



# Skin Irritation Test of Curcuminoids Facial Mask Containing Chitosan As a Binder

Srisombat Nawanopparatsakul et al.\*

## Abstract

Curcuminoid facial mask containing chitosan as a binder and other substances was developed. Curcuminoids have been reported to cause skin irritation therefore this study was conducted to explore possible toxicity of this facial mask with an acute skin irritation test. Irritation scores of various concentrations of curcuminoid extracts (0.25%, 0.50%, 0.75%, and 1.00%) from Draize technique using rabbit model revealed that there was no significant toxicity / irritability as compared to the plain facial mask. The facial mask containing curcuminoid extracts up to 1.00% did not potentially cause significant skin irritation in acute irritation test using Draize technique in rabbit model and in healthy volunteers and the curcuminoid extracts greater than 20 mg did not provide an acute skin irritation.

**Keywords :** Curcuminoids, chitosan, facial mask, skin irritation test, Draize test

---

\* Jeeratikorn Euasathien, Chuwit Eamtawecharum, Porntip Benjasirimingkol, Sakdanai Soiputtan, Photchanart Toprasri, Thawatchai Phaechamud and Srisombat Nawanopparatsakul

## Introduction

Chitosan is a biodegradable, biocompatible and mucoadhesive biopolymer. It has been notably recommended as an appropriate material for many purposes in pharmaceutical, medical, cosmetic and food industries (Chenite et al., 2003). For skin care preparation, chitosan may be employed as an alternative to collagen or hyaluronic acid (Dee et al., 2001). Furthermore, the use of chitosan in cosmetic formulations can improve their dermatological compatibility and sensorial properties. Because of its hydrophilic and retaining-water-structure property, this natural polymer can form a viscous gel at low pH (He et al., 1998). In face mask preparations, especially a hydrophilic system, chitosan could use as a binder to form water-washable protective paste with kaolin and other substances (Toprasri et al., 2003). The facial mask containing chitosan, lactic acid and curcuminoids had been developed and curcuminoids releasing from this facial mask had been evaluated (Toprasri et al., 2003). Curcuminoids are the extracts from *Curcuma longa* Linn. that have an action of anti-inflammatory, antioxidant, antimicrobial and wound healing effects (Gritsanapan et al., 2003). Curcuminoids had been reported to reduce skin irritation (Pitakvongsaporn et al., 2003) while many chemicals had been reported to have skin irritation which triggered by surfactants. Skin irritation is one of the most common adverse effects in humans depended on many factors, including the concentration, duration and frequency of exposure, exposed skin site, rate of penetration and intrinsic toxic potential of the substance. The aim of this study was to evaluate acute skin irritation of facial mask containing curcuminoids extracts at different time intervals and different the concentrations of curcuminoids extracts in the preparation.

## Materials and methods

### *Materials*

Chitosan having M.W. 40,000 from chitin of crab shell was purchased from G.T. Chemicals Co., Bangkok, Thailand. The N-deacetylation degree was  $85.91 \pm 0.75$  %, determined by colloidal titration method. Curcuminoid extracts (calculated as 70% curcumin) were prepared from turmeric rhizome. Lactic acid and other reagents were of analytical or food grade. Honey was provided by Vejpong marketing Co., Ltd. (Thailand). Cremophore RH 40 and micronized titanium dioxide were obtained from Numsaing Co., Ltd. All other chemicals were of reagent grade.

### *Preparations of facial masks*

Facial mask was prepared by using chitosan as a binder. Briefly, chitosan of 5 g was dispersed in a 3.08% w/v lactic acid solution. The mixture was stirred vigorously without heating until chitosan dissolved. Then the solution was filtered to separate the non-soluble accompanying fibers. Curcuminoids were dispersed in a solution of lactic acid, cremophore RH 40, chitosan solution and other liquid materials. Facial mask components were incorporated by a geometric tritulation technique between kaolin, titanium dioxide and liquid phase. The concentration of curcuminoid in facial mask were 0.25, 0.50, 0.70, and 1.00%. The negative control is the plain facial mask base without curcuminoid.

### *Draize test (Kirwin C.J., 1984)*

The method of Draize was selected to observe the presence of erythema and edema on the test sites. Grading of the severity of erythema and edema formation was also investigated after 24 and 72 hours.

### ***Experimental Animals***

This study employed eighteen male rabbits (New Zealand White, 1.2-2.5 kg) to test for the skin irritation. They were kept carefully following an acclimation period of 7 days to ensure their suitability for the study. Test animals were kept within a limited-access rodent facility with environmental conditions set to a temperature of  $25 \pm 2$  °C, a humidity of 60-90% RH and a 12-h light / 12-h dark cycle. Animals were provided ad libitum access to a commercial rabbit-diet and drinking water was supplied to each cage.

The area on the back of each rabbits was shaved prior to the experiment. The back was divided into six marked areas for the topical application of the facial mask containing various concentrations of curcuminoids extracts. The 0.5 g of each test product was placed on each area for 30 minutes and another group for 6 hours, and then was washed off by tap water. Scoring of the erythema and edema was performed at 24 and 72 hours with Draize technique and skin pigment was measured using a skin pigment analyzer (Courage & Khazaka electronic GmbH, Germany).

