บทความวิจัย

การดูดซึมตัวยาผ่านผิวหนังนอกกายของคาเฟอีนจากอิมัลชัน
In Vitro Percutaneous Absorption of Caffeine from Emulsions

สุนิสา มีเสน[1*] สมฤทัย จิตตภักดีบดินทร์[2]
Sunisa Meesen[1*] and Somrutai Jitpukdeebodintra[2]

บทคัดย่อ

คาเฟอีนเป็นสารเสพติดที่มีฤทธิ์ต่อระบบสำคัญของร่างกาย แต่ถูกนำมาใช้ในทางเครื่องสำอางอย่างกว้างขวางหลายรูปแบบ โดยเฉพาะในรูปแบบอิมัลชัน เช่น ครีมลดริ้วรอย ครีมลดรอยหมองคล้ำรอบดวงตา ผลิตภัณฑ์ป้องกันผมร่วงและผลิตภัณฑ์กำจัดเซลลูไลต์ วัตถุประสงค์ของงานวิจัยนี้เพื่อศึกษาการซึมผ่านผิวหนังนอกกายของคาเฟอีนจากอิมัลชัน เพื่อนำผลการศึกษามาพัฒนาผลิตภัณฑ์ให้คาเฟอีนแทรกซึมเข้าสู่เฉพาะผิวหนัง ไม่ดูดซึมไปยังอวัยวะระบบอื่นๆ ที่จะส่งผลต่อร่างกายได้ การศึกษาได้ทดลองการซึมผ่านหนังของลูกหมูที่เสียชีวิตหลังคลอดทันทีแทนผิวหนังมนุษย์ จากการทดลองพบว่า คาเฟอีนในอิมัลชันชนิดน้ำมันในน้ำซึมผ่านผิวหนังมากกว่าชนิดน้ำในน้ำมัน ดังนั้นจึงสรุปว่าการพัฒนาและเตรียมตำรับเครื่องสำอางที่มีคาเฟอีนควรใช้รูปแบบอิมัลชันชนิดน้ำในน้ำมัน

คำสำคัญ : คาเฟอีน การดูดซึมผ่านผิวหนัง อิมัลชัน

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[1] นักศึกษาปริญญาโท หลักสูตรวิทยาศาสตรมหาบัณฑิต (วิทยาศาสตร์เครื่องสำอาง) หลักสูตรนานาชาติ คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ หาดใหญ่ จังหวัดสงขลา 90112

[2] ผู้ช่วยศาสตราจารย์ ภาควิชาเทคโนโลยีเภสัชกรรม คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ หาดใหญ่ จังหวัดสงขลา 90112

[*] Corresponding author: โทรศัพท์ 074-288841, โทรสาร 074-428148, E-mail: tooktan@hotmail.com

Abstract

Caffeine is narcotic drug affects to many important systems of the body, but it is widely applied in many cosmetic forms, especially cosmetic emulsion, such as anti-wrinkles and anti-periobital hyperpigmentation products, hair lost prevention, cellulite treatment products etc. The object of the research was to study *in vitro* percutaneous absorption of caffeine, now that the caffeine should not absorb to any other system of organ and took its action. The experiment of percutaneous absorption through the newborn pig skin instead of human skin was comparatively studied between caffeine in oil in water emulsion and water in oil emulsion. The result showed that caffeine amount was absorbed from oil in water emulsion was more than water in oil emulsion. So it was concluded that water in oil emulsion should be considered for cosmetic formulation and preparation of caffeine.

Keywords : Caffeine, Percutaneous Absorption, Emulsion

Introduction

Caffeine is a natural substance found in popular beverage, tea and coffee. It acts as a central nervous system (CNS) and cardiac stimulants. It is considerably more toxic to some other human due to a much poorer ability to metabolize this compound, and to a much sensitive to caffeine. The signs of toxicity include tremor, nausea, nervousness, and tachycardia/arrhythmia. Caffeine may in some people, causes tachycardia and gastric symptoms. Caffeine has also been implicated as a cause of food allergy, a non-specific term for a disorder which includes symptoms such as headache, palpitations, vomiting, panic attacks and anxiety. Caffeine interferes with normal sleep patterns and may enhance the absorption of certain drugs. Caffeine is said to be popular in cosmetic application, it works as anti-wrinkles and anti-periobital hyperpigmentation products, hair lose prevention shampoo, Alopecia aerate treatment [1], Cellulite treatment products [2]. It's assumed that the caffeine can penetrate the skin once it's applied. But even if caffeine does enter the bloodstream via cosmetic topical product, the jury is out on whether enough of it can penetrate to make a difference in alertness. So the type of topical cosmetic base in which caffeine composed is important one to predict whether a cosmetic product is safe or risky. So the aim of this study is to comparatively study the percutaneous absorption of two typical type of emulsion, and discuss which one should be apply in cosmetic product.

