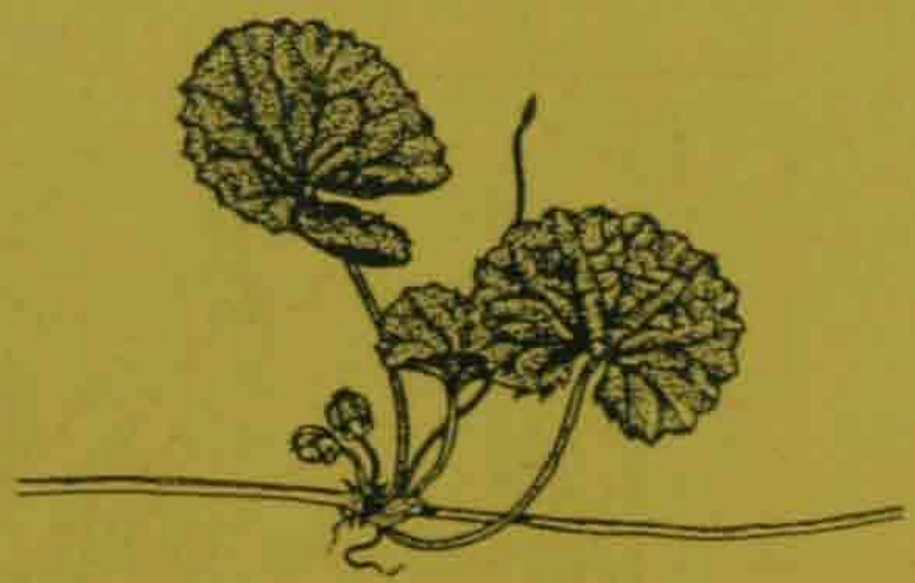
สกว

รายงานวิจัยฉบับสมบูรณ์

การเตรียมและประเมินไมโครสเฟียร์ที่ประกอบด้วยสารสกัดบัวบก

โดย

ขวัญจิต อึ้งโพธิ์ และคณะ



ธันวาคม 2545

CONTROLLED RELEASE OF ASIATICOSIDE AND MADECASSOSIDE FROM CHITOSAN-ALGINATE MICROSPHERES CONTAINING *Centella asiatica* EXTRACT

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ABSTRACT SUMMARY

Chitosan-alginate microspheres containing titrated extract of *Centella asiatica* (TECA) as wound-healing mucoadhesive delivery systems were prepared by complex coacervation/ emulsification technique. The release of asiaticoside and madecassoside, as active markers of TECA, from the microspheres in 0.2% Tween 80 solution at 37 °C over 6 hours was controlled as a function of chitosan-alginate ratios. The slowest liberation was found in 1:1 chitosan-alginate microspheres.

METHODS

Extraction: The dry powder of CA was extracted with petroleum ether, followed by chloroform and finally with ethanol.

Active constituents: The dried ethanol extract of CA was determined by HPLC and LC-MS for its active constituents.

Preparation of microspheres: The chitosan-alginate microspheres were prepared by complex coavervation/ emulsification method as shown in fig. 1.

In vitro release study: Paddle method, 100 rpm, 0.2% Tween 80, 100 ml, at 37 °C.

