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Research Article

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Catechin Liposome Gel Formulation

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ABSTRACT

The researchers have performed a research on catechin liposome gel. In addition, this research aims to form catechin liposome into gel preparation. In the process of making catechin liposome gel, the researchers used gelling agent, Carbopol 940, which is an acrylic acid polymer with the characteristics of hydrophilic and stable. Carbopol 940 forms a good viscosity for gel preparation. The test for catechin liposome gel using Franz diffusion discovers that drug delivery system by liposomes will produce better absorption than gel absorption without liposome. The result indicates that catechin liposome gel formula I gives better result than catechin gel preparation without liposome.

Keywords: Liposome; Catechin; Gel; Franz Diffusion; Carbopol 940

INTRODUCTION

Catechin is one of the substances that works as antioxidant and it is an effective component of anti-aging, however but the nature of catechins (hydrophilic and unstable in the air) causes some problems with its physicochemical characteristics [1-3]. Previous research has successfully overcome the problem of catechin physicochemical characteristics by formulating catechin into catechin liposomes [4]. The results in previous resarch were in a quite good catechin liposome form and here a further research is by making gel from catechin liposome. Gel is defined as a semi-solid preparation consists of suspensions made from small inorganic particles or large organic molecules, penetrated by a liquid, can be transparent or opaque masses and it is used topically [5,6]. Gel is a clear, translucent and semi-solid preparation contains an active substance, a colloidal dispersion that have power caused by tissue binding to each other in the dispersed phase [5,6].

Catechin liposome gel making process, gelling agent Carbopol 940 which is an acrylic acid polymer with hydrophilic and stable characteristics is used. Carbopol 940 forms a good viscosity for gel preparation. The selection of hydrophilic polymers with bio adhesive characteristics in liposomes can increase medicine delivery and it has been indicated that liposomes are compatible with acrylic acid polymers [7]. Recently, there have been many

researches on liposomes but none have reported on catechin formulation using liposome technique. Therefore, seeing the importance of medicine delivery system using liposome technique, the researchers then are interested in conducting this research.

METHOD AND MATERIALS

Materials

The materials used were catechins, DPPH (2,2-diphenyl-1-picrylhydrazyl) from Sigma (Singapore), Egg Phosphatidylcholine (EPC), cholesterol obtained from Sigma (Singapore); Potassium Dihydrogen Phosphate (brataco), Sodium Hydroxide, Carbopol 940, Propylene glycol obtained from Brataco; Chloroform and Methanol from Merck

Catechin Liposome Gel Making Process

The catechin liposome used was made based on previous research (Table 1) [4].

Table 1. Catechin Liposome Gel Formulation

Component	Formula (%)			
	F0	**F1**	**F2**	**F3**
Catechin	10	-	-	-
Catechin Liposome (1:1)	-	10	-	-
Catechin Liposome (1:2)	-	-	10	-
Catechin Liposome (2:1)	-	-	-	10
Carbopol 940	1	1	1	1
Propylene glycol	10	10	10	10
Aquadest	79	79	79	79

Gel Evaluation

a. **Homogeneity check [6,8,9]:** All gels were tested for homogeneity visually. Looking at the shape and color

b. **pH measurement [6,9,10]:** pH was measured using pH meter calibrated with pH 4 dan pH 7 standard. pH measurement was conducted in a room temperature for 4 weeks.

c. **Liposome vesicle size distribution on gel [11,12]:** This measurement process used PSA (Particle Size Analizer) method with the aimed to view the changes in vesicles size experienced by liposome when inserted on the gel.

d. **Penetration test by *in vitro* [11,13]:** The Penetration Test was conducted using Franz diffusion cells at 37 ± 1°C, the membrane used was rat skin. The receiving fluid compartment on the Franz diffusion cell device was filled with a solution of pH 7.4. The gel was applied to the rat skin placed on the Franz diffusion cell device. Stirring was conducted at 600 rpm using a magnetic stirrer. The water temperature on the glass vessel was 37 ± 1°C. The samples were taken at certain time intervals (15, 30, 45, 60, 75, 90) in minutes, the receiving medium (phosphate buffer pH pH 7.4) was taken as much as 5 mL and replaced with an external receiving medium as much as 5 mL. The sampling was equated for each test. The samples were measured for absorption using an ultraviolet spectrophotometer at 278 nm wavelength.

Data Analysis

The research results were made in the form of tables and graphs. The diffusion test was processed using SPSS 17 one-way anova.

