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# Convolution method to predict drug concentration profiles of 2,3,5,6-tetramethylpyrazine following transdermal application

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**Abstract**

The objective of this work is to predict the systemic drug concentration of 2,3,5,6-tetramethylpyrazine (TMP) following transdermal application in rabbits from the in vitro skin permeation data. The in vitro skin permeation was studied in Franz diffusion cells. Pharmacokinetic evaluation of TMP following transdermal application and bolus intravenous administration were carried out in New Zealand White (NZW) rabbits. Drug concentration–time curve following transdermal application was predicted via the convolution procedure using an in vitro skin permeation data as a weighting function, and the intravenous data as an unit impulse response. The results showed that the predicted drug concentration following transdermal application by convolution method was in good agreement with the observed drug absorption profiles. These findings indicated that in vitro skin permeation tests could be useful to predict in vivo drug absorption profiles following transdermal application.


*Keywords:* In vitro skin permeation; In vivo drug absorption; Transdermal delivery systems; Convolution

## 1. Introduction

One of the challenges of transdermal delivery research is correlating in vitro skin permeation information to the in vivo drug profiles (Guy and Hadgraft, 1985). When a drug is applied topically on the surface of the skin, a series of diffusion barriers prevent its entrance to body, such as diffusion barriers from vehicle to skin, from skin to the site of action (Guy and Hadgraft, 1986). With a better understanding of the effect of the in vitro drug release and in vitro skin penetration on the in vivo drug absorption, formulation optimization and evaluation could be made efficiently through the in vitro testing (Flynn et al., 1999; Shah et al., 1992).

2,3,5,6-Tetramethylpyrazine (TMP) is a biological active ingredient originally isolated from *Ligusticum wallichii* Franch in 1957 and currently used in China for the treatment of cardiovascular diseases (Guo et al., 1983; Chen and Chen, 1993). TMP has been investigated for improvement of the immune response,

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treatment of ischemic cardiovascular and cerebral disorders, and as an anti-inflammatory agent. TMP can increase cerebral blood flow, accelerate the velocity of blood flow, dilate the spastic artery, and decrease peripheral arterial resistance (Bensky and Gamble, 1993; Guo et al., 1983; Ojewole and Odebiyi, 1980; Ho et al., 1989). However, when delivered orally, TMP is subjected to fast first-pass metabolism with only 10–30% of bioavailability (Cai et al., 1989).

Transdermal delivery could be an alternative method to maximize the effectiveness of the drug due to its advantages of reducing the systemic toxicity and side effect profiles, and minimizing the loss of drug due to first-pass metabolism in the liver. Moreover, the release of the drug is controllable and zero-order absorption kinetics could be obtained. Thus, the systemic concentration of drug could be maintained within the therapeutic range. Overall, the ease of use would allow for a better patient compliance (Brown and Langer, 1988).

Our recent studies (Qi and Hou, 1997; Qi, 1999) on physicochemical properties and percutaneous penetration of TMP showed that TMP meets the requirement of a potential candidate for delivery across the skin (Guy and Hadgraft, 1989; Flynn and Stewart, 1988). TMP has a molecular weight (MW) of 136.2. Its melting point is 76–78 °C and its solubility in water is ~11 mg/ml. The partition coefficient ($k_{oct\text{-}water}$) of TMP was found to be 123.5, the $\log k_{oct\text{-}water}$ thus being 2.3. TMP has ~90 μg/cm$^2$/h of permeation rate through human breast cadaver skin in its aqueous solution without any skin permeation enhancer. The estimated transdermal patch to reach therapeutic level is about 40 cm$^2$ (Qi and Hou, 1997; Qi, 1999).

In this study, convolution method was used to predict drug absorption kinetics following transdermal application of TMP. The in vitro skin permeation data was used as a weighting function, and the intravenous data was used as an unit impulse response.

## 2. Materials and methods

### 2.1. Materials

TMP phosphate and TMP phosphate injection (50 mg/ml) were purchased from Liming Pharmaceutical Company (Guangzhou, China). Carmazepine (purity > 99.5%) was obtained from Shanghai Chemical Compound Company (Shanghai, China).

