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# STORAGE STABILITY AND SAFETY OF ACTIVE VITAMIN C IN A NEW DUAL-CHAMBER DISPENSER

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## Summary

As it is known vitamin C is so reactive and unstable in a fully formulated cosmetic composition that is extremely difficult, if not impossible, to maintain it active and stable in a cosmetic formulation and, of course, on the skin.
Moreover, l-ascorbic acid is one of the major antioxidant in skin and allows us to exist in an oxygen rich environment in which reactive oxygen species (ROS) are regularly generated by exposure to ultraviolet light, pollution and inflammation.
The aim of the study was to control the stability of a high concentration of l-ascorbic acid used in a normal cosmetic formulation utilizing a new two-chamber dispenser which allows to combine the cosmetic lotion pump with an air-free pump connected to the cartridge - vit. C contained in one housing. Both are simultaneously operated by a single actuator only.
L-ascorbic acid remains stable in the cartridge at 40° C, decreasing its activity of about 20% at 45°C after a storage period of 30 weeks.
Moreover, it inhibits the melanin biosynthesis of about 95% for vitamin C concentrations of 500 mmol/l, demonstrating an interesting depigmenting activity "in vitro".

## Riassunto

Come è noto la vitamina C è così reattiva ed instabile che risulta estremamente difficile, se non impossibile, mantenerla attiva, quando venga inserita in un prodotto cosmetico.
Comunque, l'acido l-ascorbico (vit. C) rappresenta il maggior antiossidante del nostro organismo che ci permette di vivere in un ambiente dove i radicali liberi dell'ossigeno (ROS) sono continuamente prodotti dai raggi del sole, dagli inquinanti ambientali e dai processi infiammatori.
Scopo di questo studio è di controllare la stabilità di un'alta concentrazione di acido l-ascorbico inserito in una normale formulazione cosmetica, utilizzando un nuovo contenitore che permette di mantenerlo separato dal resto della formulazione cosmetica e lontano da qualsiasi fonte di ossidazione.
All'atto dell'erogazione l'acido l-ascorbico ed il resto del prodotto vengono mescolati direttamente a

livello cutaneo.
Su colture di melanociti ha rivelato di possedere un'attivtà inibitoria del 95% sulla sintesi della melanina.
L'acido l-ascorbico utilizzato si è rivelato stabile al 95% dopo un periodo di 30 settimane a 40° C ed ha rivelato di possedere un'attività inibitoria del 95% sulla biosintesi della melanina prodotta da colture di melanociti.

## INTRODUCTION

In the last years the interest concerning the use of natural compounds, such as antioxidants for the skin photoprotection grew very much. That's happened also because we became more conscious about the damage sun-rays can provoke at cutaneous level (1-2). It is well known that reactive oxygen species (ROS) are normally generated by exposure to ultraviolet light, pollution, and inflammation, and contribute to mutations which result in skin cancer (4-5).
One of the best known anti-oxidant is vitamin C which has been shown to be effective in photodamage (6-7).
Moreover, as it is known, vitamin C is so reactive and unstable in a fully formulated cosmetic composition, and it is extremely difficult, if not impossible, to maintain it active and stable, both in its formulation and at skin's level.
Derivatives of vitamin C, such as ascorbyl palmitate or magnesium ascorbyl phosphate, to be effective, has to pass through the skin barrier and to be converted to l-ascorbic acid. Moreover, magnesium ascorbyl phosphate is poorly absorbed by the skin and the human skin fibroblasts appear not to be able to convert ascorbyl palmitate readily to l-ascorbic acid (8).

## AIMS

The aim of the study was to control the stability, the safety, and the activity of an high concentration of l-ascorbic acid, included in a normal cosmetic formulation utilizing a new dual-chamber dispenser that allows to split the studied formulation and to hold the active and less reactive ingredients separately.

## MATERIAL AND METHODS

The two-chamber dispenser (SYMBIO®) combines a cosmetic bottom pump and an air-free lotion pump in one housing. Both are simultaneously operated by a single actuator only.

The smaller chamber is a cartridge with a plunger in the lotion which rises during its usage, thus keeping air-free content of vitamin C or other instable active ingredients.
The second chamber is a traditional container which is connected to its own pump (fig. 1). Each pump stroke delivers 0.4 grams of formulation with a ratio of 1 :11 (cartridge - container).

## COSMETIC PREPARATION

To control the storage stability of vitamin C, it has been used a cosmetic preparation containing (w/w) :

| | |
|---|---|
| Sodium Ascorbate | 56% |
| Water | 20% |
| Glycerol | 20% |
| Beta carotene (colour) | < 0.1% |
| Hydrocolloid | < 0.1% |

Of this composition it was controlled the relative concentration of vitamin C, at two different temperature (40° and 45°C) during a period of 30 weeks using the colorimetric L-ascorbic acid test of tetrazolium salt methodology (9-10). The obtained results are reported in figure 2.

