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Permeation Study of Vitamin E Emulgels Using Carrageenan a Marine Biota Products as Gelling Agents.

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Abstract: *Carrageenan as one of marine biota products is widely used in various industries such as food, cosmetics, pharmaceuticals, printing, and textile. Application and use of carrageenan in the manufacture of gels and other gel products is still very limited especially for cosmeceutical products. Caraageenan has the ability to form gels in a thermo-reversible waymaking it widely used as a gelling agent, thickener, stabilizer and additional permeation enhancement. Thereby this study aims to conduct how permeation study of Vitamin E an anti-oxidant in emulgels preparations using Carrageenan as gelling agents compare to another gelling agents (HPMC). In vitro permeation tests by Franz Diffusion Cell showed better result for emulgels Carrageenan than HPMC. The cumulative penetrated amounts of Vitamin E emulgels with Carrageenan was 5262.64±49.37 µg/cm^2 while HPMC 4453.90±49.37, Fluks values for emulgels Carrageenan and HPMC was 29.24±0.27 and 24.75±0.27 respectively.*

Keywords: Carrageenan, Vitamin E, HPMC, Permeation Test, Franz Diffusion Cell.

INTRODUCTION

Over the past few years, marine biota products such as bacteria, microalgae and seaweed have represented a large source of promising material that can be developed [1]. Carrageenan is one of a carbohydrat polymers that derived from seaweed particularly *Eucheuma cottonii* [2], caraageenan has the ability to form gels in a thermo-reversible waymaking it widely used as a gelling agent, thickener, and stabilizer in various industries such as food, pharmaceuticals, cosmetics, printing, and textile[2]. Application and use of carrageenan in the manufacture of gels and other gel products is still very limited especially for cosmeceutical products. Therefore, it is necessary to conduct a study on the effect of the concentration of gelling agent as to provide more comprehensive data related to the physical properties of the gel produced. For this purpose carrageenan was chosen, a polysaccharide and has very interesting properties like good adhesiveness on skin which can be a benefit for topical application [3].

Another benefit of Carrrageenan is also can help permeability through the skin of active ingredient in pharmaceutical topical preparations [4], so that in this research, the aim study is to evaluate how permeability Tocopherol acetate (Vitamin E) in emulgels, using Carrageenan as gelling agent compared with another gelling agents in emulgels preparations and also evaluate its characteristics.

METHODS

1. Materials

Carrageenan was obtained as a gift sample from Padjadjaran University (Bandung, Indonesia). Tocopherol Acetate (Vitamin E) (Brataco Chemical), Span 20 (Brataco Chemical), Tween 20 (Brataco Chemical), Parrafin liquidum (Brataco Chemical), Propylene glycol (Brataco Chemical), Methyl and Propyl Paraben (Brataco Chemical), Triethanol Amine (Brataco Chemical), HPMC (Brataco Chemical), Phosphat Buffer pH 7.4, All other chemicals used were of analytical grade.

2. Emulgel Base Orientation

Base selection was done by using varying amount of Carrageenan as gelling agent as much as 0.5% (F1), 0.75% (F2), 0.85% (F3), 0.95 % (F4), 1% (F5), 1.125% (F6), 1.25% (F7), 1.5% (F8) and 2% (F9). The composition of different formulations of emulgels is shown in Table 1

Table 1. Composition of Emulgels Base Orientation

Ingredients	Concentrations (% w/w)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Carrageenan	0.5	0.75	0.85	0.95	1	1.125	1.25	1.5	2
Liquid Paraffin	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Tween 20	1	1	1	1	1	1	1	1	1
Span 20	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Propylene Glycol	10	10	10	10	10	10	10	10	10
Nipagin	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Nipasol	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
TEA	2	2	2	2	2	2	2	2	2
Aqua ad.	100	100	100	100	100	100	100	100	100