TMP transdermal delivery system (TMP-TTS, 100 mg TMP/10 cm$^2$, 200 mg TMP/20 cm$^2$, 300 mg TMP/30 cm$^2$), prepared in our lab, is a reservoir-type transdermal system using EVA membrane as rate-controlled membrane. Modified Franz diffusion systems, with a 7.0-ml capacity receiver compartment and a 2.54-cm$^2$ diffusion surface area, was made by Technical Support department, Shanghai Institute of Industrial Pharmaceutics (Shanghai, China). New Zealand rabbits (2.5–3.0 kg) were obtained from the Animal Center of Shanghai Institute of Industrial Pharmaceutics (Shanghai, China).

### 2.2. In vitro drug release

Drug release testing was performed using a cylinder apparatus for transdermal delivery systems, which is described in USP XXII as the USP Dissolution Test Apparatus IV (U.S. Pharmacopoeia, 1995). Testing was conducted at a rotating speed of 50 rpm. The dissolution medium was 500 ml purified water maintained at 37 °C. A sample was taken through a 0.8 μm filter at predetermined sampling times and replaced with an equal volume of purified water. Drug released was analyzed by a HPLC method. A Shimazdu LC-10A liquid chromatography equipped with SPD-10A UV-Vis detector was used. The mobile phase consisted of 35% methanol and 65% of water, adjusted to be at pH 5 with phosphoric acid. The flow rate was 1.0 ml/min. The UV detection wavelength was performed at 280 nm. The injection volume was 10 μl. All measurements were performed in triplicate.

### 2.3. Preparation of skin membranes

Rabbits were sacrificed by cervical dislocation. The abdominal skins were removed by blunt dissection. The abdominal furs were removed using clippers. Then the skin samples in full thickness were cut and washed with water. Fat and connective tissues were carefully removed with a scalpel. Skin was observed for any damage through a magnifying lens. The skin specimens were wrapped in plastic film and stored in a freezer at −20 °C until they were needed. The skin samples were thawed to room temperature before mounting them within the diffusion apparatus. All

frozen skin samples were used within 1 month after preparation.

### 2.4. In vitro skin permeation studies

The Franz-type diffusion cell was used to investigate in vitro skin penetration kinetics at 37 °C. A 10-$cm^2$ patch was placed between the donor and receptor compartment. The receptor compartment contained 7.0 ml of water. The diffusion surface was 2.54 $cm^2$. All of the receiver solution was withdrawn at predetermined time, and replaced with an equivalent volume of drug free medium. The samples were assayed for TMP using a previously described HPLC method. The cumulative corrections were made to determine the total amount permeated of TMP at each time interval. This experiment was repeated three times with three cells at a time.

Cumulative corrections were made to determine the total amount of TMP permeated at each time interval. As "burst" release from patch upon application onto skin membrane was found in the in vitro experiments. The flux of TMP, $J$, at certain time period (from $t_1$ to $t_2$), will be determined using the following equation:

$$J = \frac{(Q_2 - Q_1)/A}{t_2 - t_1} = \frac{V(C_2 - C_1)/A}{t_2 - t_1} \quad (1)$$

where $Q$ and $C$ are the drug mass (μg) and concentration gradient (μg/ml), respectively, in the receiver phase; $A$ is the diffusion area ($cm^2$), $V$ is the receiver solution volume (ml), and $t$ is time (min).