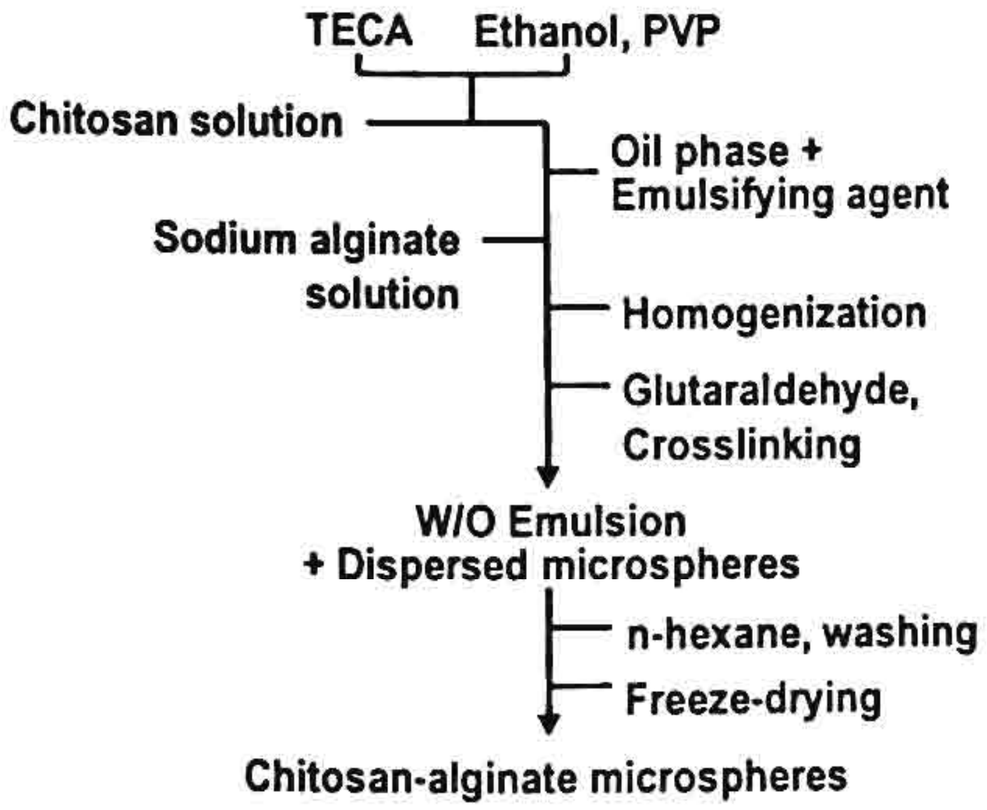

Fig. 1 Preparation of chitosan-alginate microspheres containing TECA

RESULTS

Surface morphology of chitosan-alginate microspheres containing TECA prepared by complex coavervation/ emulsification technique is shown in fig. 2. Asiaticoside and madecassoside contents as markers of TECA were determined. Release of asiaticoside and madecassoside from chitosan-alginate microspheres in 0.2% Tween 80 was controlled over 6 h (fig. 3). Chitosan-alginate ratios strongly affected the liberation of asiaticoside and madecassoside. Faster release was found when chitosan-alginate ratios were 2:1 and 1:2 respectively compared with the slowest release of 1:1 ratio.

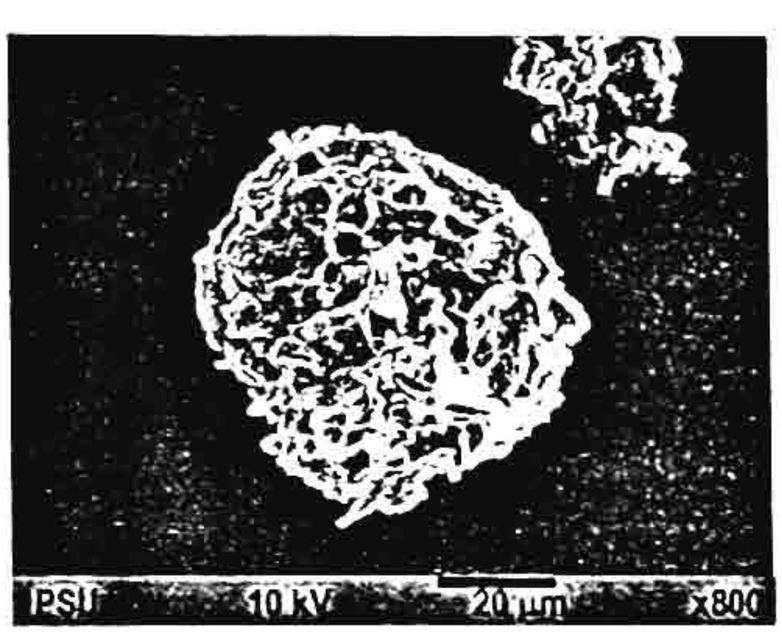

Fig. 2 SEM of chitosan-alginate microspheres containing TECA

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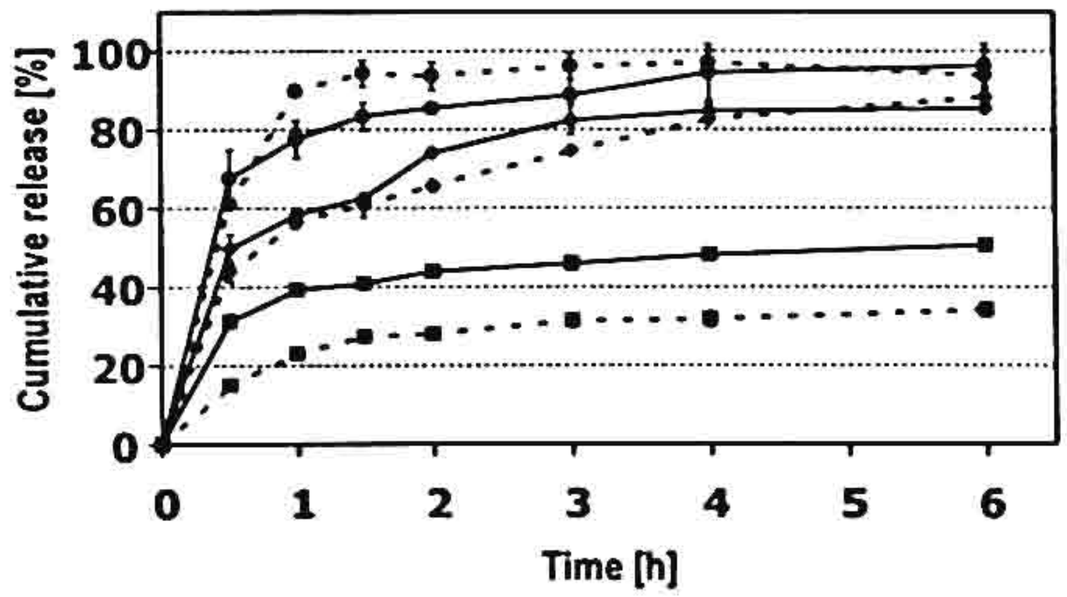