RESULT AND DISCUSSION

This research is the continuation from the previous research [4]. Catechin liposomes were made in the previous research. The previous research results fulfilled the requirements and we continue the research by making catechin liposomes into gel. Gel is chosen because it is favorite and easy to wash. In addition, we use gelling agent of Carbopol 940 which is an acrylic acid polymer with the characteristics of hydrophilic and stable in making liposome gel. Carbopol 940 forms a good viscosity for gel preparation. The selection of hydrophilic polymers with bio adhesive characteristics in liposomes can increase medicine delivery and it has been shown that liposomes are compatible with acrylic acid polymers [7]. The Carbopol 940 concentration used is 1% to form a gel with moderate viscosity, in order to facilitate the process of spreading during topical application to the skin. In the gel base formulation, only propylene glycol which functions as a humectant is added [5].

Liposome Gel Evaluation

a. **Homogeneity:** The catechin gel and catechin liposome gel in the formulation look homogeneous and transparent. This means that all ingredients are well mixed and the method is right. The homogeneity test results of catechin gel and catechin liposome gel can be seen in Table 2.

Table 2. Catechin liposome gel homogeneity observation

Characteristics	Gel F0	Gel F1	Gel F2	Gel F3
Shape	Gel	Gel	Gel	Gel
Color	White Clear	White Clear	White Clear	White Clear
Odor	No odor	Egg	Egg	Egg
Homogeneity	Homogen	Homogen	Homogen	Homogen

b. **pH measurement:** A good topical preparation should be in the skin pH range of 4.2-6.5, if it is too acidic it will cause skin irritation and if it is too alkaline it can cause scaly skin. This is due to the damage of acid mantle in the skin stratum corneum layer. Liposome gel preparation pH is measured using pH meter. pH evaluation is conducted every week for 4 weeks. The results change every week but the results obtained are still within the normal skin pH range of 4.2-6.5 [1,12]. The results of catechin gel pH and catechin liposome gel measurement can be seen in Table 3.

Table 3. Table of catechin gel and catechin liposome gel pH measurement for 4 weeks

Gel formula	Week				Mean ± SD
	I	II	III	IV	
F0	4.25	4.27	4.35	4.33	4.30 ± 0.05
F1	4.18	4.26	4.24	4.2	4.22 ± 0.04
F2	4.41	4.42	4.4	4.38	4.40 ± 0.02
F3	4.34	4.22	4.34	4.3	4.30 ± 0.06

c. **Catechin liposome gel vesicle size measuring:** This measurement uses PSA (Particle Size analysis) on the gel, and it aims to see whether the liposomes inserted in gel experience size increase or not. From the result, it can be seen that liposomes have a size increase about 70%, except formula 1 that has a slight decrease in the particle size. The increase in particle size is probably due to the interval between the making and measuring time that is too long, as a result the liposome particles aggregate with each other and experience vesicle size increase. The results are presented in Table 4 below.

Table 4. Liposome gel vesicle size distribution

Formula	Average ± SD
F1	2742.5 ± 465.4 nm
F2	1990.6 ± 1340.4 nm
F3	1363.4 ± 330.0 nm

d. **Penetration test by *in vitro*:** Penetration test by *in vitro* aims to see the liposome gel penetration power into the skin layer. The penetration test by *in vitro* uses a horizontal type Franz diffusion device. The working principle of Franz diffusion cell is by putting the rat skin membrane between the donor compartment and the receptor that applied with liposome gel, then the compounds entering the receptor fluid are measured using a UV spectrophotometer. The skin is inserted in Franz diffusion cell and no air trapped between the membrane and receiving fluid. The trapped air can inhibit the catechin liposome gel penetration because it blocks the membrane and receiving fluid. The diffusion system is set to ± 37°C with the aim to create a similar state as human body temperature, by flowing water around the receptor compartment. The compartment temperature must be maintained since it might change the catechin liposome gel diffusion rate in penetrating the membrane. The medium used in the receptor compartment is phosphate buffer of pH 7.4 because this medium describes the physiological fluid of human body and it is also suitable with human blood pH.

The stirring process used a magnetic stirrer at a speed of 600 rpm. The stirring process using magnetic stirrer aims to homogenize substances that penetrate through the membrane in order to spread evenly to the receiving liquid. The use of higher speed can cause gas bubbles to arise between the membrane and receptor compartment fluid. The amount of liquid taken as sample is 5 mL. Each 5 mL liquid sample taken will be replaced by the receiving liquid (phosphate buffer) with the same volume of 5 mL. The purpose is to keep compartment volume remains the same. In calculating the level, a correction factor will be used to minimize the error factor in the process. Before measuring the sample, there is a step to discover catechin maximum wavelength in phosphate buffer, and the result was 278 nm. Next, a catechin calibration curve is made in the phosphate buffer. The concentration on the calibration curves are 20 ppm, 30 ppm, 40 ppm, 50 ppm and 60 ppm. Each concentration is measured at 278 nm wavelength and the regression equation is y=0.0122x+0.0212 with r=0.9995. The results can be seen in Figure 1.