### 2.5. Animal studies

To reduce the risk and to minimize the cost of human study, animal were used in bioavailibility study of transdermal delivery systems. It should be noted that animal studies may not be precisely predictive of human skin permeation. In this study, a group of New Zealand White (NZW) rabbits ($n = 6$, 2.5–3.0 kg), obtained from Animal Center of Shanghai Institute of Industrial Pharmaceutics (Shanghai, China), were used. Three rabbits were randomly assigned into group 1 (transdermal application) and group 2 (intravenous administration). For the rabbits used in the transdermal application, pretreatment was needed one day prior to the experiments. In transdermal application group, the abdominal side hair was carefully shaved. No visible signs of damage on the skin surface were observed. On the following day, TMP patch was placed on the abdominal side skin of each rabbit for up to 24 h. Blood samples were obtained via marginal ear vein at 0, 0.25, 0.5, 1, 2, 4, 6, 12, 16, and 24 h after the patch application. The rabbits used in the intravenous study were dosed at 10 mg/kg of TMP phosphate injection via the marginal ear vein. Blood samples were taken via marginal ear vein at 0, 0.083, 0.167, 0.333, 0.666, 1, 1.25, 1.5, and 2 h after intravenous administration. Food but not water was withheld until the end of each study.

The plasma concentrations of TMP were determined by a HPLC method. The liquid chromatography consisted of a Shimadzu LC-10A pump, SPD-10A UV-Vis detector and a Hewlett-Packard data module. The mobile phase was a mixture of acetonitrile, 0.02 N sodium phosphate and water (30:65:5). The flow rate was 1.0 ml/min. The UV detector was set at 285 nm and 0.002 AUFS. The column temperature was performed at 35 °C. The injection volume was 10 μl. Carbamazepine was used as internal standard. To each of 200 ul of blood samples, 20 μl of the internal standard (10 μg/ml) and 100 μl of 20% ammonium hydroxide solution (pH = 8.2) were added. The mixture was shaken for 2 min and then extracted by methlyene dichloride, followed by centrifugation at 3500 rpm for 10 min. The organic phase (20 μl) was withdrawn and added to 100 μl of 10% hydrochloride acid–methanol solution. The upper layer was dried at 40–50 °C. The dried samples were stored at −80 °C until further analysis. When analyzed, the samples were reconstituted with 200 μl of acetonitrile and injected into HPLC.

## 3. Results and discussion

### 3.1. Estimation of the unit (1 mg) impulse response model parameter A, B, a, b from a bolus intravenous injection

The plasma concentration of TMP after intravenous administration plotted against time is shown in Fig. 1. The plasma concentration of TMP versus time profiles after bolus intravenous administration was well fitted by a two-term exponential equation. Therefore, the value of unit impulse parameter $A$, $B$, $a$, $b$, could be

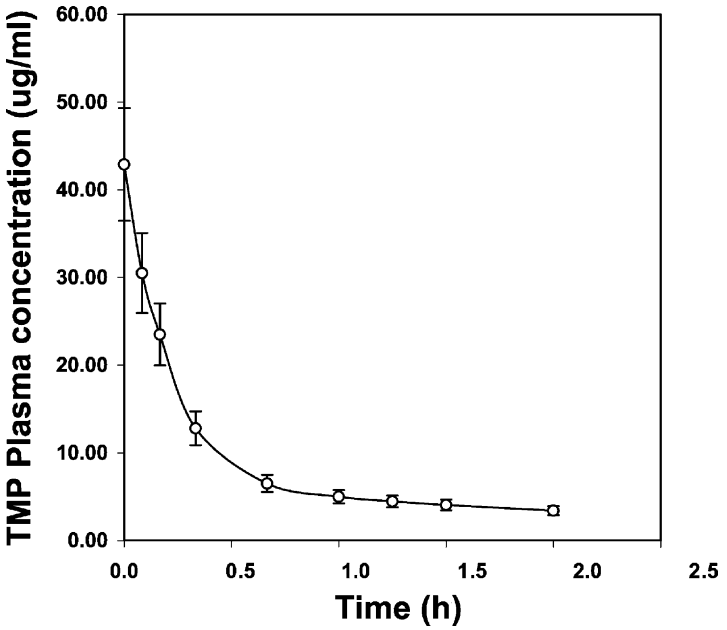


Fig. 1. Mean plasma concentration–time curve after bolus intravenous administration of tetramethylpyrazine (10 mg/kg). Each point represents average of three measurements. Error bars represent means ± S.D. for each observation.

estimated by fitting the intravenous drug concentration time data to the following two-term exponential equation:

$$C(t) = A\,e^{-at} + B\,e^{-bt} \quad (2)$$

where $A$, $B$ are the coefficients and $a$, $b$ are exponents. The WinNonlin program was used to fit the intravenous bolus plasma data. $A$, $B$ were found to be 2.55± 0.33, 0.38 ± 0.06 μg/ml mg, respectively; $a$, $b$ were found to be 5.60 ± 0.28, 0.38 ± 0.12 $h^{-1}$, respectively.