Fig. 3 Release of asiaticoside (heavy lines) and madecassoside (dash lines) from chitosan-alginate microspheres containing TECA in 0.2% Tween 80 solution at 37 °C. (●2:1, ■1:1, ♦1:2 chitosan-alginate microspheres)

CONCLUSION

Encapsulated TECA in porous chitosan-alginate microspheres can be prepared by complex coacervation/ emulsification technique. Sustained liberation of the entrapped TECA from microspheres in 0.2% Tween 80 at over 6 hours was indicated. These chitosan-alginate microspheres may be used as controlled release carriers of TECA in phytoceutical and cosmeceutical preparations.

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2. P.K. Inamdar, et al. J. Chromatograph. A. 742 (1996) 127-130.
3. P. He, et al. Int. J. Pharm. 166 (1998) 75-88.
4. J. W. Lee, et al. J. Pharm. Scl. 89 (2000) 850-866.

TITLE: **PREPARATION OF CHITOSAN-ALGINATE MICROSPHERES CONTAINING *Centella asiatica* EXTRACT**

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[2]*Faculty of Pharmaceutical Sciences, Mahidol University, Bangkok, 10400, Thailand*

Objective: To prepare chitosan-alginate microspheres containing *Centella asiatica* extract by w/o emulsification technique

Methods: The dry powder of *Centella asiatica* (CA) was extracted with petroleum ether, followed by chloroform and finally with ethanol. The dried ethanol extract of CA was determined by HPLC for asiaticoside content. The titrated dose of CA extract (TECA) in ethanol was incorporated into chitosan-alginate microspheres by emulsification technique. Various w/o emulsions with different oil types, oil content, emulsifying agents and amount of TECA were prepared by concomitant dropping the chitosan-TECA mixture and the alginate solution into the oil phase under homogenization. The dispersed microspheres in the w/o emulsions were crosslinked with glutaraldehyde. Then they were separated and washed with *n*-hexane before freeze-dried. The morphology of the microspheres was examined using a scanning electron microscope (SEM). The size and size distribution of microspheres were determined using laser diffraction technique.

Results: The asiaticoside content of the dried ethanol extract of CA was 7.70 $\pm$ 0.07%. The fine chitosan-alginate microspheres with pale straw color were obtained from all emulsion formulations. The microsphere size was varied according to oil types, oil content, emulsifying agents and amount of TECA in the formulas (1, 2). Mineral oil and Span 80 were suitable for the microspheres formation. The resulted microspheres were easily to be washed and under 50 μm in diameter. The SEM determination indicated the porous morphology of the microspheres. This morphology has been reported to have the high efficiency in the incorporation and the controlled release of the entrapped drugs (3).

Conclusion: The porous chitosan-alginate microspheres containing TECA were obtained by using w/o emulsification technique. The emulsion formulations influenced the size of the microspheres.

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1. Gaserod O, Jolliffe IG, Hampson FC, Dettmar PW and Skjak-Braek G (1998) *Int J Pharm* **175**, 237-246.
2. Takeuchi H, Yasuji T, Yamamoto H and Kawashima Y (2000) *Pharm Res* **17**, 94-99.
3. Hildebrand GE and Tack JW (2000) *Int J Pharm* **196**, 173-176.

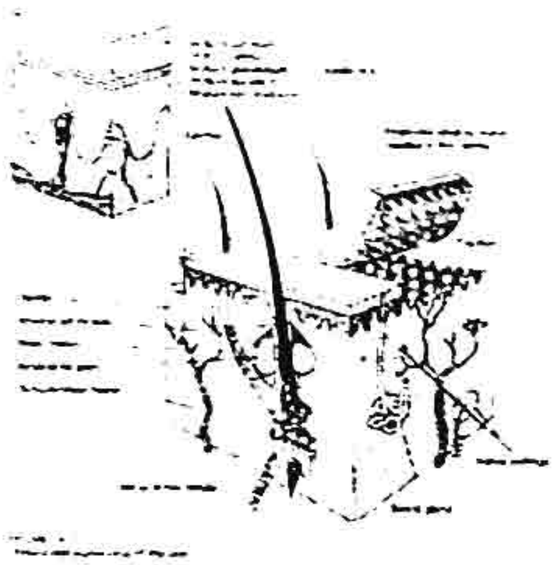
Keywords: chitosan, alginate , microsphere, *Centella asiatica*

Herbal Extracts in Novel Cosmetic Delivery

Kwunchit Oungbho

Faculty of Pharmaceutical Sciences
Prince of Songkla University
Hat Yai, Songkhla

Topical? Transdermal?