To investigate the effect of concentration of curcuminoids extracts, the aboved mentioned method was also used. The test products, the curcuminoids extract, was dissolved in absolute ethanol and then applied for 1 hour before washing off by tap water. The positive control of this experiment is 98% lactic acid and the negative control is the plain facial mask without curcuminoid for testing of facial mask and absolute ethanol for testing of curcuminoid extract.

### ***Skin Irritation Testing in Human Volunteer***

Six healthy volunteers (aged from 23-40 years) were participated in this study. The participants were briefed on the study procedures, and each was given written informed consent. Each facial mask was applied once, at a dose of 0.5 g on a surface area of 1 inch<sup>2</sup> on lateral arms. The test specimen was thereafter washed

off by tap water after 6 hrs. Subsequently, scoring by Draize technique and skin pigment analyzer was conducted.

Data from the skin irritation test was analyzed with the t' test calculation of primary irritation index, PII.

$$\text{Average Scores} = \sum \frac{\text{Erythema grade at 24,72 hrs} + \sum \text{Edema grade at 24,72 hrs}}{\text{number of subjects}}$$

Variable factor = types of skin x time of reading

PII = Average scores x variable factors

## Results

The calculated PIIs were 0.00 at 30 minutes for skin irritation test of facial mask containing various concentration of curcuminoids extracts. This result indicated that there was no irritation in all concentrations (0.25%, 0.50%, 0.75% and 1.00% curcuminoids in facial mask) in 6 rabbits compared to positive control (98% lactic acid, PII was 1.42, mild irritant, data was not shown). The facial mask was left for 6 hours in another groups of animals (in case of the antioxidant activity from curcuminoids was also needed). The similar results (no irritation) were obtained from all concentrations (PII = 0.2 in all groups, 98% lactic acid was 3.3, data was not shown). There was no irritation in normal volunteers at all concentrations used. The data from skin pigment analyzer revealed that there was no significant different result ( $\alpha=0.05$ ) before and after applied the facial mask in rabbits and in normal volunteers.

From skin irritation test by Draize technique, there was no skin irritation (PII of lactic acid was 3.6) of curcuminoid extracts (2.5, 5.0, 10, 20 mg) which were applied for 1 hour in rabbits and in normal volunteers. Additionally, significant difference was not found before and after application of the facial mask in rabbits and normal volunteers after measurement with skin pigment analyzer.

**Table 1** PII of facial mask containing curcuminoid extracts at various concentration

Curcuminoids in facial mask (%)	PII		
	Rabbit		Human
	30 mins	6 hours	6 hours
0.25	0	0.2	0
0.50	0	0.2	0
0.75	0	0.2	0
1.00	0	0.2	0

**Table 2** PII of curcuminoid extracts at various amount

Curcuminoid extracts (mg)	PII	
	rabbit	human
	1 hour	1 hour
2.5	0	0
5.0	0	0
10.0	0	0
20.0	0	0

## Discussion

The facial mask containing lactic acid (0.5%), chitosan and curcuminoid was evaluated for the acute skin irritation test by Draize test and skin pigment analyzer. There was no skin irritation from facial mask after application for 30 minutes at the first time in rabbits. The facial mask was additionally applied for 6 hours. Since this facial mask is applied on skin for longer time for antioxidant activity from curcuminoids, the effect on skin irritation should be further conducted. The results indicated that there was no irritation detected with these methods (Draize test and skin pigment analyzer) and in two species (rabbit and human). It could be concluded that the facial mask containing up to 1.00% of curcuminoid extracts did not induce an acute skin irritation. Additionally, the

concentration of curcuminoid extracts greater than 20 mg did not provide an acute skin irritation in 2 species that were measured with these two methods.

The sensitivity of the Draize test was particularly good, as proved by the scores of positive and negative control. The Draize test, however, has many disadvantages including the subjective nature of the visual rating system, arguable extrapolation to humans, its high cost and time consumption. Thus, this study attempted to standardize the visual rating system and the Draize test was used as primary screening method of acute skin irritation test. From this study, the score from Draize test was also corresponded to the score from skin pigment analyzer.

## References

- Chenite, A. et al. (2001) Rheological characteristic of thermogelling chitosan/glycerol-phosphate solutions. *Carbohydrate polymers*, 46 : 39-47.
- Dee, G. J. et al. (2001) Chitosan-multifunctional marine polymer. *Allured's Cosmetics and Toiletries magazine*, 116(2) : 39-44.
- Gritsanapan, W. et al. (2003) Antibacterial and antifungal activity of curcuminoid from *Curcuma longa* grown in Thailand (NO.55). [Abstract] Available : <http://www.mahidol.ac.th/abstract/annual/2000/0184.htm>.
- He, P. et al. (1998) *In vitro* evaluation of the mucoadhesive properties of chitosan microspheres. *International Journal of Pharmaceutics*, 145 : 231-240.
- Kirwin C.J. (1984) *Eye and skin local toxicity testing In Toxicology : Principles and practice Vol 2 edited by Sperling F. a Wiley-Interscience publication*, pp. 169-175.
- Pitakvongsaporn, P. et al. (2003) Human skin irritation study of turmeric cream. [abstract]. Available : <http://www.mahidol.ac.th/abstract/annual/2000/0185.htm>
- Toprasri, P., et al. (2003) The physical properties and release of curcuminoids from chitosan facial mask. *The 8<sup>th</sup> Pacific Polymer Conference (PPC8)*. Queen Sirikit National Convention Center, Bangkok, Thailand, November 24-27.