3.7. Evaluation of characteristic of the optimized patch

3.7.1. Uniformity of dosage units test

The amounts of drugs in per dosage unit were determined to evaluate the content uniformity of the optimized patch.

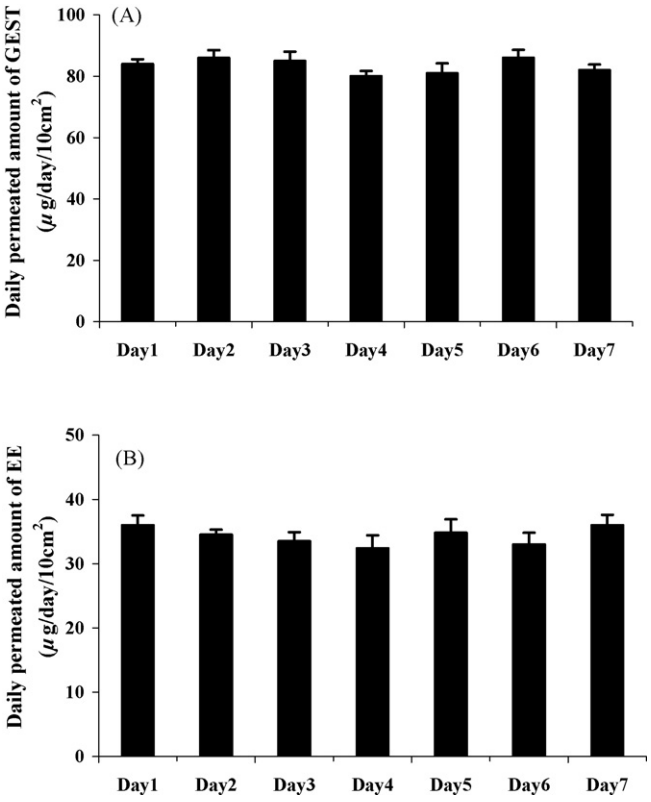

Fig. 6. Daily permeated amounts of GEST and EE through mice skin from the double-layer patch in one week. Keys: (A) daily permeated amount of GEST in one week; (B) daily permeated amount of EE in one week. Mean ± S.D. ($n = 5$).

Results in Table 4 indicated that the amounts of the drug substances in each of the 10 dosage units lay within the range of 85.0–115.0% of the patch claim, and the RSD was less than 6.0%, which met the requirements for dosage uniformity. It demonstrated that the preparation method was stable and the patch was homogenous, which was further confirmed by the polariscope result.

3.7.2. Polariscope examination

There were no signs of crystals on the surface structure of the prepared patch by polariscope examination. It meant that the patch was homogenously dense, which suggested that drugs were completely dissolved in the patch and the distribution behavior was satisfactory.

3.7.3. Adhesion test

A suitable skin adhesion is very important for patch formulation. The results of tack, shear adhesion and peel adhesion tests on the final formulation were Number 12 ball, 65 min and 4.29 N/m, which showed that the skin adhesion of patch was suitable.

3.7.4. Skin irritation study

The total irritation scores were evaluated according to Draize method (Draize et al., 1944). Overall, there were no obvious irritation effects because the total irritation score was zero in animals.

Table 4
The content percentages of GEST and EE in dosage units.

Batch	1	2	3	4	5	6	7	8	9	10	RSD
GEST	101.6%	99.1%	96.7%	96.9%	97.3%	97.1%	106.0%	94.8%	94.6%	107.2%	4.46%
EE	95.4%	101.4%	93.8%	101.9%	96.6%	97.1%	96.6%	97.7%	101.9%	103.5%	3.35%

4. Conclusions

The kinetic patterns of patches with different polymers showed that the formulation with the mixture of PVA78 and PVP (7:1) provided the regular release of drugs. As sole enhancer, PG could increase the permeation fluxes of drugs. Double-layer patch with drugs ratio of 1:4 in two layers could maintain the sustained release for 7 days. In summary, the *in vitro* transdermal permeation of both GEST and EE from the patch displayed a zero-order process and the permeation fluxes were 0.377 $\mu g/cm^2/h$ and 0.092 $\mu g/cm^2/h$, respectively. The results obtained suggested that double-layer weekly sustained release matrix transdermal patch could be a promising delivery system for non-oral contraceptive method.

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