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Moisturizing effect of Gamma-aminobutyric acid for cosmetic application

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Introduction

Human skin composes of several cell layers containing different degree of water content. The hydration of skin is essential for barrier homeostasis, desquamation and skin plasticity.[1] It is a result of natural moisturizing factors (NMFs) which are composed principally of 40% free amino acids, various derivatives of these amino acids, inorganic salts, and sugars.[2]

Moisturizers are a group of cosmetic products for improving dry skin or daily maintenance of normal skin.[3] The main functions of moisturizers are reducing trans-epidermal water loss (TEWL), attracting water to the stratum corneum, and enhancing the overall barrier function.[4] The moisturizing products can typically enhance skin hydration via three main properties: (1) Occlusivity, which physically block or reduce TEWL; (2) Emolliency, which plays a role in the skin water retention and provides the skin smooth and supple; (3) Humectancy, which are small molecule with hygroscopic property that actively withhold water and hydrate the stratum corneum. Their function is to restore the skin's ability to attract, hold, and redistribute water.[4]

Gamma-aminobutyric acid (GABA) is a non-protein free amino acid and is one of the major inhibitory neurotransmitters in the central nervous system. It is produced from glutamic acid by the action of glutamic acid decarboxylase (GAD).[5] Naturally, GABA could be found in rice, rambutan, tea leaves[6,7] and produced by microorganism such as *Lactobacillus* sp., *Rhizopus* sp.[8] GABA provides several physiological properties and has been used in medical applications to reduce anxiety[9], low density lipoprotein[11], and blood-pressure[12], to increase growth hormone secretion[10], and to prevent effect of Alzheimer's disease[13]. In addition, GABA has been reported to stimulate collagen synthesis[14] and hyaluronic acid production in human dermal fibroblasts, and to possess anti-oxidative stress[15] and wound healing properties.[16]

Since GABA contains various benefits with possible ability to retain water due to its small molecule, it might offer moisturizing property and could be applied in cosmetic product. In the present study, GABA was, thus, evaluated for water retention ability and stable topical product containing GABA was developed. The data and formulation development will provide beneficial guideline for further cosmetic uses.

Methods

Preparation of newborn pig skin

The naturally dead newborn porcines were obtained from Soonthorn farm, Nakhon Pathom. They were cleaned to remove dried blood and dirt and abdominal skin was separated. The subcutaneous fat and muscle layers were surgically excised. The prepared skin sheet was wrapped with aluminium foil and stored in freezer maintain at -20°C until furthered used. Prior each experiment, the skin was allowed to thaw at room temperature and was cut into 6.45 centimeter square.

Water desorption and water sorption test

The water desorption and water sorption of GABA was examined on newborn porcine skin with some modification.[17] Briefly, 6.45 centimeter square newborn porcine skin was soaked in the GABA test solution and excess test solution was removed. The treated skin was weighed and placed in the controlled chamber maintained at 25°C and 43% relative humidity (%RH). The remaining weight of treated skin was recorded at pre-determined time interval to obtain the water desorption phase. The treated skin was then transferred to the controlled chamber maintained at 25°C and 79%RH and was weighed at pre-determined time interval to obtain the water sorption phase. The experiment was repeated using DI water to serve as a control. The water desorption and water sorption profiles were plotted between water content (%) and time (minutes) considering the post-soaking value to be equal to 100 percent.

Development of GABA creams

Formulation of cream base:

Effect of stiffening agent*:*

The oil in water emulsion (cream) was developed. The oil phase contained glyceryl monostearate self emulsifying, capric caprylic triglyceride, jojoba oil, cyclomethicone, steareth-21 and steareth-2. Cetyl alcohol and/or stearyl alcohol was varied and included as stiffening agent(s) in the formulation. The water phase included propylene glycol, carbomer 940, EDTA, methylparaben, propylparaben and water. The cream base was prepared by beaker method.

Effect of emulsifier

The effect of emulsifier concentration was evaluated on the cream base selected from previous section. Combination of steareth-21 and steareth-2 were varied at 3, 4 and 5%w/w to serve as emulsifer3, 4, and 5, respectively. The prepared formulations were subjected to 6 cycles of heating-cooling condition (45°C for 48 hours alteration with 4°C for 48 hours for 1 cycle). The physicochemical properties including appearance, pH, viscosity and skin feel, were evaluated. The most stable formulation with good skin feel was then selected to prepare the GABA cream.

Formulation of GABA cream

GABA cream was prepared by incorporation of GABA in the water phase of the selected cream base to obtain the final concentrations of 8 and 10 %w/w. Stability of GABA creams was investigated through 6 cycles of heating-cooling condition. The changes in physicochemical properties, appearance, pH, viscosity and skin feel were monitored.

Statistical Analysis:

The experimental results are expressed as means ± SD. Data were analyzed using an analysis of variance (ANOVA). P-value of lower than 0.05 was considered to represent statistically significant difference.

Results and Discussion

Water desorption and water sorption test

The water content of treated skin being kept at 43%RH and 25°C was recorded at different time interval and the resulting water desorption phase is presented (Figure 1.). As the treated skins were stored in controlled dried condition, water evaporated and the remaining water content (%) decreased with time. Skin treated with 5 and 10% w/w GABA solution exhibited better ability to retain water compared to 1% w/w GABA and control. However, no significant difference was observed. By transferring the treated skin into more humid condition of 75% RH, the water content within the skin increased with time as the skin absorbed water from environment (Figure 2). The highest water sorption property was detected in the skin treated with 10% w/w GABA followed by 5 and 1% w/w GABA. Significant differences in skin water content compared to the control were exhibited at 60 and 240 minutes post storage of skin treated with 10 and 5% w/w GABA, respectively. No difference was observed between 1% w/w GABA and control samples. Since GABA is a small free amino acid molecule, it possibly possesses the humectancy. The result of both experiments revealed the effects of water desorption and water sorption which are related to the water retention and hygroscopic properties, respectively. Both effects are concentration dependent. However, it is obviously that its hygroscopic effect is the predominant factor.

