Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 5, 2014

Original Article

# PERCUTANE TRANSPORT PROFILE OF CAFFEINE AND AMINOPHYLLIN AS ANTI-CELLULITE AND THE INFLUENCES OF OTHER SUBSTANCES ON *IN VITRO* PENETRATION

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*Received: 01 Apr 2014 Revised and Accepted: 07 May 2014*

**ABSTRACT**

**Objective:** The aim of the study is to investigate the effect of various dosage forms and substances such as Tretinoin, α-hydroxy acid, Vitamin C and Vitamin E on percutane transport profile of caffeine and aminophylline through rat skin using Franz Diffusion Cell.

**Methods:** Aminophylline and caffein were formulated into three different dosage forms, which were cream, gel, and ointment. Tretinoin, α-hydroxy acid, Vitamin C and Vitamin E were added into formulations as penetration enhancer. All dosage forms were physically evaluated and penetration of active ingredient through the skin were tested.

Results: Gel has the highest caffeine and aminophylline penetration followed by cream and ointment. Tretinoin and AHA can enhance the caffeine and aminophylline percutaneous penetration. Vitamin C, Vitamin E and the combination of both substances can enhance the caffeine and aminophylline percutaneous penetration. where Penetration enhancement by vitamin C is higher than penetration enhancement by vitamin E in caffeine penetration, but in aminophylline, penetration enhancement by vitamin E is higher. On the other side, aminophylline penetration on cream containing vitamin C+E combination did not significantly doubled the penetration of each one as caffeine penetration did.

**Conclusion:** The gel is the highest eighth-hour caffeine or aminophylline penetration, followed by cream and ointment. Tretinoin, α-hydroxy acid, Vitamin C and Vitamin E enhanced the eighth-hour caffeine or aminophylline skin penetrations from cream, gel, and ointment. All formula**s** were physically stable.

**Keywords:** Percutane transport profile, Caffeine, Aminophylline, tretinoin, α-Hydroxy acid, vitamin C and E.

## INTRODUCTION

Skin penetration is of great interest to personal care formulators for many reasons, from toxicology concerns to innovative delivery opportunities. With the introduction of nanotechnology to the industry, issues surrounding skin penetration have become more complex. The medical industry has spent much time focused on the mechanisms to increase and decrease skin penetration. This investigation is focused on 'Cellulite', the superficial pockets of trapped fats cause uneven lumps, bumps and dimples, or "orange peel" skin found at the thighs, buttocks and abdomen of post-adolescent women, but rarely seen in men [1], is defined as a localized metabolic disorder of the subcutaneous tissue which provokes an alteration in the female body shape caused inferiority because of ugly body appearance at the swimming pool or other fit center. Cellulite appears when there is a damage on blood vessel and lymph vessel, decreasing microcirculation of blood flow, increasing lymph fluid on dermis and accumulation of fat on subcutaneous adipose tissue and change of collagen matrix surrounded [2,3]. Lifestyle (sport, diet, alcohol, smoking, stress) and age also cause cellulite formation. The solution to overcome this problem is to use the topical dosage forms (cream, gel, or ointment) containing agents capable of distributing or reducing local fat accumulation by lipolytic action, thereby improving the aesthetic appearance of the skin. Among the common agents for treatment of cellulite as slimming agents are xanthine derivates such as caffeine or aminophylline which can block the antilipolytic action of adenosine, a potent endogenous inhibitor of lipolysis [1,4]. Other known method in lipolysis stimulation are achieved by inhibiting phosphodiesterase in order to prevent or at least limit the degradation of cAMP. Xanthine based adenosine antagonists such as caffeine or aminophylline are also known to be effective phosphodiesterase inhibitors. Other existing methods for the treatment of cellulite have been the stimulation of adenylate cyclase to increase cAMP levels (beta adrenergic agonists) or to block the antilipolytic inactivation of adenylate cyclase (alpha-2-adrenergic antagonist) [4]. Other than methods previously described, methyl xanthine can induce in vitro lipolysis by stimulating of beta adrenergic receptor abundant found at the thighs, and buttocks of post-adolescent women [2]. This stimulation induces hydrolysis of triglycerides into glycerol and free fatty acids. Methyl xanthine can also induce cellular dehydration by transporting Na+ ion into plasma and interstitial fluid [3]. Caffeine can also induce blood capillary dilatation lead to vasodilatation of peripheral blood vessel together with increasing cardiac output which increasing the peripheral blood stream, then metabolism waste and local fat accumulation can be distributed out of the periphery [5]. From the previous investigation, in vitro percutaneous caffeine absorption is relatively small, only 9.0 % [6]. On the other side, the structure of stratum corneum consisted of lipid lamellar and corneocytes make the skin being a very strong natural barrier to prevent any substances including drugs to penetrate into body. This become a special challenge in designing the delivery system in order to get therapeutic absorption level. Penetration enhancers are molecules which can reversibly reduce the resistance of skin barrier, can be introduced into anti cellulite formulation to enhance and accelerate the drug penetration [7]. These substances can enhance the drug penetration by changing the physical and chemical properties of stratum corneum lead to reduce the resistance against diffusion, i.e. enhancing the moisture of stratum corneum, disrupting the stratum corneum to be opened, to wider the intercellular space of stratum corneum lead the drug molecule to easier penetrate [7,8]. The substances used in this investigation are AHA (α-hydroxy acid: lactic acid) which can moist the skin and accelerate the keratinization process of stratum corneum, tretinoin which can irritate the skin and accelerate the keratinization process of stratum corneum, vitamin C and vitamin E to make the skin being easily penetrated by active ingredient.