Trends in Cosmetics

Pharmaceutics

⋮

Cosmeceutics

↑

Cosmetics

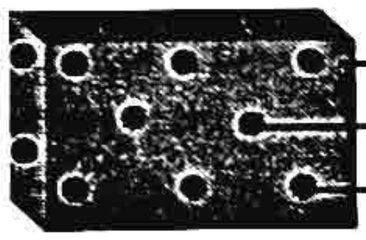
Novel Cosmetic Delivery Systems

- Vesicular ; Liposomes, Niosomes
- Molecular ; Cyclodextrins
- Particulate ;Microcapsules, Microspheres

Advantages of novel cosmetic delivery

- Enhanced skin penetration
- Increased stability
- Decreased toxicity
- Decreased skin irritation
- Intensified activity
- Advertising strategy

Microspheres in Cosmeceutics



Herbal Extracts in Cosmeceutics

- Herbal extracts
- Biopolymers

Le Natural Product Designer

Which one can be the best one?

Biopolymers

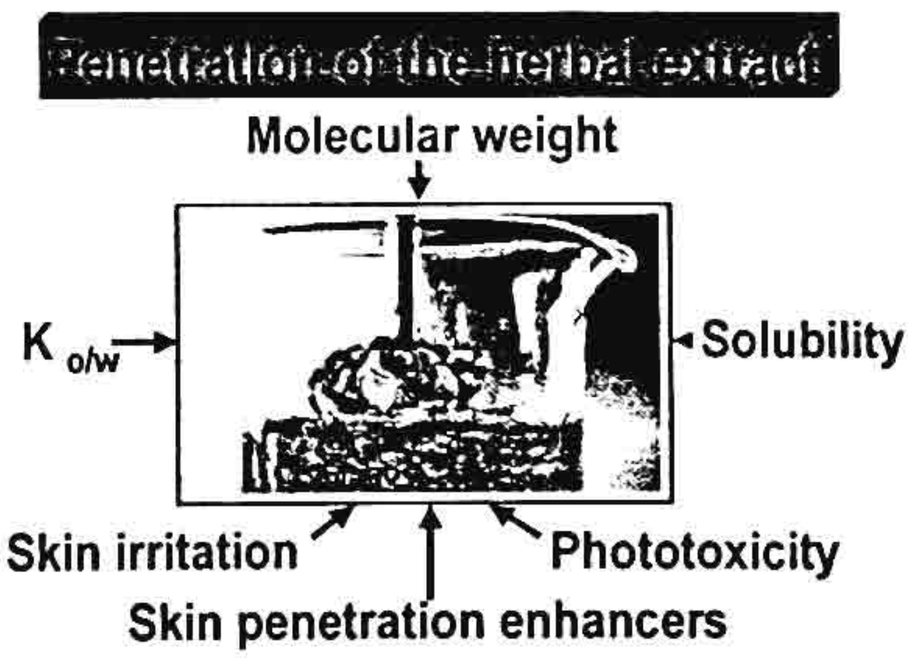
Chitosan ; Deacetylated chitin
Soluble in diluted acid solution
Positively-charged polymer

Alginate ; Sodium salt of alginic acid
Soluble in water
Negatively-charged polymer

How to make the polymers insoluble?

Polyion complexation ; Chitosan-Alginate

Crosslinking ; Glutaraldehyde, Carbodiimide



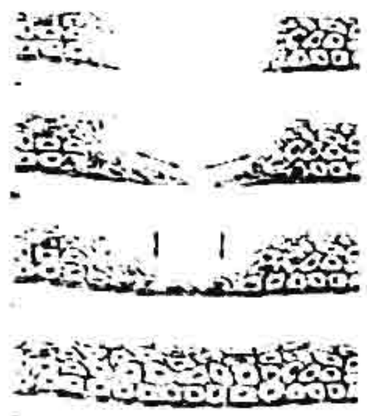
Centella asiatica Linn. urban

Centella asiatica (Umbelliferae)

- Creeping plant
- Polymorphism

Botanical garden
Fac. Pharm. Sci.
Prince of Songkla U., Hat-yai
10.8.00

Biological Activities



Collagen synthesis
Wound healing properties

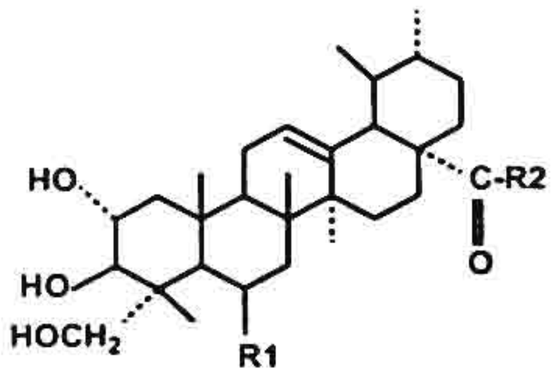
Clinical Applications of *Centella asiatica*

- Systemic scleroderma
- Abnormal scar formation
- Keloids

Cosmetic Applications of *Centella asiatica*

- Anti-wrinkles, anti-aging
- Sunburn healing
- Healing wounds
- Firming and tensing
- Dark under-eye circles

