CHAPTER IV

RESULTS AND DISCUSSION

Gas Chromatography – Mass Spectrometry was used to identify main components of tea tree oil. Main components of tea tree oil were found, α-terpinene, 1,8-cineole, γ-terpinene, terpinolene and terpinen-4-ol.

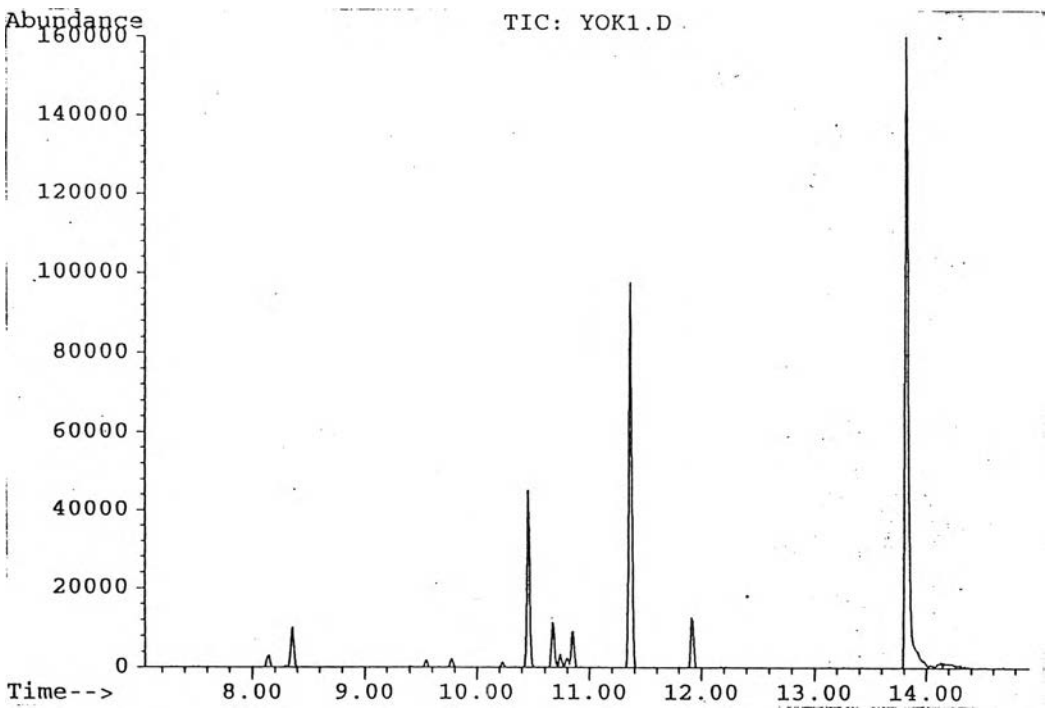

Figure 5 Peaks of main components of tea tree oil from Gas Chromatography – Mass Spectrometry

α-terpinene was found at 10.454 minute, which has structure as figure 6.

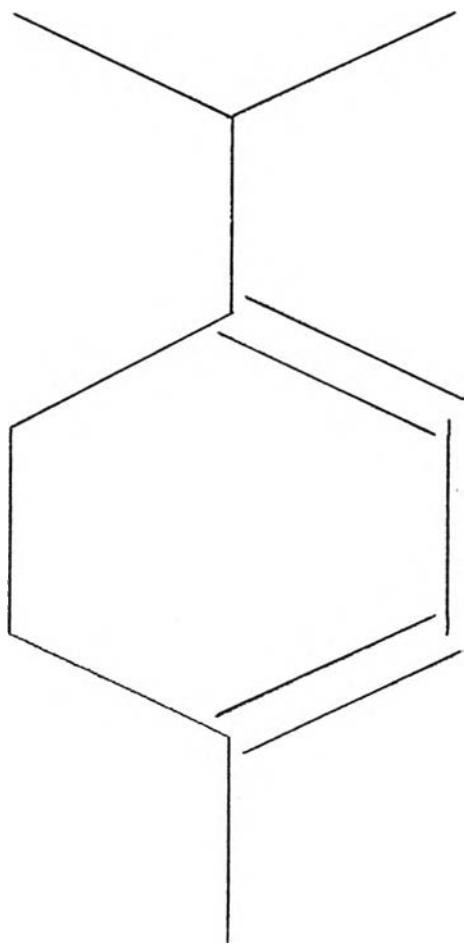

Figure 6 Structure of α-terpinene

1,8-cineole was found at 10.842 minute, which has structure as figure 7.