### 3.2. Estimation of weighting function from in vitro skin penetration data

As shown in Figs. 2 and 3, the in vitro skin permeation and the in vitro release of TMP from TMP-TTS have multi-phasic patterns. As shown in Table 1, the multi-phasic pattern of the in vitro skin permeation of TMP from TMP-TTS is characterized by a rapid release phase during the first 4 h, followed by a slow release phase from 4 to 6 h (~20% decrease in permeation rate), and further slower release phase from 6 to 24 h (~20% decrease in permeation rate). The burst release from patch upon application onto skin was due to that the dissolved TMP rapidly diffused from membrane to skin. The burst-release phenomenon has been reported by Roy et al. (1996) in polymer-based transdermal delivery systems and by Burns et al. (1982) in EVA-based intrauterine devices and Lesser et al. (1996) in EVA-based implants.

In order to elucidate the effect of diffusion change, a multiple-constant inputs in convolution procedure was used.

### 3.3. Convolution method to predict of the plasma profiles of TMP following transdermal application

Convolution method has been employed to predict drug level profiles from the in vitro data (Culter, 1978;

Table 1
The transdermal fluxes of TMP across rabbit skin membranes from 10 $cm^2$ TMP-TTS

| Time, $t$ (h) | $J$ (μg/$cm^2$/h) | Ratio of permeation rate |
|---|---|---|
| 0–4 | 121.3 ± 29.4 | |
| 4–6 | 95.3 ± 20.3* | 78.5% compared to $J_{0-4}$ |
| 6–24 | 78.8 ± 15.5** | 82.6% compared to $J_{6-24}$ |

Each value represents average of three measurements, $X$ ± S.D.
* $P < 0.01$, compared to $J_{0-4}$.
** $P < 0.01$, compared to $J_{4-6}$.

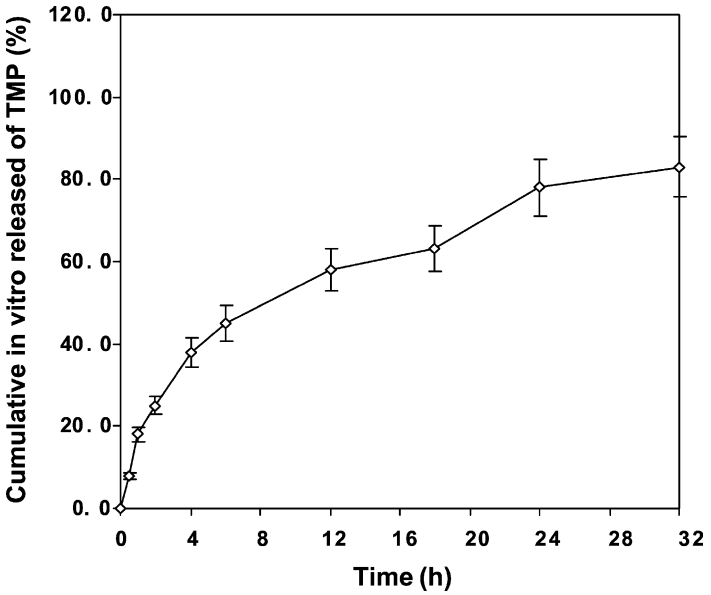


Fig. 2. The in vitro drug release profiles of TMP-TTS (300 mg/30 $cm^2$). The mean fraction of TMP released from TMP-TTS was determined by USP XXII apparatus for in vitro dissolution testing of transdermal delivery systems. Testing was conducted at a rotating speed of 50 rpm. The dissolution medium was 500 ml purified water maintained at 37 °C. Each time point represents the mean fraction released of three patches. Error bars represent means ± S.D. for each observation.