3. Preparation of Vitamin E Emulgels

Different formulations were prepared using varying concentration of carrageenan as a gelling agent. The preparation of emulsion was same in all the formulations. The gel bases were prepared by dispersing Carrageenan in heated distilled water (75oC) with constant stirring at a moderate speed using mechanical shaker and adjusted to 5.5 to 6.5 using tri ethanol amine (TEA), The oil phase was prepared by dissolving certain amount of span 20 in liquid paraffin, while the aqueous phase was prepared by dissolving the required amount of tween 20 in purified water. 0.03 g of methyl paraben and 0.01 g propyl paraben were dissolved in 10 g of propylene glycol and both were mixed with aqueous phase. Tocopherol Acetate was dissolved in oil phase. Both the oily and aqueous phases were separately heated to 70-80°C. Then, the oil phase was added to the aqueous phase with continuous stirring until it got cooled to room temperature. The emulsion was poured into a gel with gentle stirring until homogenous emulgel was obtained [14,15].

The antimicrobial activity of methyl paraben and others is considerably reduced in the presence of nonionic surfactants, as a result of micellization. However, propylene glycol (10%) has been shown to potentiate the antimicrobial activity in the presence of nonionic surfactants [16].

4. Evaluation of emulgels

a. Physical parameters of prepared formulations

All the prepared formulations were visually checked for the color, appearance, homogenicity, phase separation and freeze thaw test.

b. Determination of pH

The pH measurements were done using a digital pH meter (Mettler Toledo). Gel (1 g) was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation until the constant reading was observed. The measurement determination of pH of each formulation were measured in three replicate [14].

c. Determination of spreadability

A weighed quantity (350 mg) of emulgel was taken on a glass plate (10x5 cm). Another glass plate (10x5 cm and 5.8±1 g) was dropped from a distance 5 cm. The diameter of the circle of spread was measured after 1 min [15]. Types of gels based on spreadability are given in Table 2.

Table 2. Types of gels based on spreadability [17]

Type of gels	Measurement (in cm)
Fluid gel	More than 2.4
Semi-fluid gel	1.9-2.4
Semi stiff gel	1.9-1.6
Stiff gel	1.6-1.4
Very stiff gel	Less than 1.4

5. PermeationnTest

The formulation tested is the best formula using Carrageenan and emulgels using HPMC as other gelling agents for comparation. The composition can be seen in table 3 below.

Table 3. The composition of the Vitamin E Emulgels With Carrrageenan and HPMC

Component	Formula Carrrageenan	Formula HPMC
Vitamin E	5	5
Carrageenan	1	-
HPMC	-	8
Liquid Paraffin	7,5	7,5
Tween 20	1	1
Span 20	1,5	1,5
Propylene glycol	10	10
Propyl Paraben	0.01	0.01
Metyl Paraben	0.03	0.03
Aquadestilata ad	100	100

Permeation tests of emulgel preparations were carried out using membrane dorsal python skin (Python reticulatus) with Franz diffusion cells (diffusion area 3.14 cm2, compartment volume 21 ml, receptor compartment filled with phosphate buffer pH 7.4 with a temperature of 37±0.5°C). Membrane is first cleaned with aquadest, then dried at room temperature and placed between the donor and the receptor compartment with the horn layer facing up. The Franz Diffussion Cell apparatus can be seen in Figure 3 below.

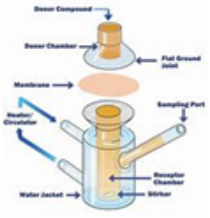


Fig. 3. Franz Diffussion Cell apparatus [18]

Emulgel Vitamin E with Carrrageenan and without Carrageenan (HPMC) weighed 1 gram each and applied to the membrane. A total of 0.5 ml of sample was taken from the receptor compartment periodically for 3 hours using a syringe and replaced by the same amount of phosphate buffer pH 7.4. The sample obtained was measured absorption using a UV-Vis Spectrophotometer at aEmulgel Vitamin E with Carrrageenan and without Carrageenan (HPMC) weighed 1 gram each and applied to the membrane. A total of 0.5 ml of sample was taken from the receptor compartment periodically for 3 hours using a syringe and replaced by the same amount of phosphate solution pH 7.4. The sample obtained was measured absorption using a UV-Vis Spectrophotometer at.a.wavelength.of.212.nm.