## EFFICACY

Inhibition of melanin biosynthesis was tested in Bib melanoma cells, according to the method described by K. Tomita et al, respectively with 5 mg/l of arbutin (preparation B), with 25 mg/l of linoleic acid (preparation C) or with a third preparation of vitamin C phosphate (11).
The efficacy of vitamin C alone was compared with combinations of vitamin C with 5 mg/L-arbutin.

## MELANIN SYNTHESIS INHIBITORY ACTIVITY TEST

B16-F1 melanoma cells are suspended in a monolayer in Eagle's minimum essential medium

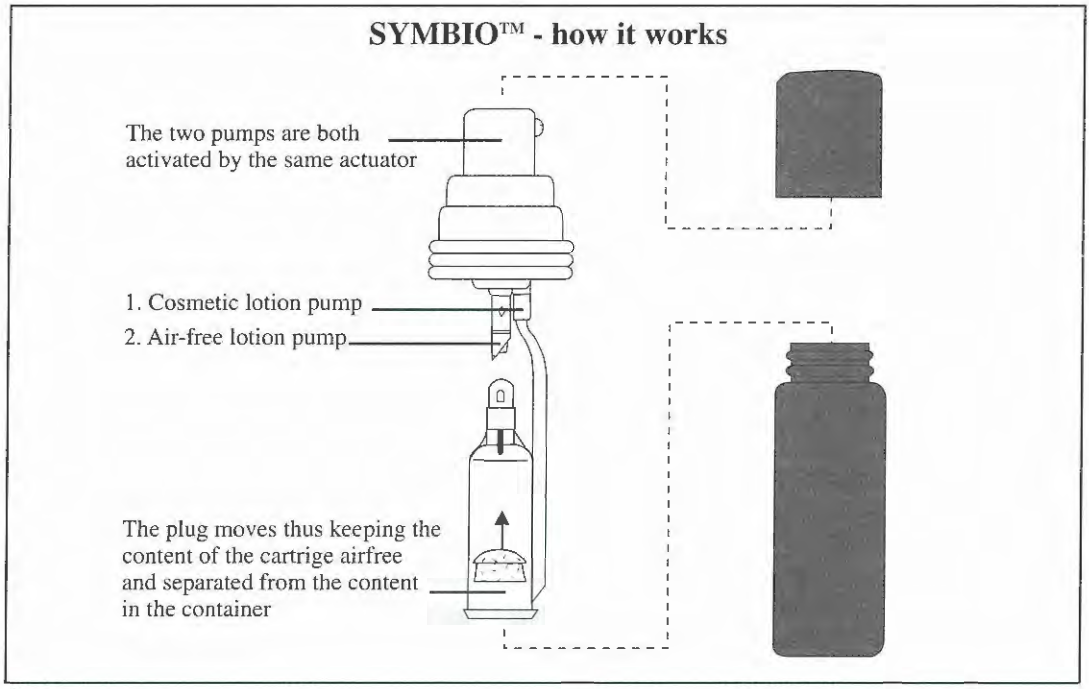
SYMBIO™ - how it works
The two pumps are both activated by the same actuator
1. Cosmetic lotion pump
2. Air-free lotion pump
The plug moves thus keeping the content of the cartrige airfree and separated from the content in the container