The previous investigation proved that vehicles and penetration enhancers influence the aminophylline in vivo penetration from

cream dosage form [9], but in vitro investigations have not yet found in current references.

This study is undergone to investigate the effect of various dosage forms and the effect of tretinoin, α-hydroxy acid, Vitamin C and Vitamin E on percutaneous transport profile of caffeine and aminophylline through *Sprague Dawley* rat skin as the membrane, using Franz Diffusion Cell instrument. All experiments which involving animal (rat) were conducted based on local ethic requirement.

## MATERIALS AND METHODS

### Materials

Caffeine anhydrate (Jilin, Cina), aminophylline (Cina), tretinoin (DSM Nutritional Products Ltd., Indonesia), theophylline anhidrate (Jilin, Cina), lactic acid, sodium ascorbic, tocopheryl acetate, strain Sprague Dawley female rat aged 2-3 months weight ± 150 gram (Bogor Institute of Agriculture, Indonesia).

### Preparation of cream, gel and ointment

Caffeine and aminophylline cream were prepared by mixing water phase and oily phase. All oil-soluble materials were heated at 70$^0$C, while all water-soluble materials (3% of caffeine or 2% of aminophylline, surfactant, preservatives, penetration enhancer) were dissolved in warmed water of 70$^0$C. Both oily and water phase were mixed in homogenizer at temperature of 70$^0$C. Caffeine and aminophylline gel were prepared using HPMC as gelling agent. All material were mixed in a homogenizer. Unlikely cream, this gel formulation did not contain vitamin C and vitamin E.

Caffeine and aminophylline ointment were prepared by mixing water phase into melted oily phase. All oil-soluble materials were melted at 70$^0$C, while all water-soluble materials (3% of caffeine or 2% of aminophylline, preservatives, penetration enhancer) were dissolved in warmed water of 70$^0$C. Both oily and water phase were mixed in homogenizer at temperature of 70$^0$C and then stored to room temperature. Lanolin anhydrate was used as ointment base to adsorb water phase.

### Evaluation of dosage forms

Evaluation were conducted toward 23 formula of three dosage forms (cream, gel and ointment). The evaluation included measurement on organoleptic (odor, color, phase separation), homogeneity, pH, viscosity and rheology, consistency, mean globule diameter.

### Physical stability test

Physical stability test were conducted by storing the dosage forms under several condition: temperature of ±29˚C, 40±2˚C, and 4±2˚C for 8 weeks. Freez-thaw methods/cycling test (6 cycles) and centrifugation test were also performed to measure physical stability of formula. Physical evaluation included measurement on organoleptic (odor, color, phase separation), homogeneity, pH, viscosity and rheology, consistency, mean globule diameter.