RESULT AND DISCUSSION

Emulgel Base Orientation Result

Based on the result in Table 4 and Table 5. Formulas F5, F6 and F7 show the beter results in consistency, phase separation, and *freeze thaw* test compare to formulas F1, F2, F3, F4, F8 and F9, only formulas F5, F6 and F7 have condition easy to spread and no phase separation after *freeze thaw* test, meanwhile F8 and F9 have no phase separation but their consistency harder and very stiff.

Table 4 Physical evaluation of emulgel base

Formulas	Color	Odor	Consistency	Phase Separation
F1	White	Odorless	Thinner	Separated
F2	White	Odorless	Thinner	Separated
F3	White	Odorless	Thinner	Separated
F4	White	Odorless	Thinner	Separated
F5	White	Odorless	Viscous, easy to spread	None
F6	White	Odorless	Viscous, easy to spread	None
F7	White	Odorless	Viscous, easy to spread	None
F8	White	Odorless	Harder	None
F9	White	Odorless	Harder	None

Table 5 Freeze Thaw test Result

Formulas	Phase separation at the cycling-					
	1	2	3	4	5	6
F1	(-)	(+)	(+)	(+)	(+)	(+)
F2	(-)	(+)	(+)	(+)	(+)	(+)
F3	(-)	(-)	(+)	(+)	(+)	(+)
F4	(-)	(-)	(+)	(+)	(+)	(+)
F5	(-)	(-)	(-)	(-)	(-)	(-)
F6	(-)	(-)	(-)	(-)	(-)	(-)
F7	(-)	(-)	(-)	(-)	(-)	(-)
F8	(-)	(-)	(-)	(-)	(-)	(-)
F9	(-)	(-)	(-)	(-)	(-)	(-)

Note : (-) No Phase Separation
(+) Phase Separation

Formulation Vitamin E Emulgels

The *freeze thaw* test show that the F5, F6 and F7 have good stability in the concentration 5%. The whole Formulation can be seen in Table 6.

Table 6 Formulation of Vitamin E Emulgels

Component	Concentration (% w/w)

	F5	F6	F7
Carrageenan	1	1.125	1.25
Vitamin E	5	5	5
Liquid Paraffin	7.5	7.5	7.5
Tween 20	1	1	1
Span 20	1.5	1.5	1.5
Propylene glycol	10	10	10
Metyl Paraben	0.03	0.03	0.03
Propyl Paraben	0.01	0.01	0.01
TEA	2	2	2
Aqua ad.	100	100	100

Physical Stability Study of Vitamin E Emulgels

Table 7 and figure 1 show the physical properties of the emulgels in F5, F6, and F7. The result shows that F5 has the better formula based on parameters such as pH measurement and spreadability test. The F5 continues to stability test and figure 4 indicating stability studies data of F5 formulas.

Table 7 Physical characteristic of Tocopherol Acetate Emulgel Formulations

Formulation Code	Organoleptic characteristics	Time Storage (days) 0	7	14	21	28
F5	Phase separation	No	No	No	No	No
	Color	White	White	White	White	White
	Odor	Odorless	Odorless	Odorless	Odorless	Odorless
	Texture	Smooth	Smooth	Smooth	Smooth	Smooth
	Consistency	Viscous	Viscous	Viscous	Viscous	Viscous
	Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous	Homogenous
F6	Phase separation	No	No	No	No	No
	Color	White	White	White	White	White
	Odor	Odorless	Odorless	Odorless	Odorless	Odorless
	Texture	Smooth	Smooth	Smooth	Smooth	Smooth
	Consistency	Viscous	Viscous	Viscous	Viscous	Viscous
	Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous	Homogenous
F7	Phase separation	No	No	No	No	No
	Color	White	White	White	White	White
	Odor	Odorless	Odorless	Odorless	Odorless	Odorless
	Texture	Smooth	Smooth	Smooth	Smooth	Smooth
	Consistency	Viscous	Viscous	Viscous	Viscous	Viscous
	Homogeneity	Homogenous	Homogenous	Homogenous	Homogenou	Homogenous