Gillespie, 1997; Veng-Pedersen, 2001). Assuming a linear relationship between the rate of the in vitro skin permeation, $J$, and resulting systemic plasma level, $C(t)$, the relationship could be expressed as follows:

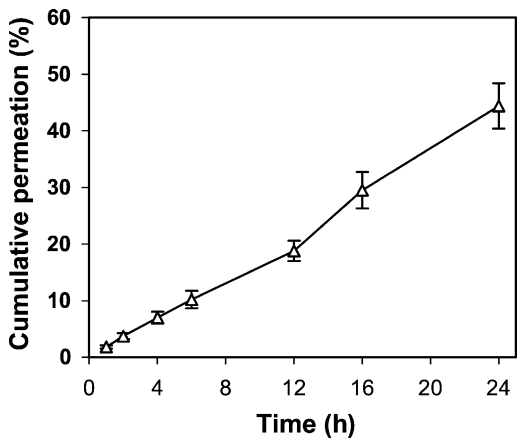


Fig. 3. The in vitro skin permeation profiles of TMP across rabbit full-thickness skin membrane from 10 $cm^2$ TMP-TTS. Each point represents average of three measurements. Error bars represent means ± S.D. for each observation.

$$C(t) = \int_0^T J(u)C_\delta(t-u)\,\mathrm{d}u = J(u) \times C_\delta(u), \quad \text{initial condition}: t = 0, C(t) = 0 \tag{3}$$

where $C_\delta(u)$ is the plasma drug concentration resulting from the bolus intravenous injection of unit amount of TMP, $J(u)$ is the corresponding rate, namely as $J_1(u)$ (the input rate during 0–2 h), $J_2(u)$ (the input rate during 2–4 h), $J_3(u)$ (the input rate during 4–6 h), and $J_4(u)$ (the input rate during 6–24 h).

Taking the Laplace transform of each side:

$$\bar{C}(s) = \bar{J}(s)\bar{C}_\delta(s) \tag{4}$$

As $\bar{J}(s)$ is constant, the equation can be rewritten as

$$\bar{C}(s) = \frac{J}{s}\left(\frac{A}{s+a} + \frac{B}{s+b}\right) \tag{5}$$

This equation can be readily solved by

$$C(t) = \frac{JA}{a}(1 - \mathrm{e}^{-at}) + \frac{JB}{b}(1 - \mathrm{e}^{-bt}) \tag{6}$$

Therefore, the drug concentration following transdermal application can be readily obtained from Eqs. (7)–(9), as shown in Fig. 4.