Centella asiatica Purified Triterpenes



R_1 = H; R_2 = OH : Asiatic acid
R_1 = OH; R_2 = OH : Madecassic acid
R_1 = H; R_2 = glu-glu-rhamn : Asiaticoside
R_1 = OH; R_2 = glu-glu-rhamn : Madecassoside

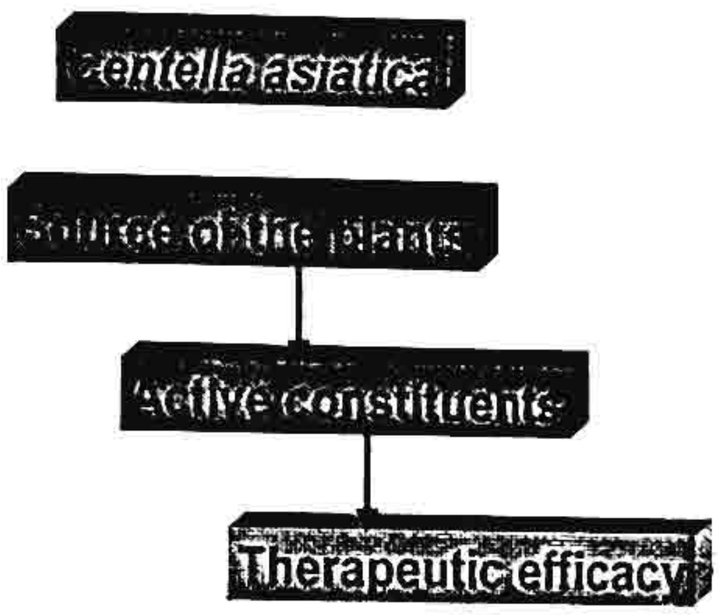
Solubility of *Centella asiatica* Extracts in Cosmetic Solvents

Extract	Solubility				Use level
	Ethanol	Water	Glycerol	Butylene glycol	
Asiatic acid	Sparingly soluble	Very slightly soluble	Slightly soluble	Sparingly soluble	0.03-0.05%
Asiaticoside	Sparingly soluble	Very slightly soluble	Practically insoluble	Sparingly soluble	0.05-0.1%
Titrated extract of *Centella asiatica*	Sparingly soluble	Very slightly soluble	Slightly soluble	Sparingly soluble	0.1-0.2%

Three botanical varieties of *Centella asiatica*

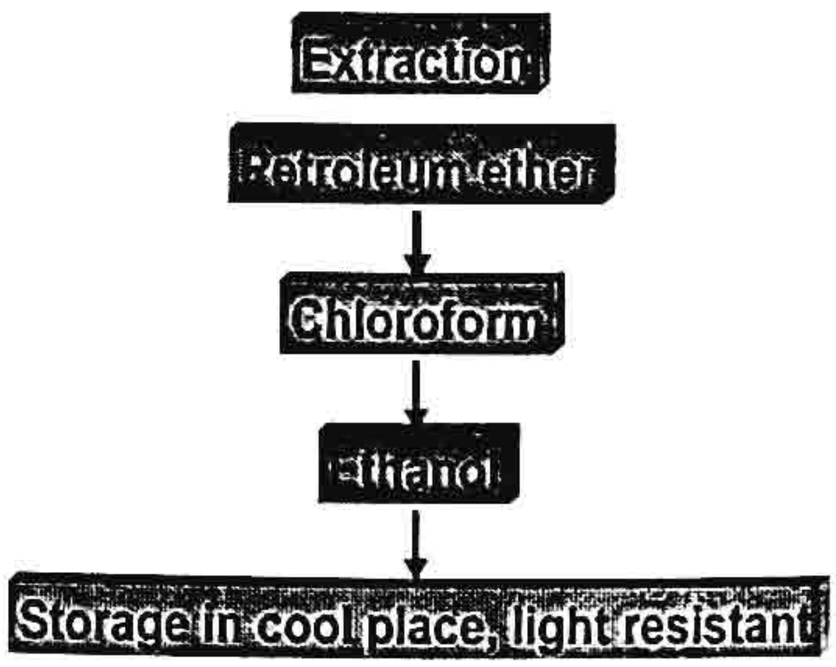
The basis of their location ;