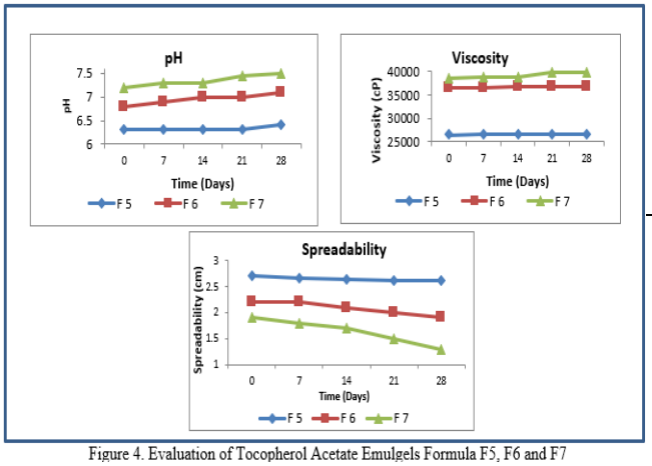


Figure 4. Evaluation of Tocopherol Acetate Emulgels Formula F5, F6 and F7

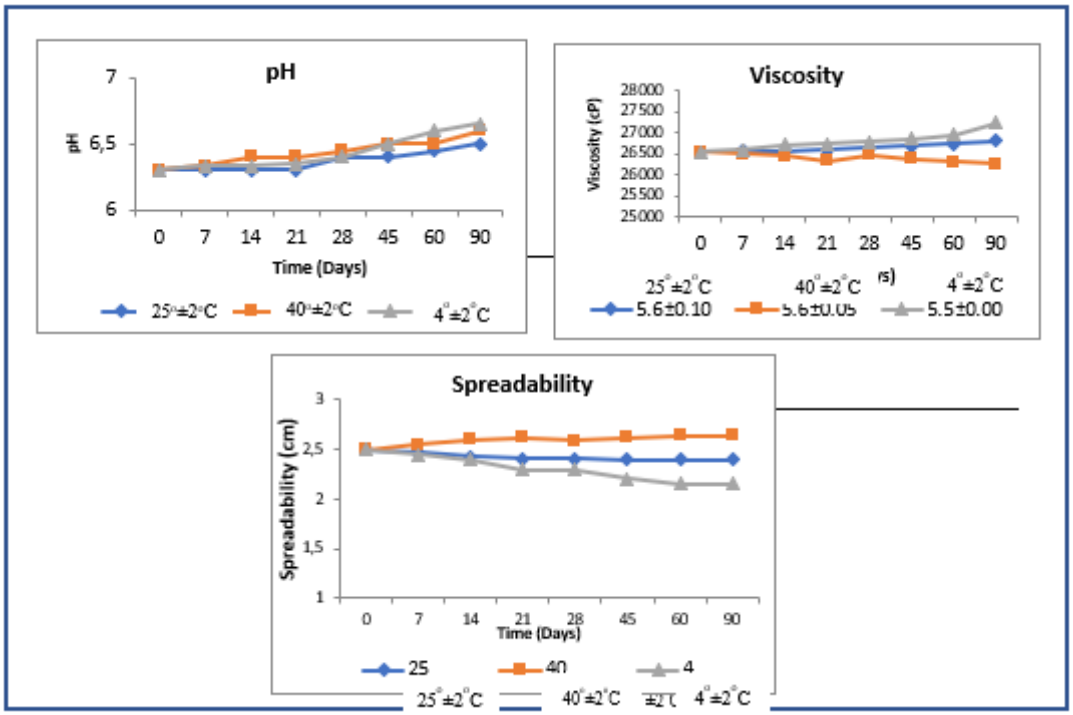


Figure 5. Stability Study of promising formula F5 during storage time at ambient temperature (25oC±2oC),at climatic chamber (40oC±2oC), and at cold temperature (4oC±2oC)

Permeation Study of Vitamin E Emulgels

The Permeation test show for that Emulgels formula with Carrageenan showed better cumulative amount penetrated than HPMC, and also better fluks values that can be seen in Figure 6 and 7 below.