**Table 1: Formulation of Caffein Cream**

| Material | CC | CC+T | CC+AHA | CC+VitC | CC+VitE | CC+VitC+VitE |
|---|---|---|---|---|---|---|
| Caffeine | 3 | 3 | 3 | 5 | 5 | 5 |
| Tretinoin | | 0.05 | | | | |
| AHA | | | 5 | | | |
| Vit C | | | | 2 | | 2 |
| Vit E | | | | | 2 | 2 |
| Cetyl alcohol | 7 | 7 | 8 | 7 | 7 | 7 |
| IPM | 8 | 8 | 4 | 5 | 5 | 5 |
| Prop.glycol | 15 | 15 | 10 | 10 | 10 | 10 |
| Steareth-21 | 3.9 | 3.9 | | 4.29 | 4.29 | 4.29 |
| Steareth-2 | 1.1 | 1.1 | | | | |
| Tween 20 | | | 3 | | | |
| Span 60 | | | 1 | 0.71 | 0.71 | 0.71 |
| Citric acid | 0.27 | 0.27 | | | | |
| Sodium citric | 2 | 2 | | | | |
| TEA | | | 4 | | | |
| BHT | 0.01 | 0.01 | | 0.05 | 0.05 | 0.05 |
| Propyl paraben | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Methyl paraben | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Aquadest ad | 100 | 100 | 100 | 100 | 100 | 100 |

**Table 2: Formulation of Aminoplylline Cream**

| Material | AC | AC+T | AC+AHA | AC+VitC | AC+VitE | AC+VitC+VitE |
|---|---|---|---|---|---|---|
| Aminophyllin | 2 | 2 | 2 | 2 | 2 | 2 |
| Tretinoin | | 0.05 | | | | |
| AHA | | | 5 | | | |
| Vit C | | | | 2 | | 2 |
| Vit E | | | | | 2 | 2 |
| Cetyl alcohol | 7 | 7 | 8 | 7 | 7 | 7 |
| IPM | 8 | 8 | 4 | 5 | 5 | 5 |
| Prop glycol | 15 | 15 | 10 | 10 | 10 | 10 |
| Steareth-21 | 3.9 | 3.9 | | 4.29 | 4.29 | 4.29 |
| Steareth-2 | 1.1 | 1.1 | | | | |
| Tween 20 | | | 3 | | | |
| Span 60 | | | 1 | 0.71 | 0.71 | 0.71 |
| Citric acid | 0.27 | 0.75 | | | | |
| Sodium citric | | | | | | |
| TEA | | | 3 | | | |
| BHT | 0.01 | 0.01 | | 0.05 | 0.05 | 0.05 |
| Propyl paraben | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 | 0.2 |
| Methyl paraben | 0.2 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 |
| Aquadest ad | 100 | 100 | 100 | 100 | 100 | 100 |

**Table 3: Formulation of Caffeine & Aminophylline Gel**

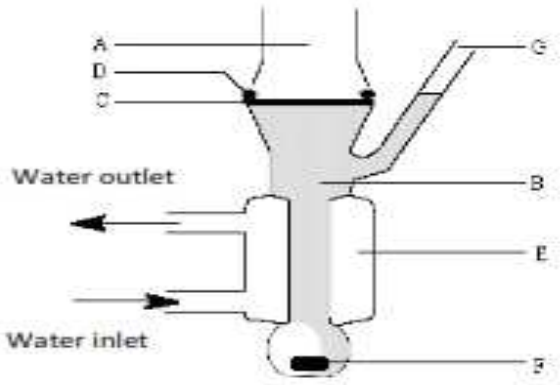
| Material | CG | CG+T | CG+ AHA | AG | AG+T | AG+AHA |
|---|---|---|---|---|---|---|
| Caffeine | 3 | 3 | 3 | | | |
| Aminophillyne | | | | 2 | 2 | 2 |
| Tretinoin | | 0.05 | | | 0.05 | |
| AHA | | | 5 | | | 5 |
| Citric acid | 0.27 | 0.27 | 0.75 | | 0.75 | |
| Sod citric | 2 | 2 | | | | |
| HPMC 4000 | 4 | 4 | 4 | 4 | 4 | 4 |
| Ethanol 90% | 10 | 10 | | 10 | 10 | |
| Propilenglycol | 15 | 15 | 10 | 15 | 15 | 10 |
| BHT | 0.01 | 0.01 | | 0.01 | 0.01 | |
| TEA | | | 4 | | | 3 |
| Propyl paraben | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Methyl paraben | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Aquadest ad | **100** | **100** | **100** | **100** | **100** | **100** |