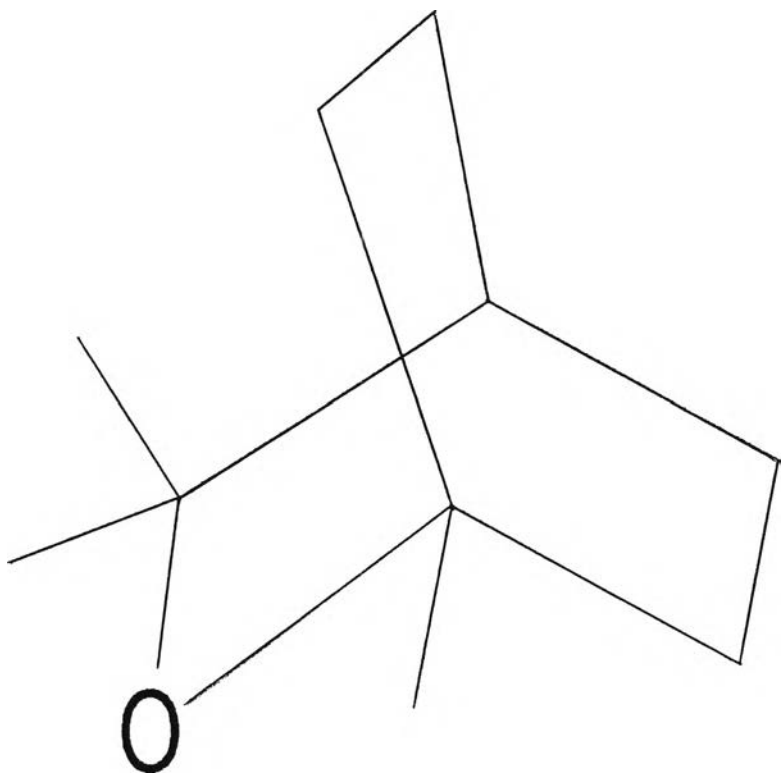

Figure 7 Structure of 1,8-cineole

γ-terpinene was found at 11.354 minute, which has structure as figure 8.

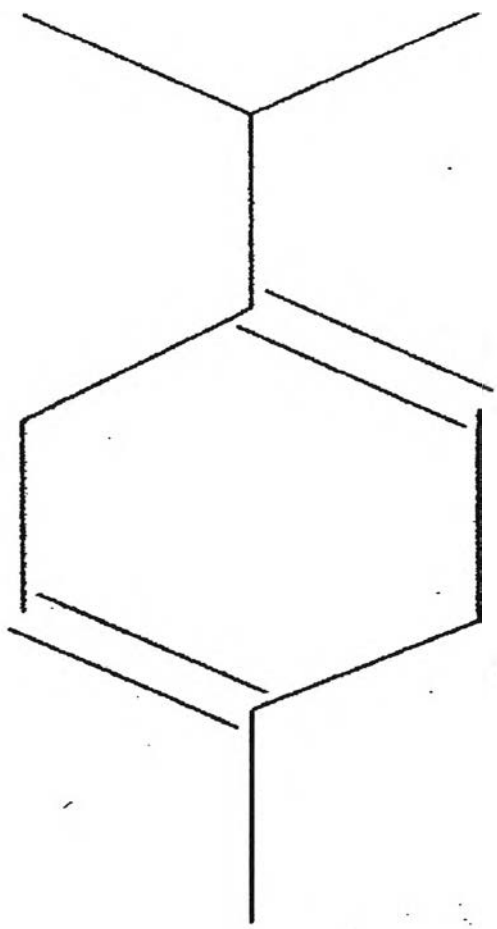

Figure 8 Structure of γ-terpinene

Terpinolene was found at 11.913 minute, which has structure as figure 9.

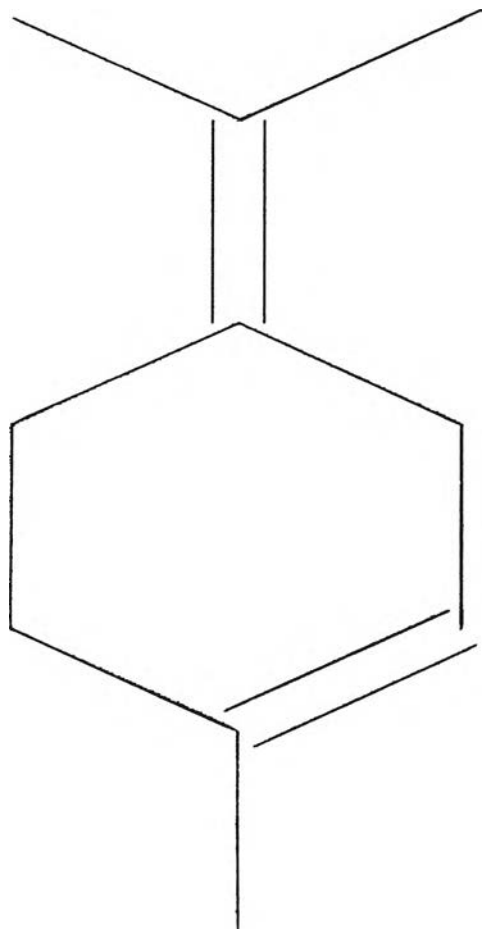

Figure 9 Structure of terpinolene

Terpinen-4-ol, the main component of tea tree oil, was found at 13.835 minute, which has structure as figure 10.