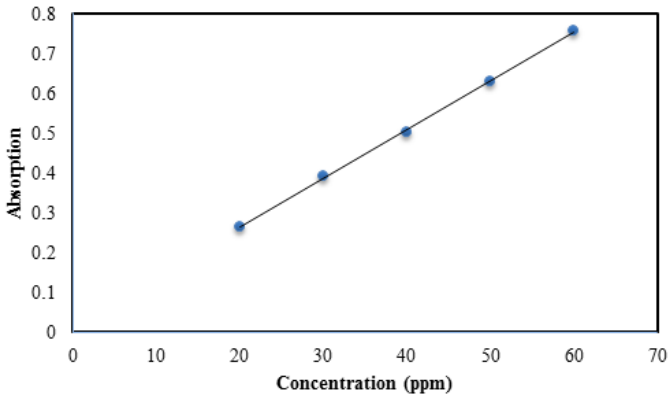

Figure 1. Catechin calibration curve in phosphate buffer of pH 7.4

Note: y=0.0122x+0.0212

r=0.9995.

The existing samples then are measured for absorption on 278 nm wavelength. The absorption obtained is very small and below 0.2. However, after the LOD and LOQ calculation, the values are 0.001241 mg/ml and 0.004136 mg/mL. If we input the LOD value into the regression equation, then y value is 0.021. The absorption from all above samples is 0.021, so the sample absorption meets the detection limit requirement. The % test result of diffusion from gel F0, gel F1, gel F2 and gel F3 can be seen in Table 5, and the % result of gel diffusion efficiency of gel F0, gel F1, gel F2 and gel F3 can be seen in Table 6 and Figure 2.

Table 5. Results of catechin gel and catechin liposome gel diffusion calculation

Time (minute)	% diffusion			
	Gel F0	Gel F1	Gel F2	Gel F3
15	23.16 ± 11.80	21.86 ± 12.24	7.59 ± 8.65	6.62 ± 1.95
30	44.90 ± 13.54	42.57 ± 11.43	16.02 ± 9.19	15.01 ± 6.25
45	55.55 ± 12.35	72.32 ± 36.31	26.11 ± 9.35	28.01 ± 8.13
60	51,14 ± 5,02	78.12 ± 11.12	34.32 ± 13.09	35.70 ± 11.19
75	59.38 ± 8.64	84.53 ± 12.48	45.70 ± 16.90	43.81 ± 13.16
90	88.92 ± 22.28	101.67 ± 24.20	49.42 ± 20.15	49.99 ± 16.13

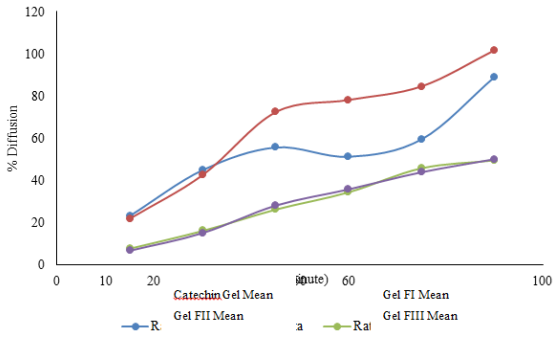

Figure 2. Diffusion profile on each formula

Table 6. Results of catechin gel and catechin liposome gel diffusion efficiency calculation

Repetition	Diffusion efficiency (%)			
	Gel F0	Gel F1	Gel F2	Gel F3
1	39.619	57.158	22.358	16.851
2	38.084	69.604	38.179	28.339
3	49.837	48.353	16.685	31.877
Mean ± SD	$42.513^{a,b}$ ± 6.89	58.372^{b} ± 10.68	25.741^{a} ± 11.14	25.689^{a} ± 7.86

Note: (Mean followed by the same letter in the column showed no significant difference in the Duncan test with 95% confidence interval [a=0.05]).

It can be seen that formula I liposome gel has a higher % diffusion than the catechin gel, therefore gel preparation using liposome medicine delivery system will provide better result to penetrate the skin stratum corneum. Liposome gel formula I is also better than liposome gel formula II and liposome gel formula III. This result indicates that medicine delivery system by liposomes will produce better absorption than gel absorption without liposomes. The results of active substance efficiency analysis then are processed statistically by SPSS 17 using one-way Anova. The results of ANOVA statistical calculation indicate a sig value <0.05, which is 0.007. Followed by Duncan test, the results show that there is a significant difference between gel F1 and gel F2 and gel F3. It means that there is an effect on the differences in phosphatidylcholine and cholesterol compositions in each formula.

CONCLUSION

1. Catechin liposome can be formulated into catechin liposome gel preparation.
2. Franz diffusion test results indicate that catechin liposome gel formula I gives a better diffusion result.

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