Materials and methods

The caffeine used in formulations was anhydrous powder of commercial grade bought from Vita Co.Ltd., a local company in Bangkok. Other chemicals listed in Table 1 were cosmetic grade, bought from P.C.Drug Center Co.Ltd., a local company in Bangkok too.

Table 1 Chemical composition of the investigated formulations

Ingredients	Content (%w/w)		Categories
	o/w emulsion	w/o emulsion	
Mineral oil	20	56	Emollient agent
Cetyl alcohol	5	7	Stiffening agent
Tween 60	2.45	-	Emulsifier (o/w)
Span 60	7.55	-	Emulsifier (o/w)
Arlacel 83	-	10	Emulsifier (w/o)
Methyl paraben	0.5	0.5	Preservative
Propyl paraben	0.1	0.1	Preservative
Caffeine	3	3	Active ingredient
Purified water to	100	100	Vehicle

The emulsion prepared by the beaker method; two phases of substances would be separately heat to 70°C. Then mixed the two phases together with continuing stirring until the mixture cooled down and congealed at room temperature. The cream with pH 5.5 then was attained.

The percutaneous absorption of caffeine from Emulsions was studied by Franz diffusion model, which followed the experiment of Songkro [3]. Full thickness skins of newborn pigs, weighing 1.4-1.8 kg, was prepared, cut into 4.5x4.5 cm^2 pieces, placed in phosphate buffer pH 7.4 and hydrated at room temperature for 1 hour before use. The receptor compartment of the modified Franz diffusion cell was filled with 11 ml of phosphate buffer (0.1 M, pH 7.4), which was continuously stirred. The modified Franz diffusion cell was placed in a circulating water bath with magnetically stirred at 300 rpm that maintained a constant temperature of the receptor fluid at 37°C. One gram of each formulation was put in a donor compartment, spread on the surface of the skin. Sample in the receptor compartment was withdrawn at 0.5, 1, 2, 4, 8, 10, 12, and 24 hours and collected in the vial for caffeine content analysis by HPLC method calibration with standard caffeine curve. An equal volume of fresh phosphate buffer would be immediately added to the receptor solution after each sampling. All experiments were repeated three times.

After 24 hours, the caffeine content on the donor side surface was washed 10 times with 1 ml each of fresh receptor liquid, and the excess washing liquid was absorbed on cotton swabs. Then all the obtained washing liquid was collected in the vial and analyzed the caffeine content by HPLC method calibration with standard curve. The full thickness skin was cut into small pieces with a scalpel

and collected in a vial, homogenized with acetonitrile, centrifuged 15,000 g for 5 min room temperature, and filtered through cellulose acetate filter; pore size of 0.2 µm (Sartorius, AG, Germany). The filtrate then was analyzed by HPLC method and calibration with standard curve.[4]

The HPLC analysis method modified from the method of Potard [5] using HPLC analysis. The condition of HPLC analysis would be a reversed phase column (RP8 4.6x250 mm–5 µm) and UV detector working at 271 nm wavelength. The mobile elution solvent was water/ acetonitrile/ acetic acid (85:15:1 v/v, pH 2.5). The solvent flow rate is 1 mL/ min at 35°C.

The amount of caffeine in various part of the skin was analyzed, and calculated using arithmetic mean ± S.D. triplicate done, then plotted against time. Statistical comparisons was made using analysis of variance (ANOVA, single factor). A difference was considered with significant at $p \leq 0.05$.

Results and discussions

The transfer of caffeine through full thickness skin by Franz diffusion cell method, from the two types of emulsions was first assessed and compared. The results were shown in Fig. 1. After 24 hr exposure, amount of caffeine diffused from oil in water (o/w) emulsion through the full thickness skin was 75.75 ± 24.84 µg/ml while caffeine diffused from water in oil (w/o) emulsion through the full thickness skin was 35.07 ± 6.05 µg/ml. Now that caffeine diffuses from o/w emulsion through the skin more than w/o emulsion in the same condition, So caffeine from o/w emulsion was more absorbed into the bloodstream than w/o emulsion.

However caffeine in the skin was also assesses to assure the percutaneous absorption of caffeine into the body. The result was shown in Fig.2.