abyssinica	East Africa
typica	Southern Asia
floridana	America



Cultivation of *Centella asiatica*

Drying

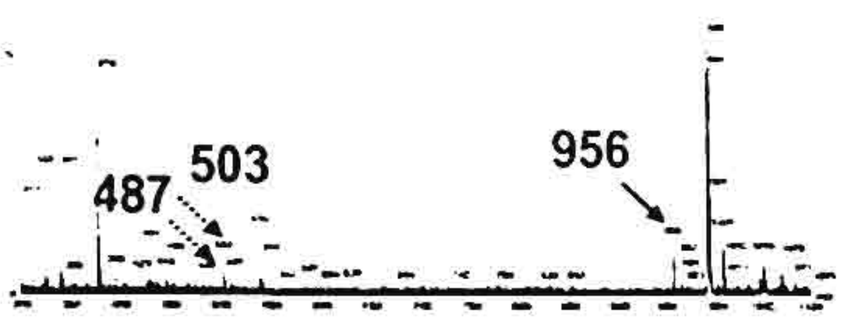


Determination of Active Constituents in *Centella asiatica* extracts

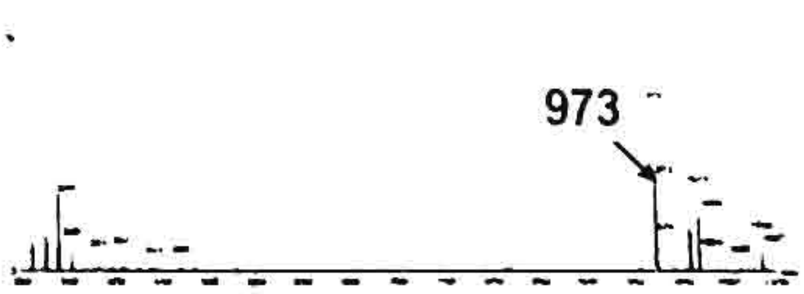
HPLC : Reverse phase, C18, 30x0.39 cm
Acetonitrile : water, gradient
UV detection at 220 nm

LC-MS : water-methanol (50:50)
electrospray ionization
mass spectrometry (ESI-MS)