Fig.1

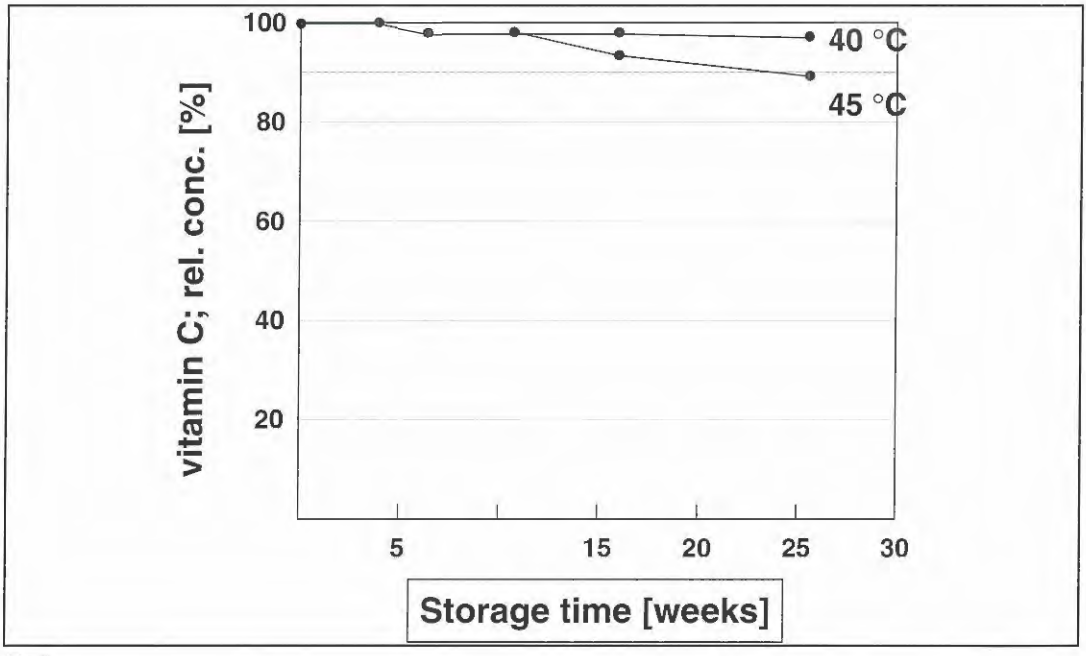
100
80
60
40
20
vitamin C; rel. conc. [%]
40 °C
45 °C
5
15
20
25
30
Storage time [weeks]

Fig.2

(MEM) containing 10% fetal calf serum (FCS) and 0,1% glucosamine hydrochloride at 5 ˜ 103 cells/ml.

One ml of the cells suspension is transferred in each well of a 6-well culture plate (350 mm i.d., Sarstedt) and incubated at 37°C in a 5% CO2 - 95% air atmosphere.

After 5 days of incubation, cells are washed twice with phosphate buffered saline (PBS) and renewed with fresh medium containing 10-6 M a-malanocyte stimulating hormone (a-MSH) or 5 mg/ml rolipram and different concentrations of the compounds to be tested.

After 3 days of incubation the adherent cells are washed with PBS (1ml) and scraped with a cell scraper. The cell suspension is centrifuged at 2000 rpm for 10 minutes. The colour and volume of the pellet are compared with those of controls.

One ml of 1 N NaOH is added to the pellet and vortexed vigorously. After incubation at room temperature over night, melanin concentration is determined by measurement of OD475 and compared with a standard curve obtained using synthetic melanin (Sigma).

The obtained results are reported in fig. 3 and fig.4.