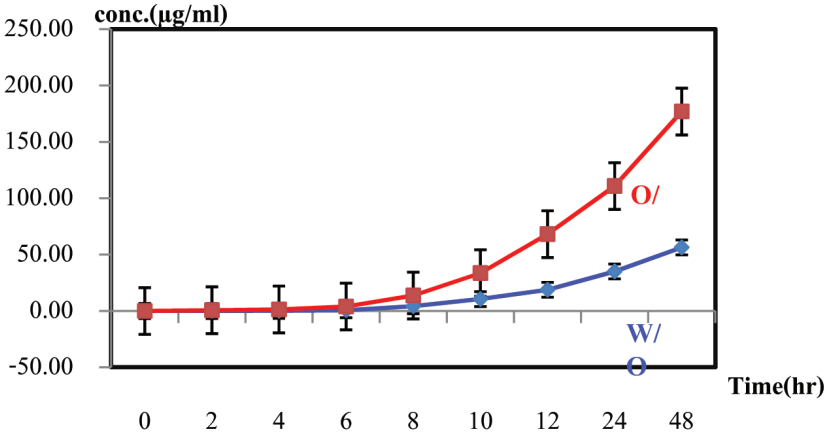

Fig. 1. Comparative caffeine diffused from water in oil (W/O) emulsion and oil in water (O/W) emulsion through full thickness skin after 24 hrs application. (caffeine assessed from receptor compartment of franz cell)

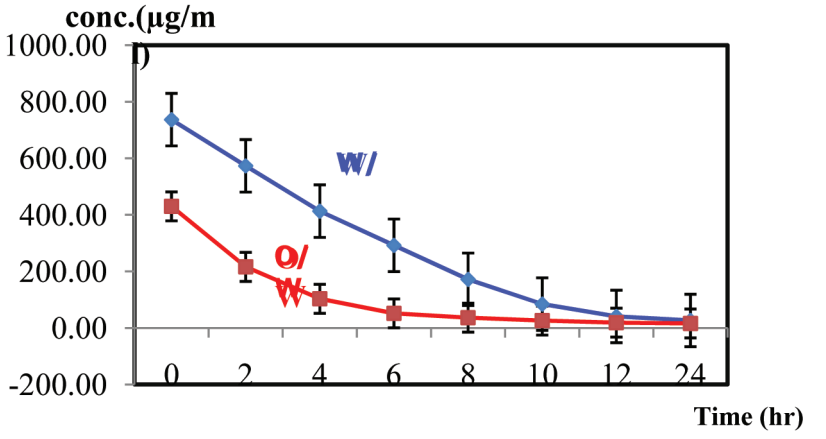

Fig. 2. Comparative caffeine from water in oil (W/O) emulsion and oil in water (O/W) emulsion accumulated in full thickness skin after 24 hrs application. (caffeine assessed from recepyor cell of franz cell)

It showed that caffeine from o/w emulsion was accumulated in skin less than caffeine from w/o emulsion. In conclusion caffeine from o/w emulsion was more penetrate through the skin into the bloodstream than w/o emulsion.

Moreover caffeine did still not penetrate through skin was measured as shown in table 1, which showed that caffeine in both types of emulsion did still remain on the top of the skin, where they were applied. The quantity of caffeine from o/w emulsion on the skin did not differ from w/o emulsion. The caffeine from both type of tested emulsion did penetrate through the skin not so well, because there were not any enhancers in the formulation of both types of emulsion. So the safety caffeine cream to be formulated might be w/o emulsion, which caffeine was not so much penetrated into blood stream. However the formulation must be further developed for a better penetration into cutaneous layer of active anti-cellulite caffeine.

Table 1 Content of caffeine from both type of emulsion on the various layers of the applied skin. After 24 hr of testing (origin concentration of caffeine was 30 mg/ml and n = 3)

Location of caffeine quantified	w/o emulsion	o/w emulsion
Caffeine on the donor side surface (mg/ml)	29.92 ± 1.25	29.86 ± 6.82
Caffeine in the skin (µg/ml)	40.55 ± 0.36	18.79 ± 2.59
Caffeine in the receptor compartment (µg/ml)	35.07 ± 6.05	75.75 ± 24.84

Conclusion

On the basis of these results, the caffeine absorption in the skin was measured after 24 hours exposure.The total amount that has penetrated the skin is the sum of amounts found in the skin and receptor compartment. After 24 hr the receptor fluid found the amount of caffeine from oil in water emulsion more than water in oil emulsion that mean caffeine absorbed through skin and flow in the blood stream that not still in the skin for reduce cellulite in the subcutaneous layer. Whatever the vehicle used, the necessary time to reach a steady state was long, ranging from 16 to 18 h massaging. Now that caffeine is a small hydrophilic molecule. So water in oil emulsion should be considered for cosmetic formulation and preparation. Because caffeine permeate into the blood stream less than oil in water emulsion. a hydrophilic molecule. Caffeine exists as higher aggregates in water

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