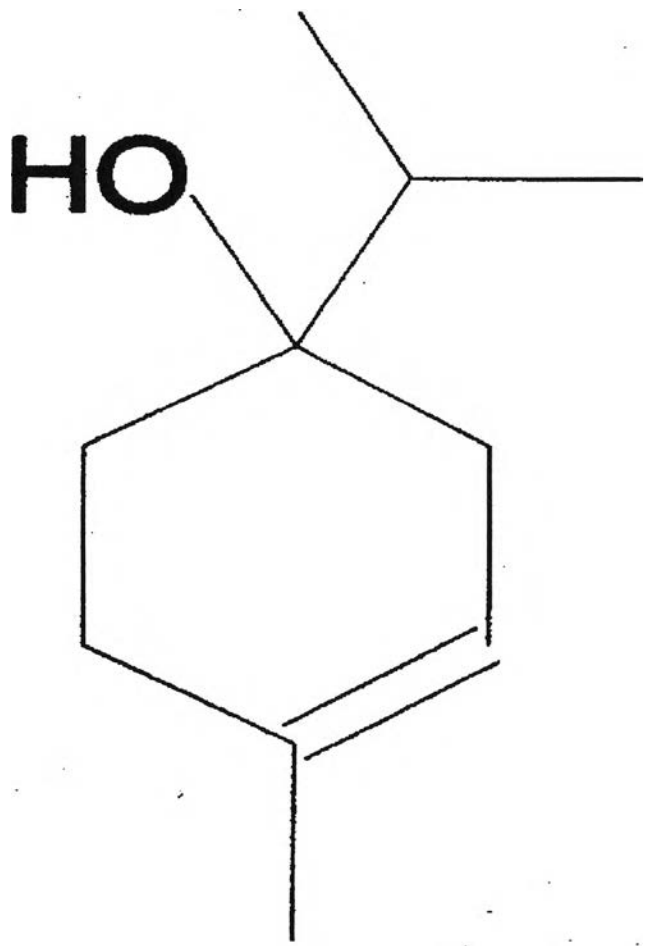

Figure 10 Structure of terpinen-4-ol

From analysis procedure, the standard curve was plotted between response and concentration of terpinen-4-ol, main component of tea tree oil, which is the analyzed component.

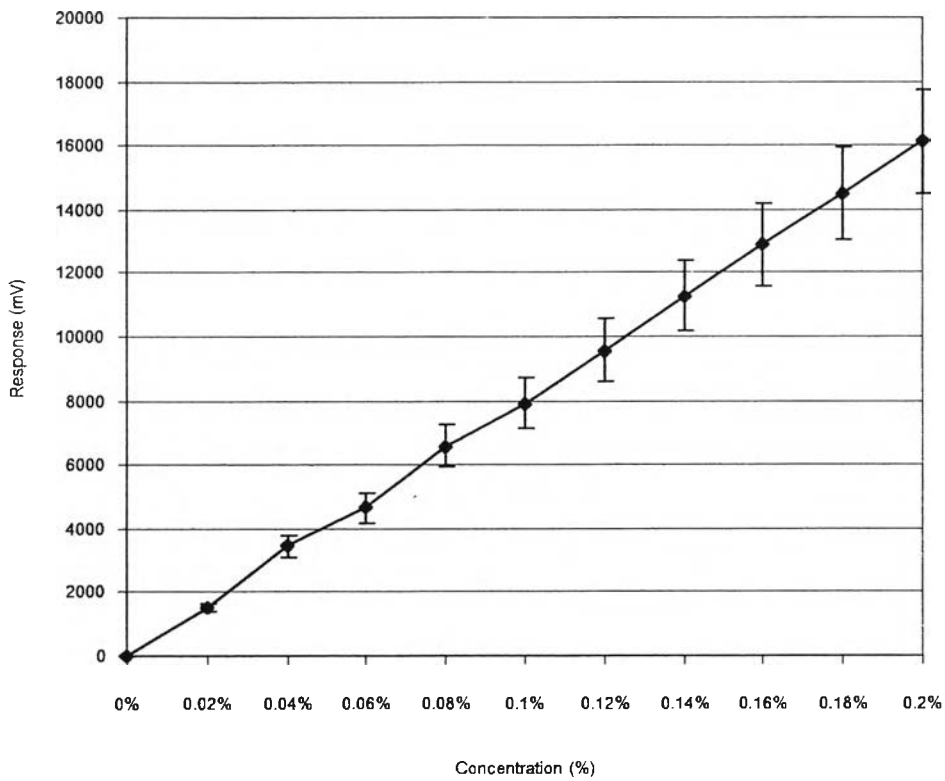

Figure 11 The standard curve plot between response (mV) versus concentration (%v/v) of terpinen-4-ol

Accumulative Concentration

The accumulative concentration (%v/v) of main component of tea tree oil (terpinen-4-ol) in ethanol, from the receptor compartment of franz diffusion cell between 1 hour to 6 hour are represented in the following graphs.

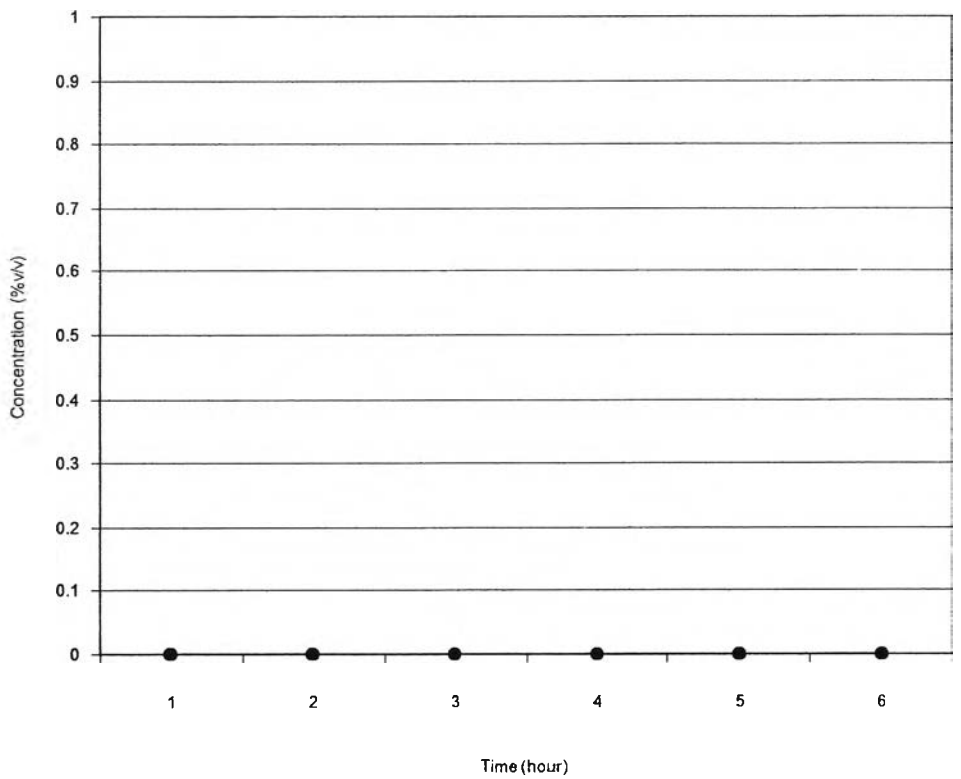

Figure 12 Concentration (%v/v) of terpinen-4-ol release from tea tree oil gel formulation (without emulsifier) versus time (hour)

From the figure 12, negligible results are reported in every hour.