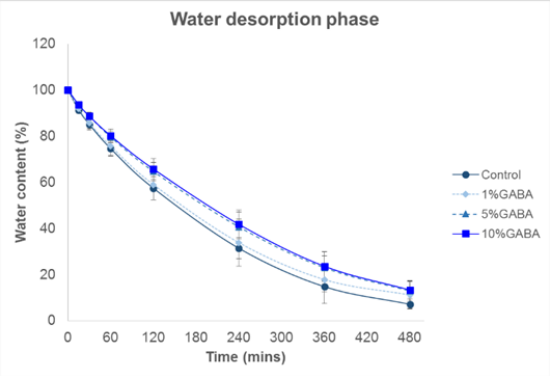

Figure 1. The water desorption test showing water content (%) of treated skin kept at 43%RH, 25°C for 8 hrs. Data are expressed as mean±standard deviation; *statistical difference with $p<0.05$; (n=3)

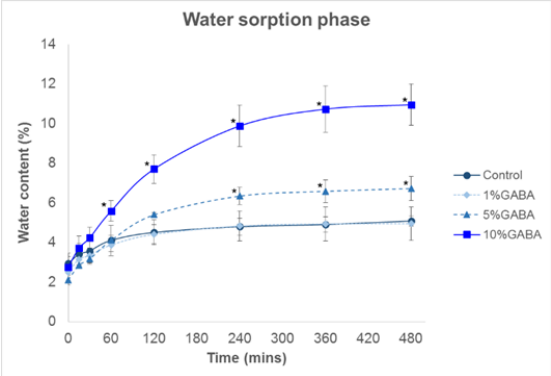

Figure 2. The water sorption test showing water content (%) of treated skin after transferring from the condition of 43%RH to 79%RH. Data are expressed as mean±standard deviation; *statistical difference with $p<0.05$; (n=3)

Development of GABA creams

Formulation of cream base:

Effect of stiffening agent: Formulations of cream base were prepared by varying stiffening agents and were evaluated for appearance and skin feel. The result revealed slight differences in viscosity, transparency, firmness, thickness and stickiness. The formulation containing cetyl alcohol as a stiffening agent provided the least in viscosity, turbidity, firmness, thickness and stickiness. Increase in stearyl alcohol in the combination yielded thicker and stiffer cream base which is due to longer hydrocarbon chain length compared to cetyl alcohol. Cream base containing 3%w/w cetyl alcohol which provided the best skin feel was, therefore, selected for further development.

Effect of emulsifier: The selected cream base was further developed by varying emulsifier concentration and their stabilities were evaluated. All cream bases were white in color with pH in the range of 6.51-6.59. Increase in emulsifier concentration resulted in the higher viscosity since the emulsifiers were in solid state. Following 6 cycles of heating-cooling condition, their physicochemical properties were evaluated and the result is presented in Table 1. No phase separation was detected. All tested formulations were stable with physicochemical properties comparable to their initial condition. Emulsifier at 3% w/w was sufficient to emulsify the system of 13% w/w oil phase. However, the formulation with 4% w/w emulsifier was selected for further preparation of GABA cream to ensure the stable product (Figure 4(a)).

Table 1. Effect of emulsifier concentration on the physiochemical properties and stability of cream base

Formulation of cream	Initial			After 6 cycles of heating-cooling condition		
	Color	pH	Viscosity (cPas)	Color	pH	Viscosity (cPas)
Emulsifier 3	White	6.54 ± 0.03	55802 ± 2972	White	6.56 ± 0.05	55769 ± 2903
Emulsifier 4	White	6.53 ± 0.02	61765 ± 2118	White	6.57 ± 0.02	62093 ± 2756
Emulsifier 5	White	6.56 ± 0.03	135785 ± 10075	White	6.57 ± 0.05	136375 ± 1718

Figure 3. Appearance comparison of (a) Cream base (b) 5%w/w GABA cream and (c) 10%w/w GABA cream.

Formulation of GABA cream

GABA creams containing GABA at the concentration of 8 and 10% w/w were prepared and physical appearance are presented in Figure 3(b) and 3(c), respectively. Both GABA creams were in light yellow color and more viscosity than cream base. The changes in viscosity and color were, therefore, contributed by the addition of GABA. After 6 cycles of heating-cooling stability test, the physicochemical properties including appearance, pH and viscosity were not changed (Table 2). They were stable with comparable properties to their initial condition.

Table 2. Physiochemical properties and stability of cream containing 8 and 10% w/w GABA.

Cream	Initial			After 6 cycles of heating-cooling condition		
	Color	pH	Viscosity (cPas)	Color	pH	Viscosity (cPas)
Cream base	White	6.53 ± 0.02	61765 ± 2118	White	6.57 ± 0.02	62093 ± 2756
8% w/w GABA	Light Yellow	6.51 ± 0.04	164358 ± 10622	Light Yellow	6.51 ± 0.05	164816 ± 8281
10% w/w GABA	Light Yellow	6.53 ± 0.05	190505 ± 3751	Light Yellow	6.53 ± 0.06	190112 ± 5473

Conclusions

The water desorption and water sorption tests revealed the water holding capacity and hygroscopic properties, respectively, of GABA. It is, therefore, suspected to offer the moisturizing effect which is dependent on concentration. Stable creams containing 8 and 10% w/w GABA were successfully developed. Such formulations might be used as alternative moisturizers.

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