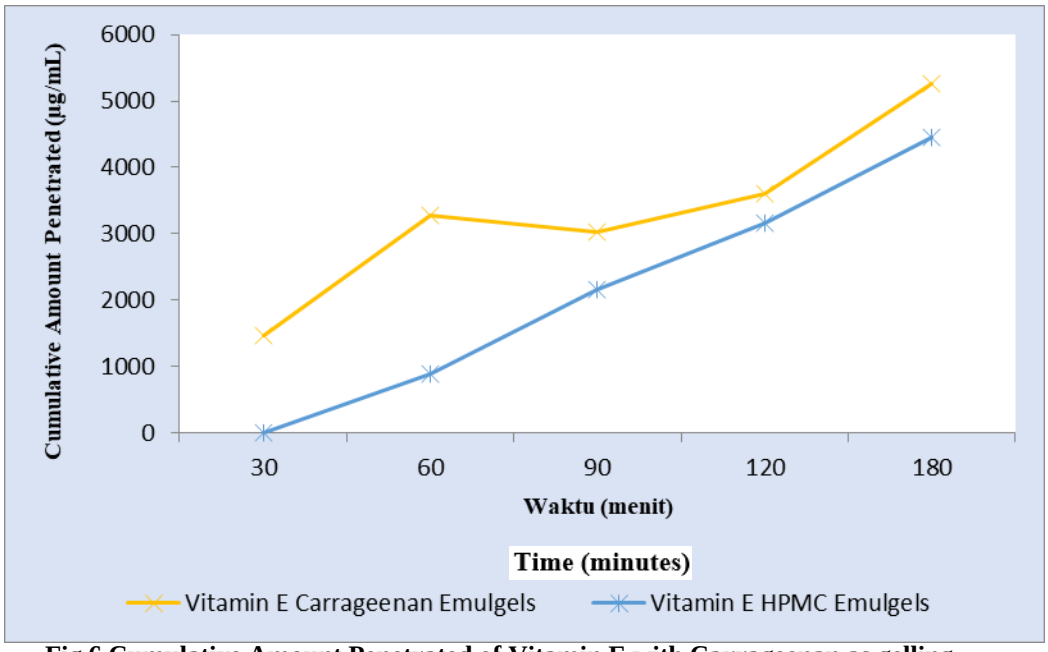
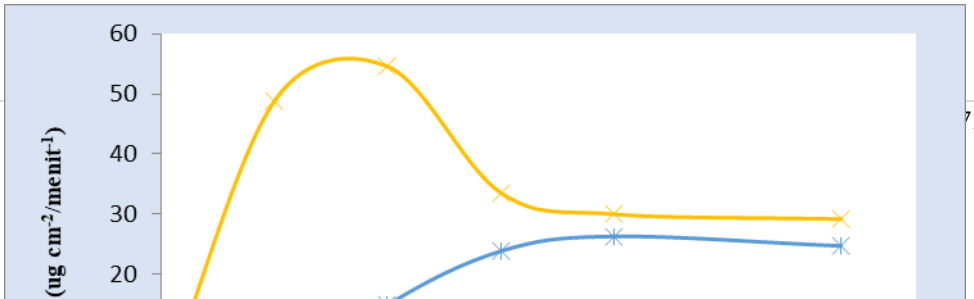


Fig.6 Cumulative Amount Penetrated of Vitamin E with Carrageenan as gelling agents and HPMC



Time (minutes)

Fig.7. Fluks values of Vitamin E with Carrageenan as gelling agents and HPMC

Discussion

1. Result of Emulgels Base Optimation

Nine formulations of emulgel with carrageenan as gelling agent were prepared as indicated in Table 1. From preliminary trials it was found that when carrageenan was used beyond 1.25% the gel base obtained was highly viscous making it unfavorable to used. Similarly at concentrations below 1% the viscosity was very poor. Hence carrageenan was used in the range of 1-1.25% in formulations F5-F7.