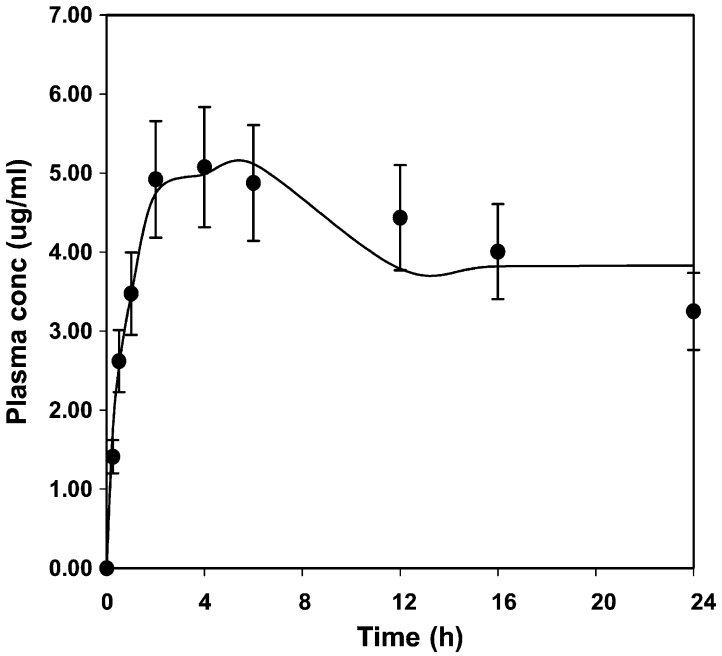


Fig. 4. Fitted (—) and observed (●, ±S.D.) mean TMP plasma concentration vs. time after a single 24 h application of TMP-TTS (300 mg/30 $cm^2$). Each point represents average of three measurements. Error bars represent means ± S.D. for each observation.

If $0 \le t \le t_1$,

$$C(t) = \frac{J_1 A}{a}(1 - e^{-at}) + \frac{J_1 B}{b}(1 - e^{-bt}) \quad (7)$$

If $t_1 \le t \le t_2$,

$$C(t) = \frac{J_2 A}{a}[1 - e^{-a(t-t_1)}] + \frac{J_2 B}{b}[1 - e^{-b(t-t_1)}] + \frac{C(t_1)}{A+B}[A\, e^{-a(t-t_1)} + B\, e^{-b(t-t_1)}] \quad (8)$$

If $t_2 \le t \le t_3$,

$$C(t) = \frac{J_3 A}{a}[1 - e^{-a(t-t_2)}] + \frac{J_3 B}{b}[1 - e^{-b(t-t_2)}] + \frac{C(t_2)}{A+B}[A\, e^{-a(t-t_2)} + B\, e^{-b(t-t_2)}] \quad (9)$$

As shown in Table 1, $t_1$, $t_2$, $t_3$ is 4, 6, and 24 h, while the corresponding $J_1$, $J_2$, $J_3$ is 121.3, 95.3, and 78.8 μg/$cm^2$/h, respectively.

In order to evaluate the internal predictability for this convolution model, percent prediction error (%PE), as shown in Eq. (10), was calculated at each time point.

$$\%PE = \frac{\text{observed} - \text{predicted}}{\text{observed}} \times 100 \quad (10)$$

The %PE factor has been adopted by the Center for Drug Evaluation and Research (FDA) and by Human Medicines Evaluation Unit of the European Agency for the Evaluation of Medicinal Products (EMEA, 1999). The %PE was used as a criterion for the assessment of the internal and external predictability in advance of in vitro–in vivo correlation (IVIVC) models for regulatory submissions. In the current study, only internal predictability was assessed since new formulation prototype with different dissolution/permeation profiles were not available to test external predictability.

As shown in Table 2, the absolute %PE values from 0.5 to 16 h was in the range of 0.5–15% and the absolute %PE from 0.5 to 6 h did not exceed 5%. Such low values of %PE suggest that the predictions of in vivo drug level from the in vitro permeation data are in good agreement with the actual observed concentration data, indicating a good prediction performance of the proposal convolution method.

Table 2
The percent prediction error (%PE) value at each time point for convolution model following administration of TMP-TTS

| Time, $t$ (h) | Observed TMP plasma concentration (μg/ml, $X \pm$ S.D., $n = 3$) | Predicted TMP plasma concentration (μg/ml) | %PE |
|---|---|---|---|
| 0.25 | 1.41 ± 0.21 | 1.73 | −24.5 |
| 0.5 | 2.62 ± 0.39 | 2.50 | 3.4 |
| 1.0 | 3.48 ± 0.53 | 3.44 | 0.5 |
| 2.0 | 4.92 ± 0.74 | 4.73 | 3.6 |
| 4.0 | 5.08 ± 0.76 | 5.01 | 1.8 |
| 6.0 | 4.88 ± 0.73 | 5.13 | −5.1 |
| 12.0 | 4.44 ± 0.67 | 3.81 | 14.6 |
| 16.0 | 4.01 ± 0.60 | 3.80 | 4.6 |
| 24.0 | 3.25 ± 0.49 | 3.80 | −17.6 |

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