**Table 4: Formula of Caffeine & Aminophylline Ointment**

| Material | CO | CO+T | CO+AHA | AO | AO+T |
|---|---|---|---|---|---|
| Caffeine | 3 | 3 | 3 | | |
| Aminophylline | | | | 2 | 2 |
| Tretinoin | | 0.05 | | | 0.05 |
| AHA | | | 5 | | |
| Citric acid | 0.27 | 0.27 | | | |
| Sodium citric | 2 | 2 | | | |
| Triethanolamine | | | 4 | | |
| Paraffin liquid | 3 | 3 | 3 | 3 | 3 |
| Lanolin anhydrate | 65 | 65 | 65 | 65 | 65 |
| BHT | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Propyl paraben | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Methyl paraben | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Aquadest ad | 100 | 100 | 100 | 100 | 100 |

**In-vitro Diffusion test**

Diffusion study were performed using Franz diffusion cell system as shown in Figure 1.




A = donor compartment, B = receptor compartment, C = membrane, D = *O-ring* , E = *water jacket*, F = magnetic stirrer, G = sample port

**Fig. 1: Franz Diffusion Cell**

The rat skin membrane was clamped into a Franz diffusion cell system, then 1 gram of cream/gel/ointment was put on the membrane. The glass cell had a 1.876 cm2 surface area of exposed skin, which was maintained at 37°C using Lauda Heating Circulator (Lauda, West Germany). The receptor fluid, 14.0 mL with pH 7.4 phosphate buffer, was stirred at constant rotation. Receptor fluid samples as much as 0.5 mL were collected every 1h up to 8 h. After each withdrawal of 0.5 mL sample, the receptor fluid was substituted with the same volume of phosphate buffer. Each sample was accurately made into 10 mL volume with phosphate buffer and the absorbance was measured by spectrophotometer at maximum wavelength [10].

The experiments were done by 3 times repeats. The data of analysis diffusion study was done by the equation:

$$Q = \left\{ C_n V + \sum_{i=1}^{n-1} C_i S \right\} / A$$

Q= cumulative amount penetrated (µg/cm$^2$)

V= cell volume = 14.0 mL

S= sample volume = 0.5 L

A= membrane surface area = 1.8376 cm$^2$

$C_n$= amount penetrated at n$^{th}$ sampling (µg/ml)

$\Sigma C_i$= amount penetrated from the first sample withdrawal interval up to (n-1)$^{th}$

**RESULTS AND DISCUSSION**

The result of physical evaluation of each dosage forms were summarized in Table 5.

Physical stability test were performed at room temperature (29±1ºC), high temperature (40±1ºC), and low temperature (4±1ºC) for 8 weeks and it showed no significant change in color, odor, homogeneity, and pH for all formula. There was also no crystal growth and phase separation for cream nor syneresis for gel during freeze-thaw test.

The results of diffusion study showed by Figure 2 to Figure 7.