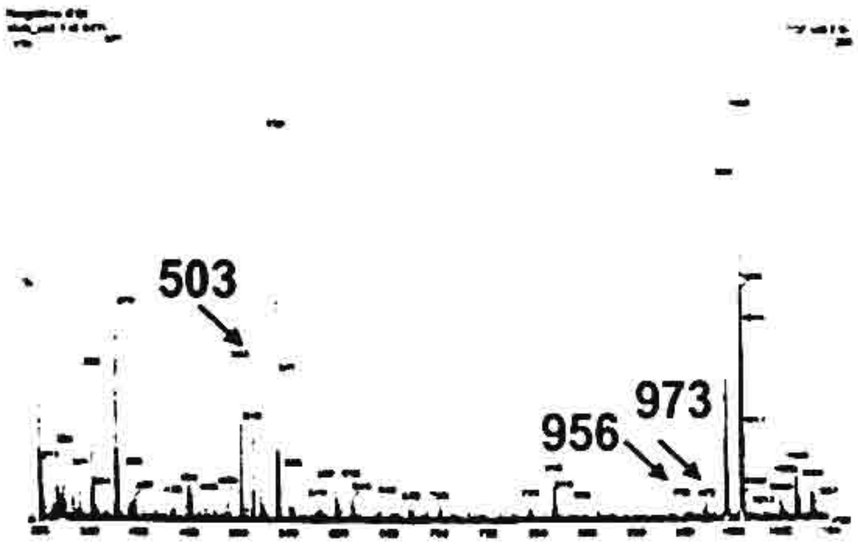
Negative Mass Spectrum of Asiaticoside



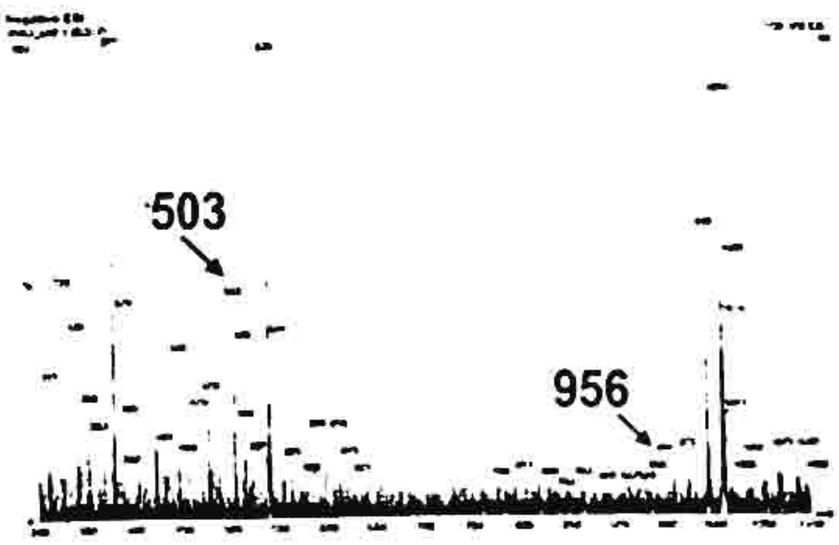
Negative Mass Spectrum of Madecassoside



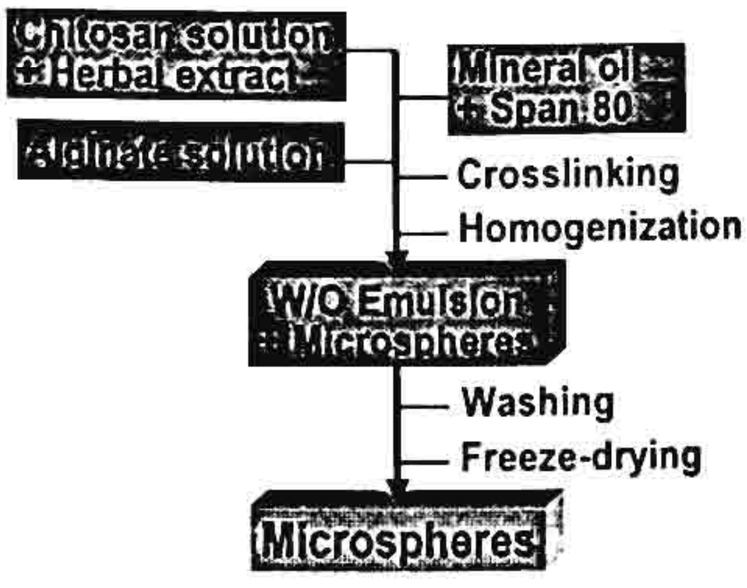
Negative Mass Spectrum of Ethanol Extract of *Centella asiatica*



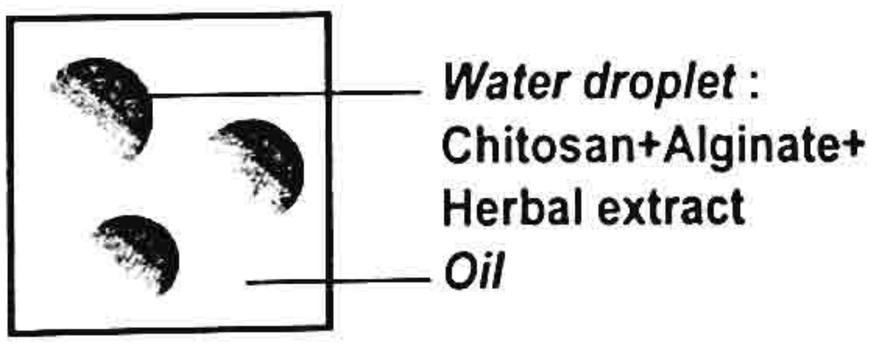
Negative Mass Spectrum of Chloroform Extract of *Centella asiatica*



Preparation of Chitosan-Alginate Microspheres



W/O Emulsion



Variation Parameters

Oil types **; mineral oil, rice barn oil,**

corn oil, palm oil etc.

Oil : Water ratio

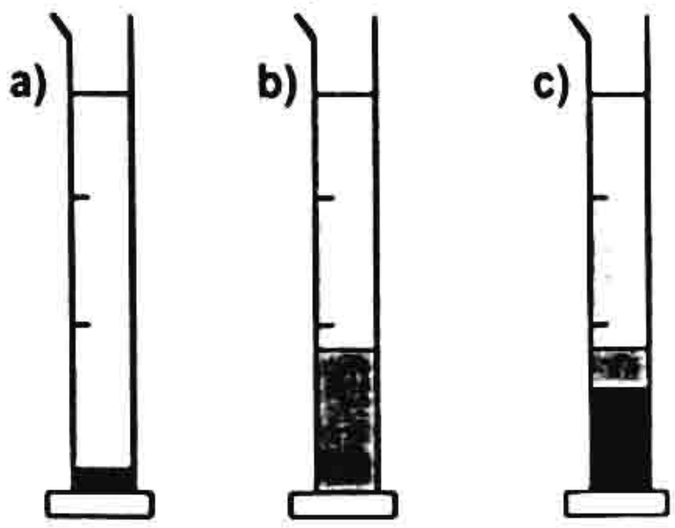
Emulsifying agent **: type, concentration**

Hydrocolloids **; type, concentration**

pH, etc.