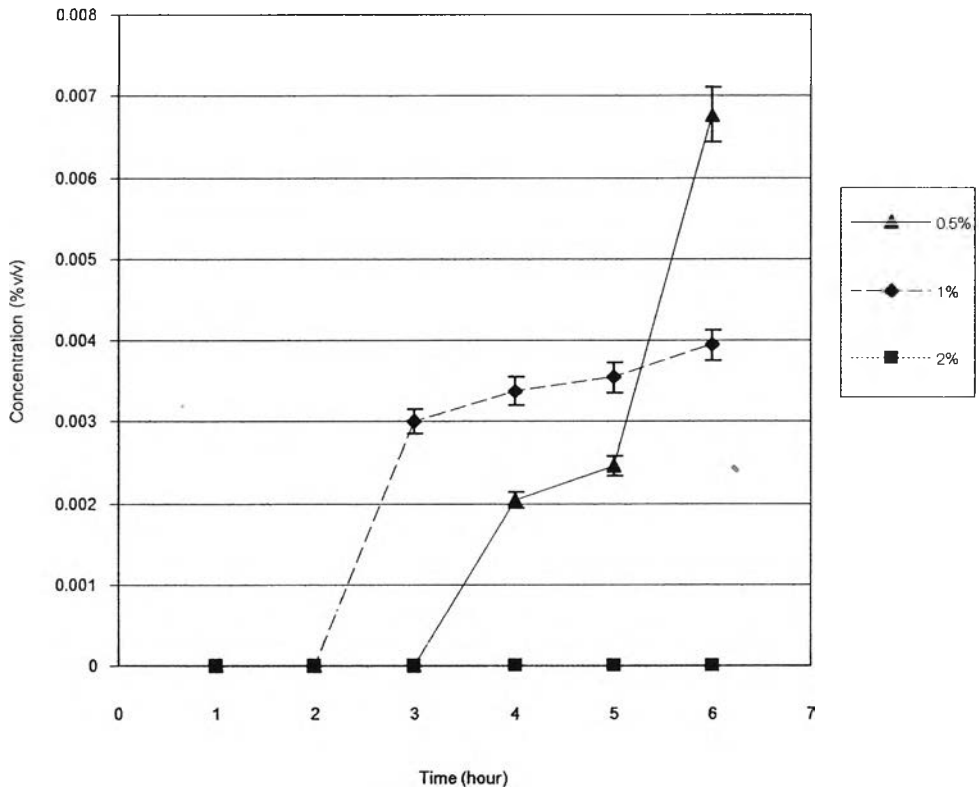

Figure 13 Concentration (%v/v) of terpinen-4-ol release from different concentration of Span 20 formulations versus time (hour)

In all Span 20 formulations, the results are as following.

1% Span 20 formulation is the best release formulation that initially release at 3 hour, which has concentration not too far off 0.003%. After that, it gradually increases and reaches 6 hour with concentration nearly 0.004%.

0.5% Span 20 formulation initially release at 4 hour, which accounts for precisely 0.002%, and marginally increases to just over 0.002% at 5 hour. At this point, the release is dramatically increased to getting on to 0.007% at 6 hour.

2% Span 20 formulation shows the negligible results in every hour.

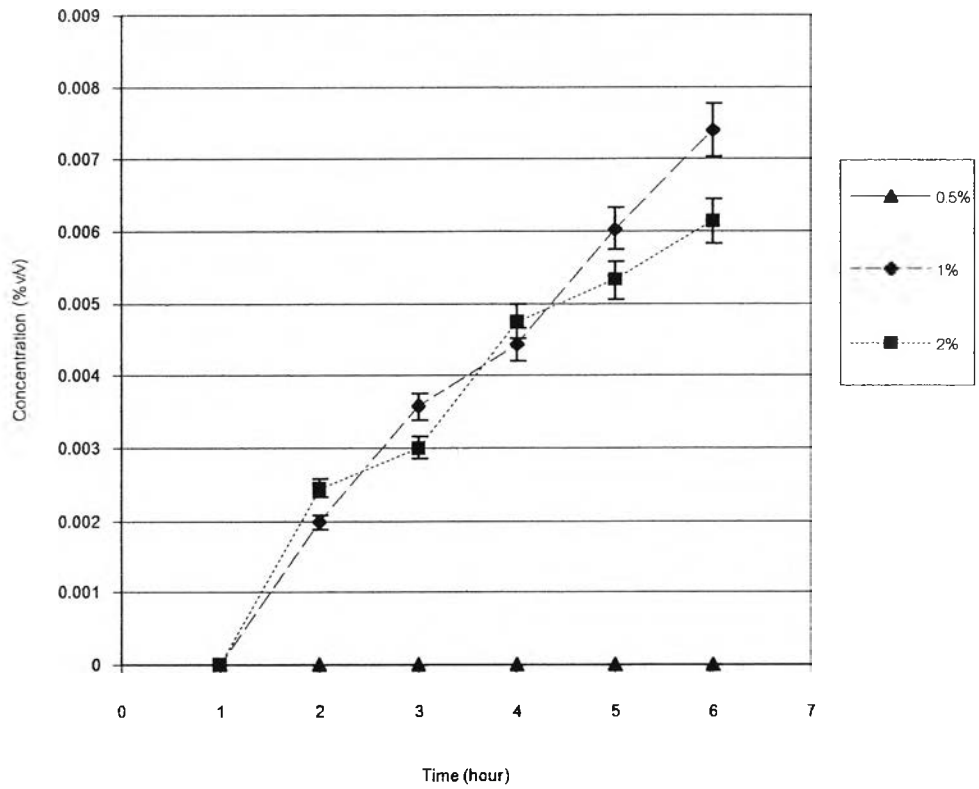

Figure 14 Concentration (%v/v) of terpinen-4-ol release from different concentration of Tween 20 formulations versus time (hour)

As is shown in figure 14, the results are as following.