**Table 5: Physical evaluation result of anti-cellulite dosage forms**

| Parameter | Cream | Gel | Ointment |
|---|---|---|---|
| Organoleptic | white cream with specific odor | transparent gel without odor | yellow ointment with specific odor |
| Homogeneity | homogen | homogen | homogen |
| pH | 5,45 – 6 | 5,45 – 6 | 5,45 – 6 |
| Viscosity and rheology | pseudoplastic-thixotropic | pseudoplastic-thixotropic | plastic thixotropic |
| Consistency | 384-404 | 604-625 | 386-421 |
| Mean globule diameter (µm) | 137 | - | 113 |

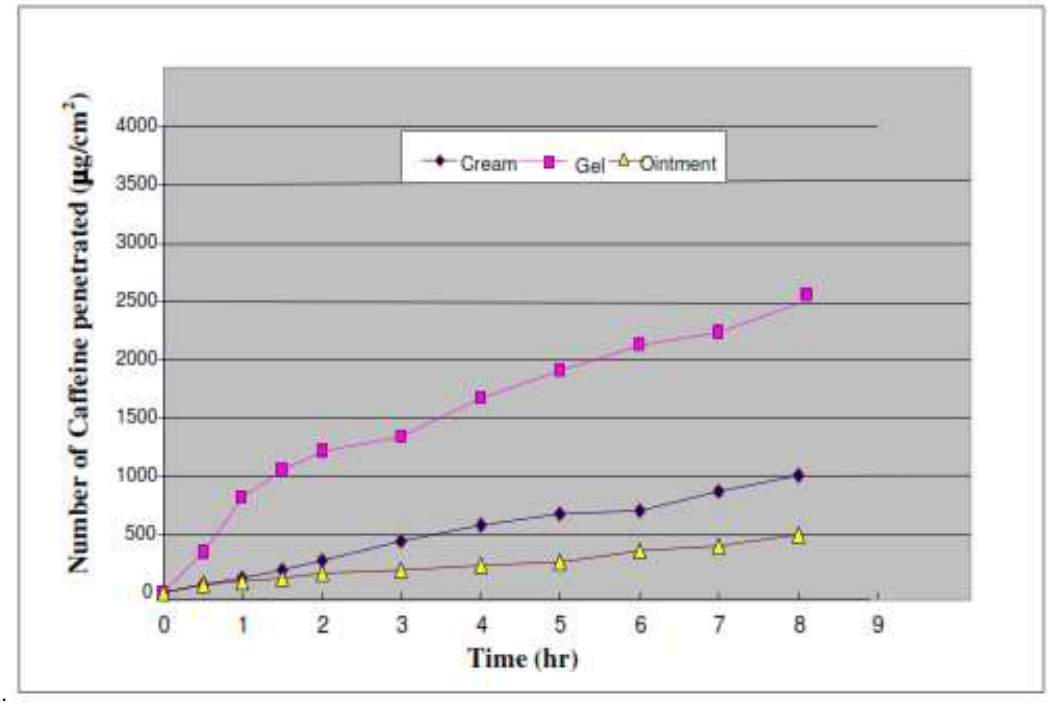



**Fig. 2: Penetration Profile of Caffeine from Cream, Gel and Ointment**

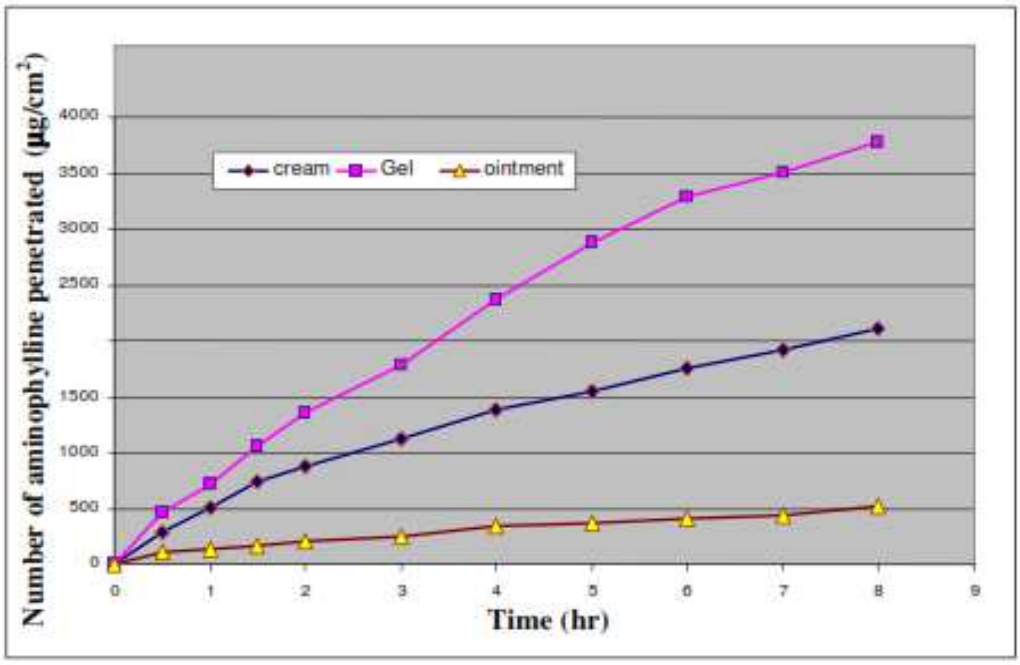



**Fig. 3: Penetration Profile of Aminophylline from Cream, Gel and Ointment**

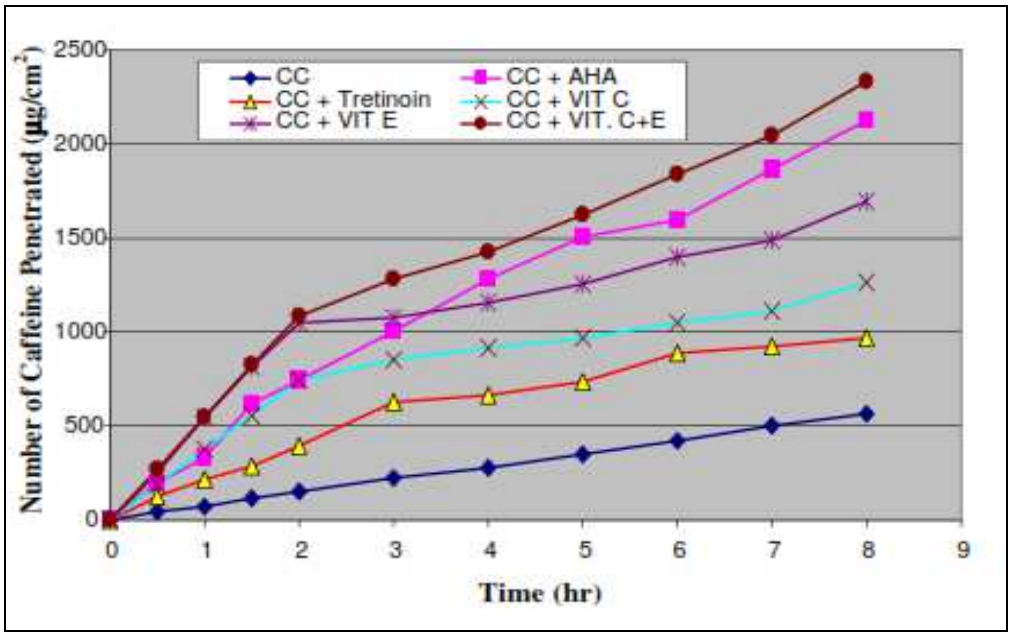