Effect of Types of Herbal Extracts on Formation of Microspheres

Effect of Types of Herbal Extracts on Microsphere Formation

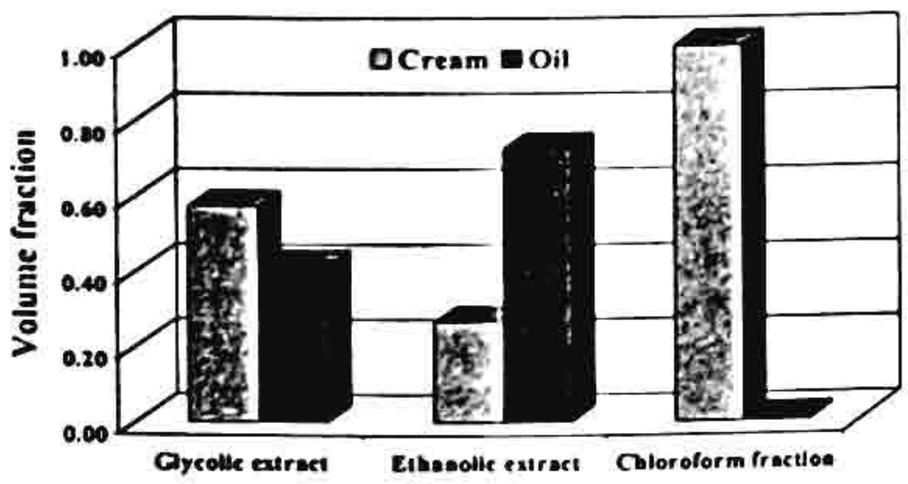


Effect of Types of Herbal Extracts on Formation of Microspheres

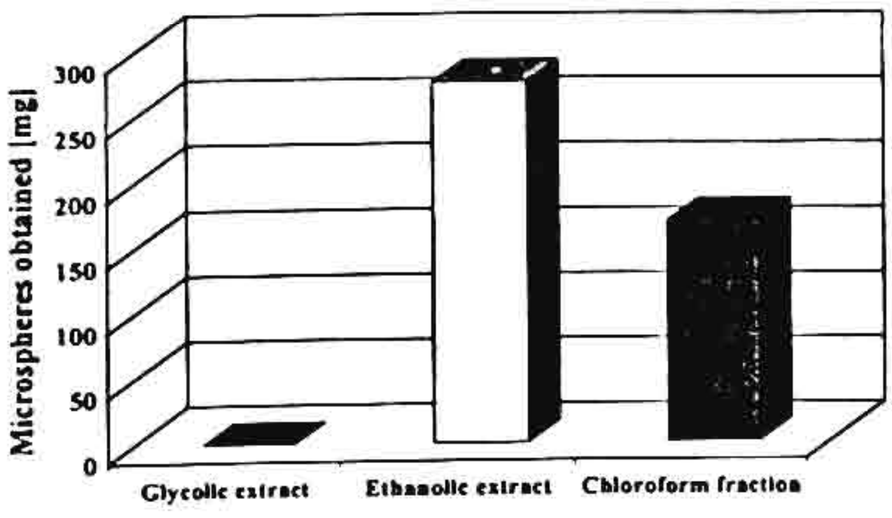
Left ; Ethanol extract

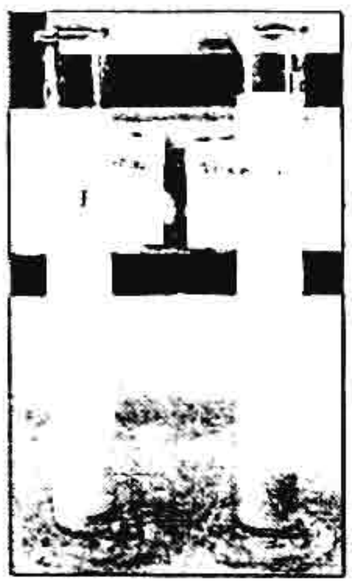
Right ; Glycolic extract

Effect of Types of *Centella asiatica* Extracts on Cream and Oil Volumes of W/O Emulsions



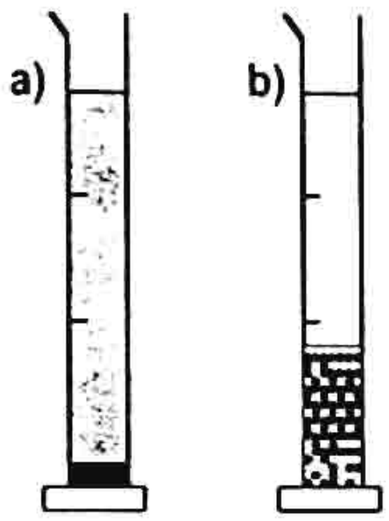
Effect of Types of *Centella asiatica* Extracts on Microsphere Yield





Effect of Amount of Herbal Extract on Microsphere Formation

Effect of Amount of Herbal Extract on Microsphere Formation

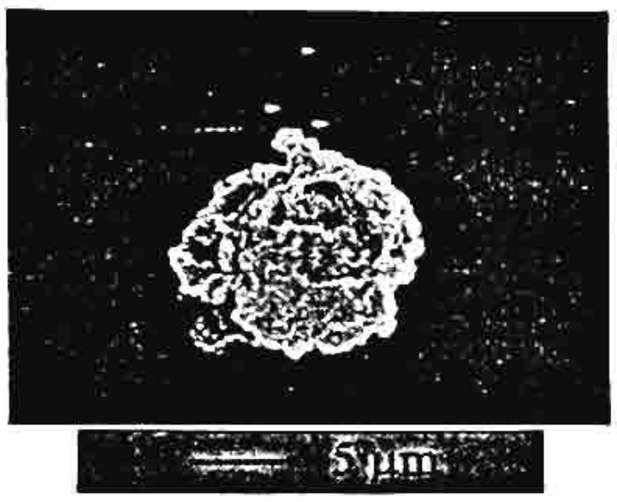


Herbal extract in ethanol

a) 10 g

b) 15 g

SEM Photo of Chitosan-Alginate Microsphere containing *Centella asiatica* Extract



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Rossukon Kongcharoen
Khanaporn Tangpongsirikul
Botanical gardeners