1% and 2% Tween 20 formulations show similar result in releasing main component of tea tree oil (terpinen-4-ol) to the receptor compartment of franz diffusion cell. However, at 6 hour, the release of 1% Tween 20 formulation, which constitutes well over 0.007%, which is a little higher than of 2% Tween 20 formulation that close to 0.006%.

0.5% Tween 20 formulation shows negligible results in every hour.

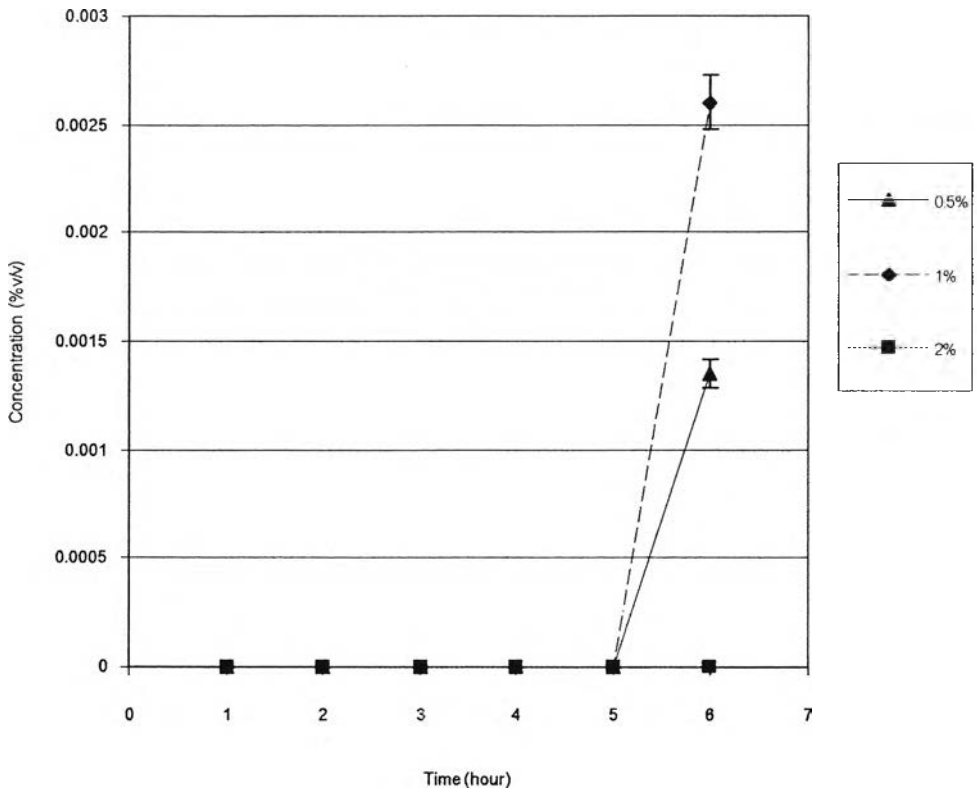

Figure 15 Concentration (%v/v) of terpinen-4-ol release from different concentration of Brij 97 formulations versus time (hour)

It can be seen from the figure 15 that in all concentration of Brij 97 formulations, negligible results are reported from 1 hour to 5 hour. However, at 6 hour, 1% Brij 97 formulation shows the highest release at just slightly over 0.0025%. The release of 0.5% Brij 97 formulation is substantially lower than that of 1% Brij 97 formulation by approximately 0.001%, whereas, 2% Brij 97 formulation shows negligible result.