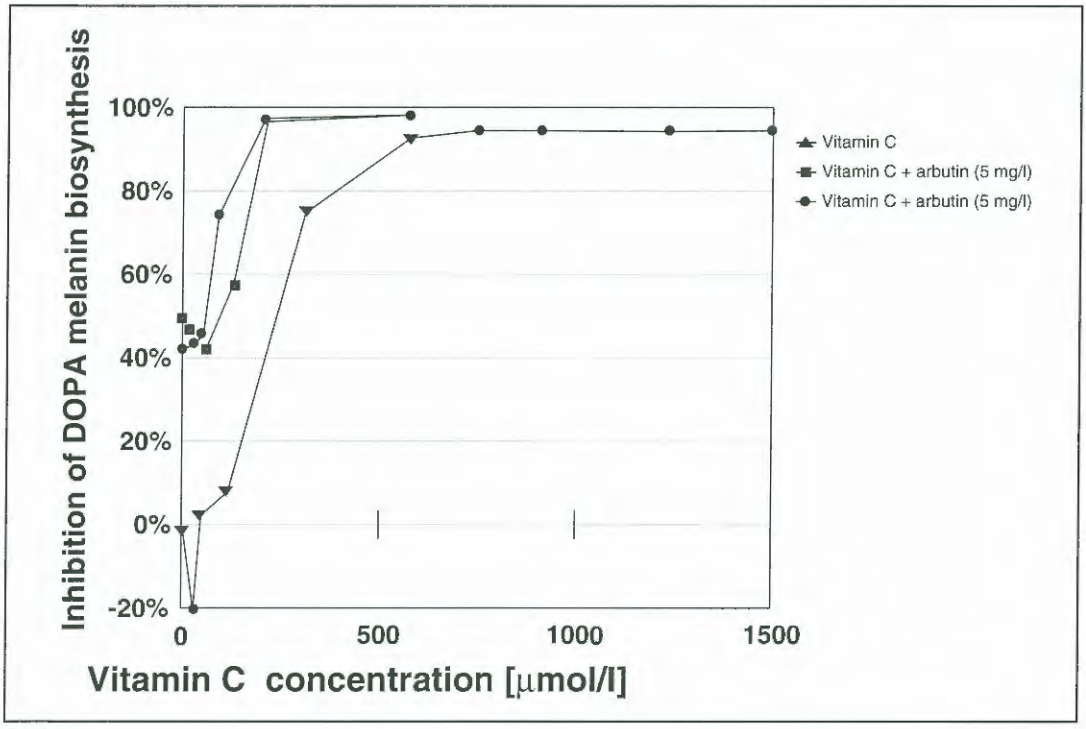


*Fig.3*

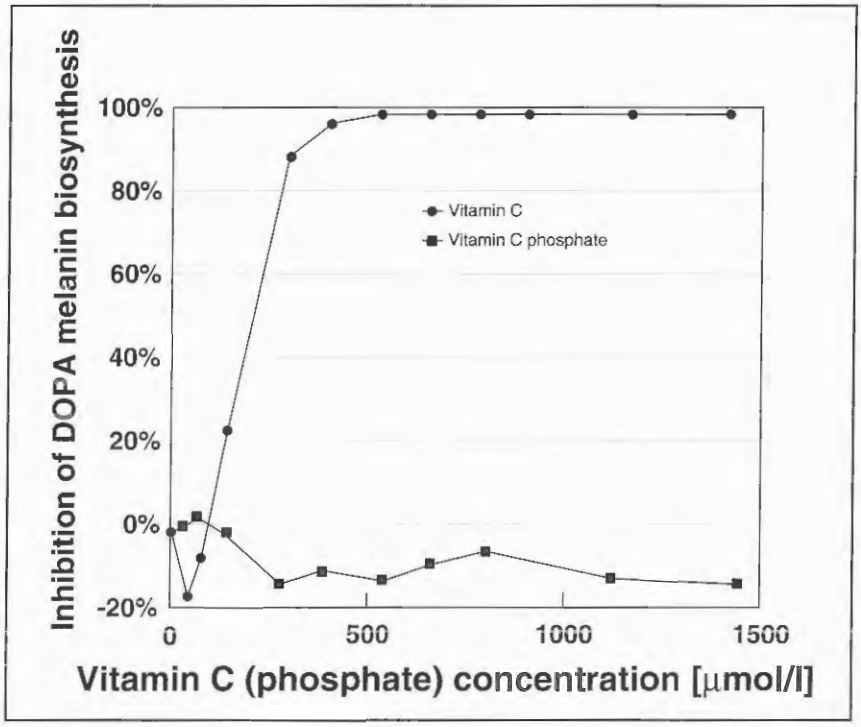


*Fig.4*

## SAFETY CONTROL

### *Clinical evaluation*

The irritancy of the used formulation was controlled on 26 female volunteers, age-range 38-62, by the Duhring chamber-test methodology, according to Frosh and Kligman (12).
20 mg of the product was applied occlusively by the Duhring chamber and occlusive tape to the back of the volunteers for 24h. on the first day, and 6h. on the 4 following days, with scoring on the 8 days by an expert dermatologist.
The product did not lead to any unwanted cutaneous reaction, demonstrating its complete local safety.

## RESULTS AND COMMENTS

How it is possible to see from figure 2, vitamin C remains stable in the cartridge for the first 10 weeks of storage. From the 15th week until the 25th , it remains practically unchanged the sample maintained at 40 ° C, exhibiting an unrivalled shelf stability of 95%, meanwhile at 45°C it can be registered a decreasing activity of about 20%.
Observed in its complex, the product seems to remain stable and active for 1 year at least, loosing a very small amount of its own activity even in not very good conditions.
What is really interesting to observe is the remarkable depigmentative activity showed "in vitro" (fig.3).
As a matter of fact, it was possible to obtain a melanin biosynthesis inhibition of about 95% for vitamin C concentrations of 500 mmol/l , whether used as it is or added with arbutin in quantity of 5mg/l or with linoleic acid (25mg/l).

The increase in concentration of 1000/1500 nmol/l does not raise its activity that resulted practically unchanged.
Even more interesting is vitamin C activity if compared to the vitamin C phosphate one (fig.4).
As a matter of fact, while vitamin C demonstrated to have an inhibitory melanin biosynthesis activity at 100%, in concentration of 500 mmol/l or of 1000/1500 mmol/l, it was not be possible to obtain the same result with vitamin C phosphate, which is active neither in concentration of 500mmol/l nor at 1500mmol/l.
Moreover, the used composition demonstrated itself to be safe in the dermatological usage, showing no irritative side effect according to Kligman and Frosh method.
Since interesting results have been obtained "in vitro" on the inhibition of melanin biosynthesis through the vitamin C usage, and since the product, thanks to the use of this new Symbio container, has showed a high storage stability, the studies to evaluate, also "in vivo", the depigmentative activity are going on.

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