**Fig. 4: Penetration Profile of Caffeine from Cream with effects of Tretinoin, AHA, Vit C, Vit E and Vit C + Vit E**

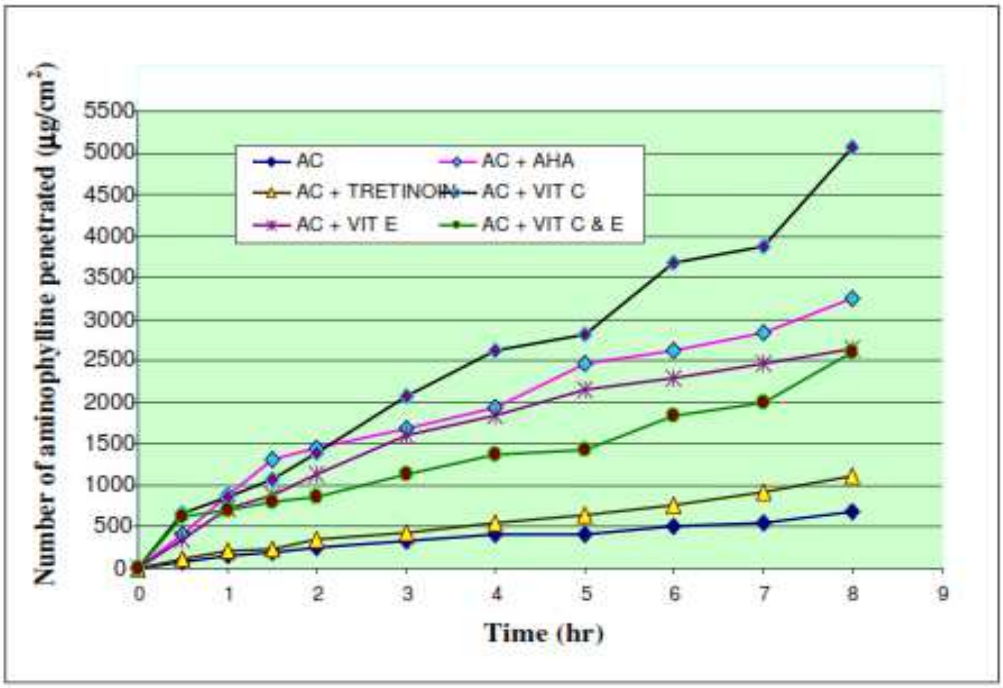



**Fig. 5: Penetration Profile of Aminophylline from Cream with effects of Tretinoin, AHA, Vit C, Vit E and Vit C + Vit E**

Based on the results of the study it was shown that there is an enhancement of caffeine and aminophylline penetration from cream and gel with the influence of tretinoin compared to cream and gel without tretionin. This enhancement is due to tretinoin which can irritate and keratolyze the skin lead to increasing the permeability of stratum corneum and enhancing the active ingredients penetration then. The keratolysis happened due to breaking the covalent bond of sulfhydril lead to disintegration and releasing the stratum corneum. Topical retinoid can enhance the Transepidermal Water Loss (TEWL) lead to decreasing the barrier function of stratum corneum, where TEWL is the indicator of stratum corneum function. The influence of penetration enhancer on caffeine or aminophylline from ointment dosage forms did not conducted, since preliminary study showed very limited amount of caffeine and aminophylline penetration.

AHA can enhance the percutaneous penetration of caffeine as well as aminophylline compared to one without the influence of AHA. In this study we used lactic acid, which can increase the cell proliferation of stratum corneum lead to enhancement of cell turn over, decreasing the thickness of stratum corneum and enhancing the thickness of viable epidermis. AHA can also decreasing the cohesion of corneocyte by ionic bond.