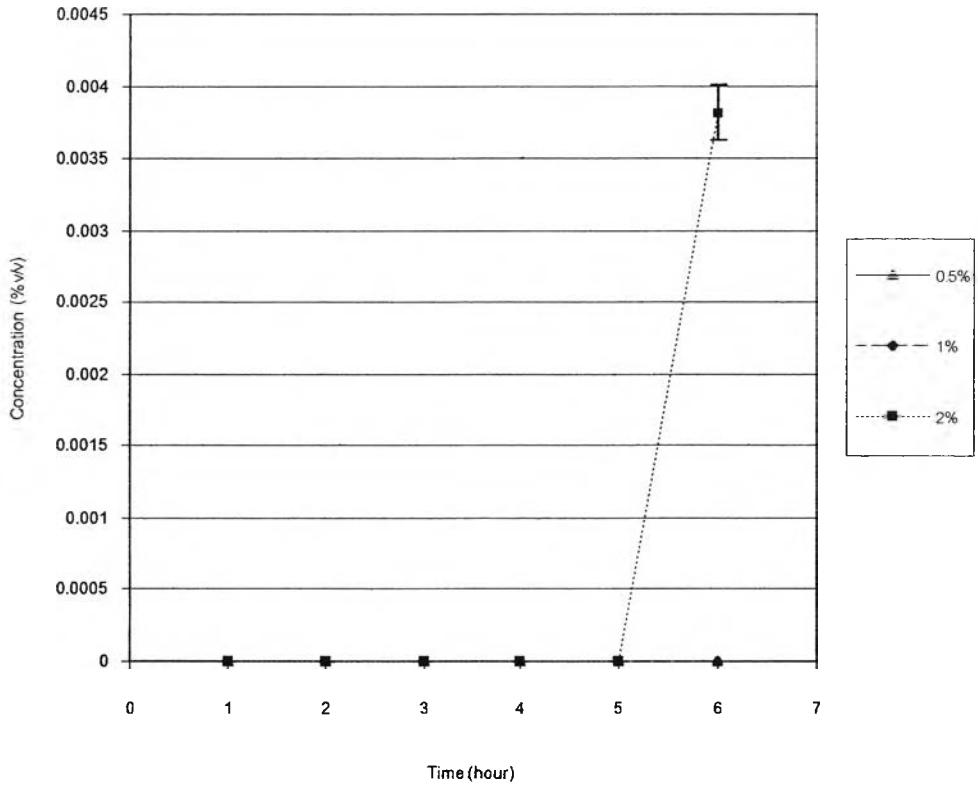

Figure 16 Concentration (%v/v) of terpinen-4-ol release from different concentration of Tween 80 formulations versus time (hour)

According to figure 16, every concentration of Tween 80 formulations shows negligible result. However, at 6 hour, 2% Tween 80 formulation illustrates the release of just under 0.004%.

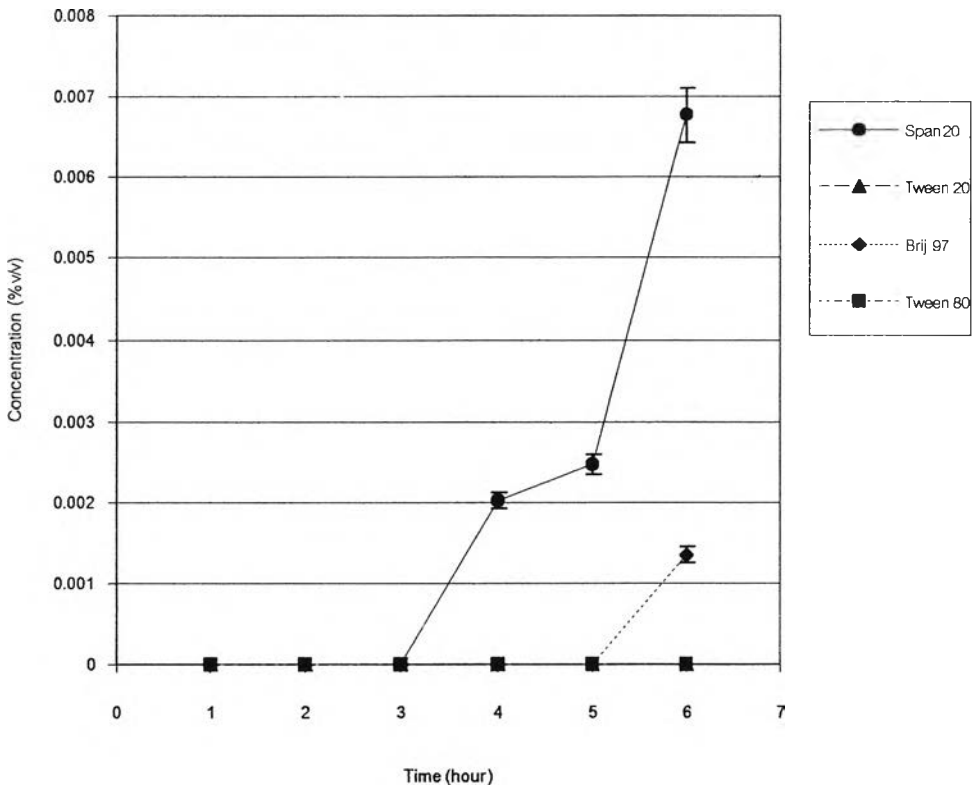

Figure 17 Concentration (%v/v) of terpinen-4-ol release from 0.5% different emulsifiers versus time (hour)

According to figure 17, Span 20 illustrates initially release at after 3 hour, which is precisely 0.002% at 4 hour, and slightly increases to over 0.002% at 5 hour. From this point, the release considerably increases to nearly 0.007% at 6 hour. Formulation of Brij 97 represents the release only at after 5 hour, which constitutes well over 0.001%, whereas formulation of Tween 20 and Tween 80 show negligible release in every hour.