The result of various physical parameters evaluated are given in Table 4,5. Formulations F1-F4 were fluid due to the presence of low carrageenan concentrations. Formulations F8-F9 were thick due to higher carrageenan concentrations. Formulations F5-F7 have creamy appearance and no phase separation was observed at centrifugation test ant freeze thaw test.

Based on viscosity, spreadability and pH measurement result of formulas F5-F7, formula F5 was found to be the best for formulating tocopherol acetate emulgel from parameters pH measurement F5 that has pH values between 6-6.5, meanwhile F6 and F7 have pH values above 6.8, that slightly beyond safe level for skin topical requirements which was at the range 4.5-6.8 [19]. For spreadability test F5 belonged to fluid gel category, and formulations F6 and F7 with higher concentration of carrageenan belonged to semi fluid gel and semi stiff gel to stiff gel respectively. With an increase in gelling agent concentration in formulation, the spreadability of formulations decreases [15]. The result are reported at figure 4.

Accelerated stability studies were performed for formulation F5 for 3 months. The samples were analyzed for 0,7,14,28.45.60 and 90 days for physical appearance, viscosity, pH and spreadability.

2. Result of Physical appearance determination

The result of organoleptic observation shows in Table 6, that during strorage time emulgel does not shows any changes in odor and color.

3. Result of Viscosity determination

The result of viscosity measurement during storage time was shown on Figure 5. The viscosity value at room temperature storage is more stable than that stored at high temperature at climatic chamber and at cold temperature. This corresponds to the Arrhenius kinetics equation where the viscosity is inversely proportional to the temperature. The higher the temperature the lower the viscosity value. In addition to temperature, storage time affects stability of dosage form. The longer the preparation is stored at climatic chamber

then the viscosity value will be lower because of the influence of the high humidity so that the preparation to absorb water vapor and cause increased volume of preparations.

4. Result of pH measurement

The pH of emulgel preparation that has been made were still on safe level for skin topical requirements which was at the range 4.5-6.8 [19]. The measured values are presented in figure 5 that indicated the suitable of emulgel for topical application.

5. Result of spreadability measurement

The result of spreadability measurement during storage time was shown on figure 5. Spreadability it is observed that by increasing viscosity spreadability is decreased. The spreadability value at room temperature storage is more stable than that stored at high temperature at *climatic chamber* and at cold temperature.

6. Result of Permeation Test

In vitro permeation test of emulgel preparations using Franz diffusion cells aims to determine the amount of Vitamin E that can penetrate through the skin during a certain time interval. The cumulative amount of penetrating Vitamin E and its flux value over 3 hours. From the graph above showed that emulgel with Carrageenan as gelling agents has a higher cumulative number of penetrated values compared to emulgel preparations with HPMC as gelling agents. The penetration result of emulgel preparation with Carrageenan as gelling agent is 5262.64 ± 49.37 µg/cm2 and emulgel preparation with HPMC is 4453.90±49.37 µg/cm^2. The values obtained for the flux of emulgel with Carrageenan is 29.24±0.27 µgcm^{2}minutes^{-1} meanwhile the flux value of HPMC emulgels is 24.75 ± 0.27 µgcm^{-2}minutes^{-1}.

The data are given as the mean and standard deviation of each parameter at each temperature and after each storage period. All results were compared by analysis of variance (ANOVA) for a 95% confidence level to identity significant difference.

CONCLUSION

This research showed that emulgels based on carrageenan as the gelling agents with of active ingredient Vitamin E has better permeation parameter into skin compared to HPMC, therefore the presented formulations as well as mixtures with carrageenan might be promising alternative to enhance drug carrier systems for topical pharmaceuticals.

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