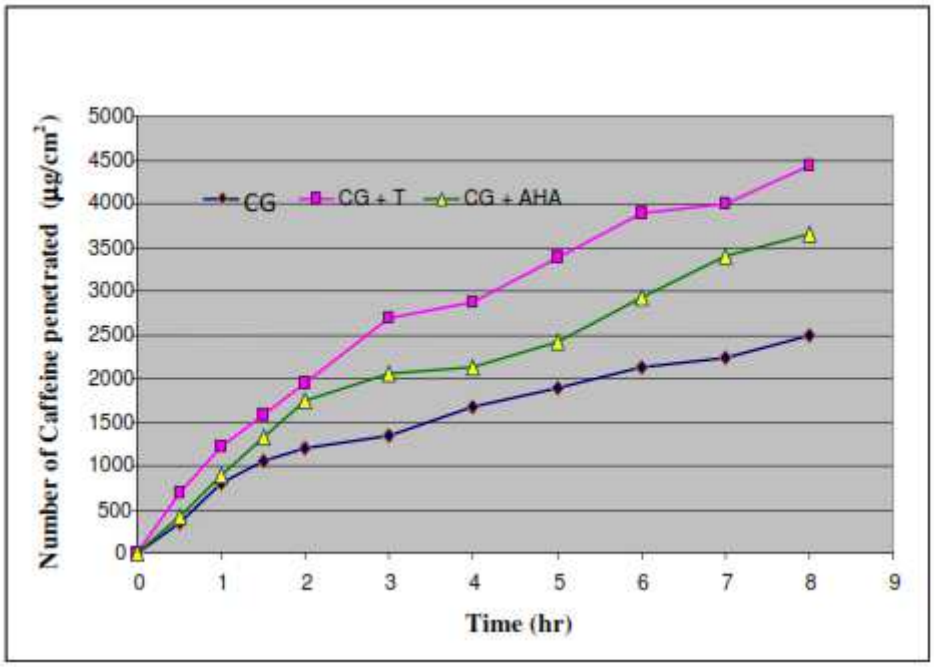



**Fig. 6: Penetration Profile of Caffeine from Gel with effects of Tretinoin and AHA**

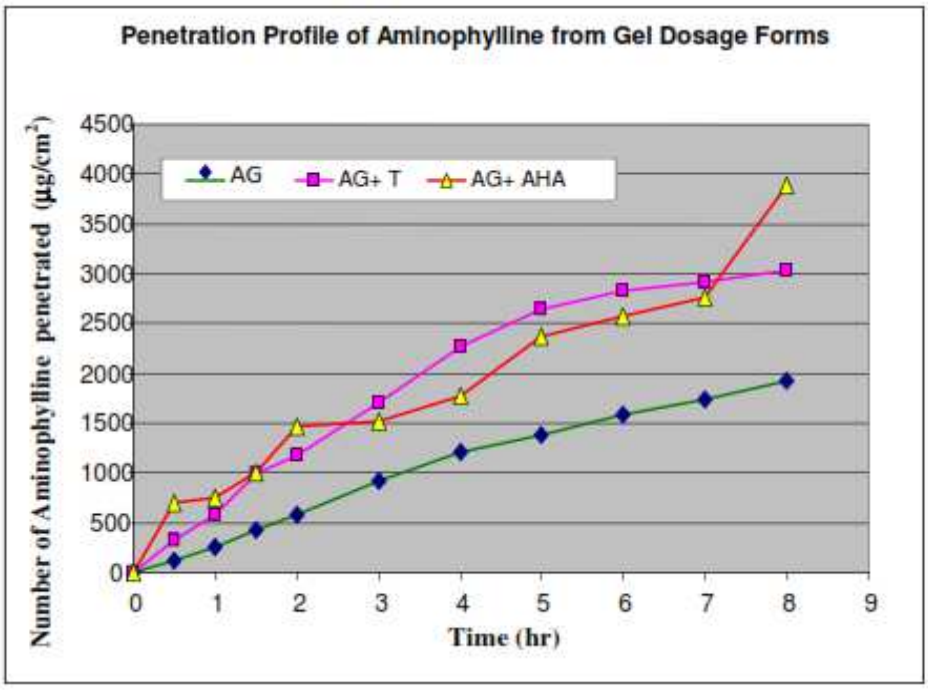



**Fig. 7: Penetration Profile of Aminophylline from Gel with effects of Tretinoin and AHA**

**CONCLUSION**

- Gel has the highest caffeine and aminophylline penetration followed by cream and ointment.
- Tretinoin and AHA can enhance the caffeine and aminophylline percutaneous penetration.
- Vit C, Vit E and combination can enhance the caffeine and aminophylline percutaneous penetration where vitamin C is higher than vitamin E in caffeine penetration, but in aminophylline penetration vitamin E is higher. On the other side, aminophylline penetration on cream containing vitamin C+E combination did not significantly doubled the penetration of each one as caffeine penetration did.
- Physical stability test under several temperatures showed no significant change in color, odor, homogeneity, and pH for all formula.
- There was no crystal growth and phase separation for creams, nor syneresis for gels at freeze-thaw test

**AKNOWLEDGEMENT**

This research is donated by the grant of Dana RUUI DRPM-UI Year 2008 Contract no. 240G/DRPM-UI/N1.4/2008 Direktorat Riset dan Pengabdian Masyarakat Universitas Indonesia. The author thanks to PT Cognis Indonesia, PT Perdoni (Uniqema), PT Kimia Farma for supporting the materials.

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