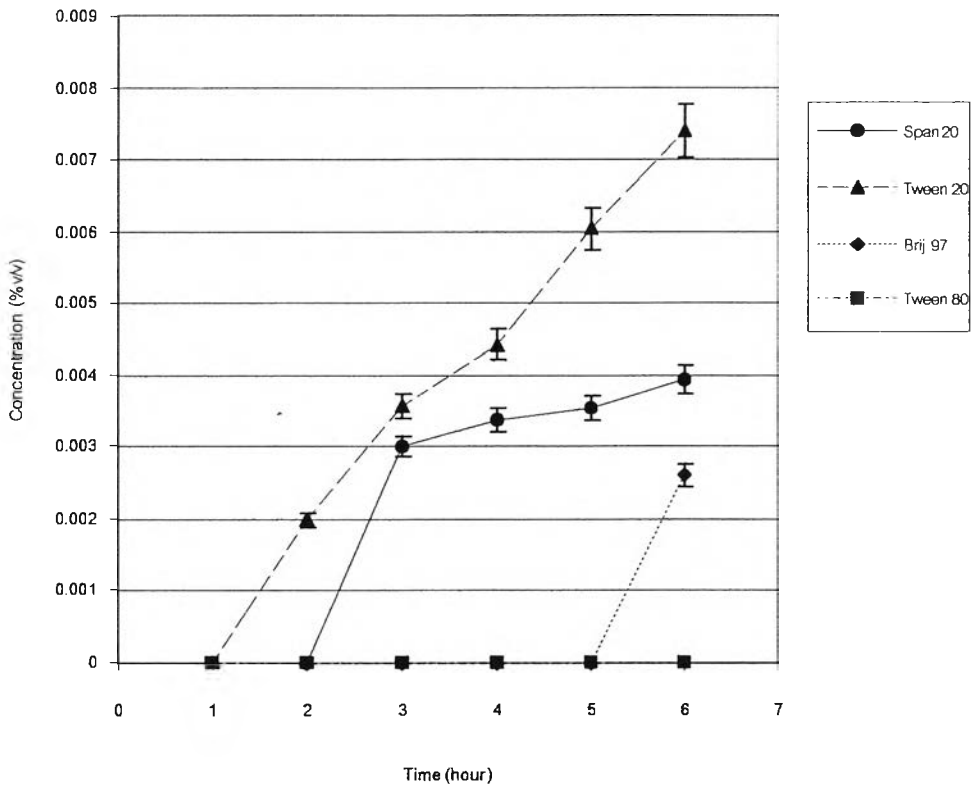

Figure 18 Concentration (%v/v) of terpinen-4-ol release from 1% different emulsifiers versus time (hour)

From figure 18, formulation using Tween 20 as emulsifier is the best release formulation, which steadily release from 1 hour to 6 hour.

Formulation of Span 20 shows initially release at 3 hour, which is precisely 0.003%, and slowly release to nearly 0.004% at 6 hour.

Formulation of Brij 97 represents the release only at 6 hour, which makes up well under 0.003%.

Formulation of Tween 80 illustrates negligible result in every hour.

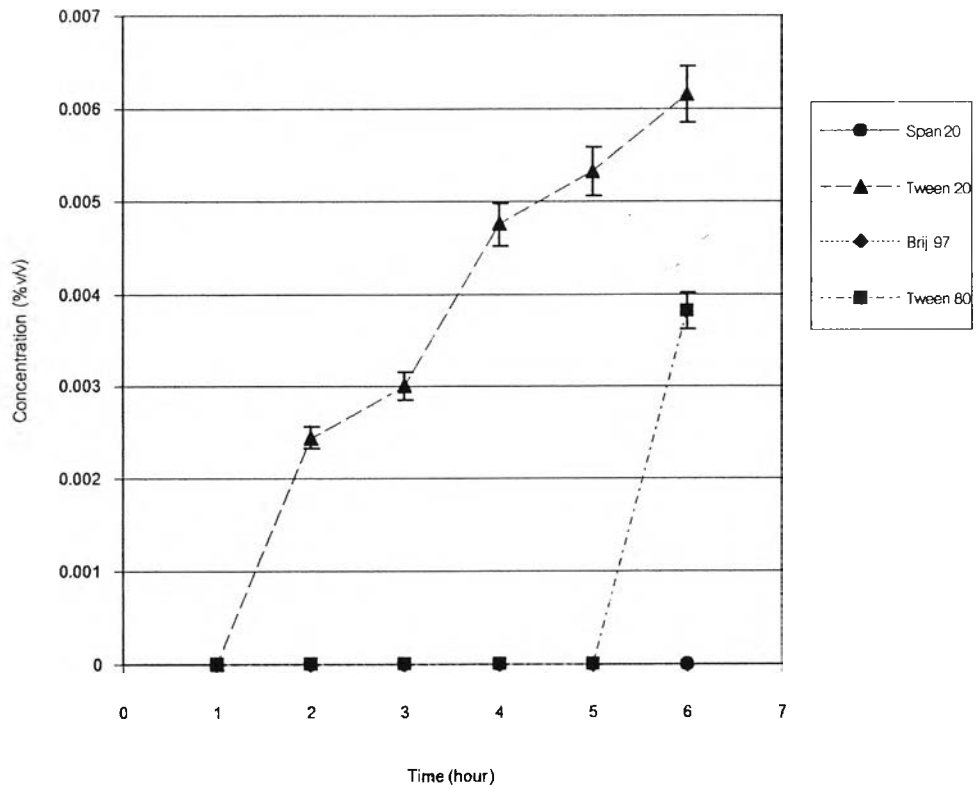

Figure 19 Concentration (%v/v) of terpinen-4-ol release from 2% different emulsifiers versus time (hour)

As is shown in figure 19, formulation of Tween 20 shows the best release characteristic with steadily release from 1 hour to 6 hour. The release of Tween 80 formulation appears only at 6 hour, which is slightly under 0.004%. Formulation of Span 20 and Brij 97 represent negligible release in every hour.

Stability of formulations

Table 3 Stability of tea tree oil gel formulations

Formulations	Appearance	After 3 months	After 6 months	After 9 months
Tea tree oil gel (without emulsifier)	Translucent gel	Separation	Separation	Separation
Tea tree oil gel with 0.5% Span 20	Opaque gel	Stable	Separation	Separation
Tea tree oil gel with 1% Span 20	Opaque gel	Stable	Separation	Separation
Tea tree oil gel with 2% Span 20	Opaque gel	Stable	Separation	Separation
Tea tree oil gel with 0.5% Tween 20	Opaque gel	Separation	Separation	Separation
Tea tree oil gel with 1% Tween 20	Opaque gel	Stable	Stable	Stable
Tea tree oil gel with 2% Tween 20	Translucent gel	Stable	Stable	Stable
Tea tree oil gel with 0.5% Brij 97	Translucent gel	Separation	Separation	Separation
Tea tree oil gel with 1% Brij 97	Translucent almost opaque gel	Separation	Separation	Separation
Tea tree oil gel with 2% Brij 97	Translucent almost opaque gel	Separation	Separation	Separation
Tea tree oil gel with 0.5% Tween 80	Translucent gel	Separation	Separation	Separation
Tea tree oil gel with 1% Tween 80	Translucent gel	Separation	Separation	Separation
Tea tree oil gel with 2% Tween 80	Translucent gel	Separation	Separation	Separation

From table 3, the translucent gel of tea tree oil without emulsifier was unstable as it separated within 3 months.

Tea tree oil gel with Span 20 in all three formulations were stable in the first three months, but separated after leaving for 6 months.

Tea tree oil gel with 0.5% Tween 20 formulation separated within 3 months due to less emulsifier, in contrast, 1% and 2% Tween 20 formulations are stable in all time of determination.

All formulations of Brij 97 and Tween 80 separated within 3 months, since the emulsifiers are not suitable for the system.

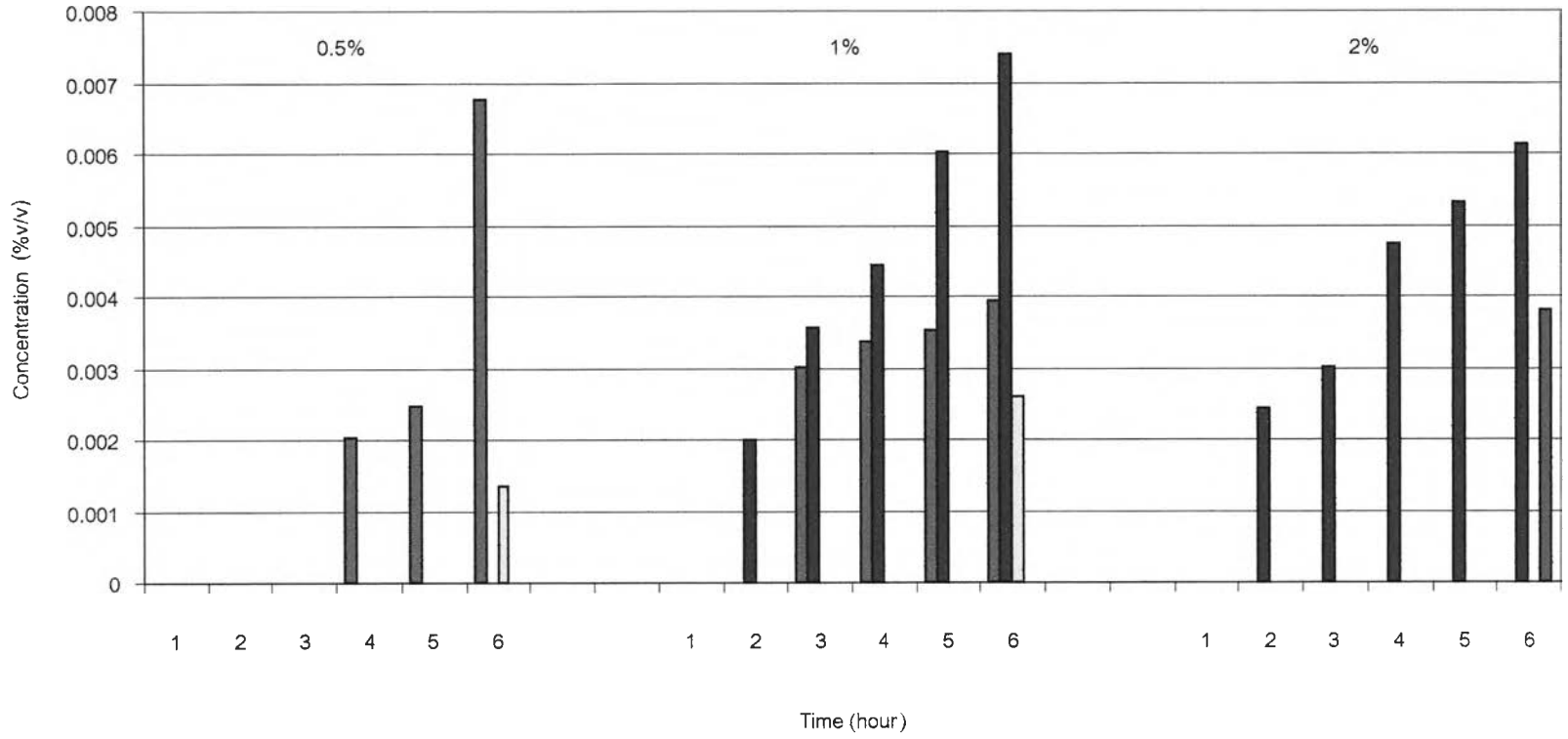

Figure 20 Concentration (%v/v) of terpinen-4-ol release from different emulsifiers with different concentrations of emulsifier in formulations versus time (hour): ■=Span 20; ■=Tween 20; ■=Brij 97; ■=Tween 80

According to figure 20, 1% and 2% of Tween 20 formulations show steadily release in every hour. In contrast, 1% and 0.5% Span 20 formulations illustrate some inconsistent release. As it can be seen in 1% Span 20 formulation, there is no release at 1 hour and 2 hour, but first release appears at 3 hour. In addition, 0.5% Span 20 formulation represents the first release at 4 hour, which is similar to the release at 5 hour, whereas, the release at 6 hour is dramatically higher than that at 5 hour. It can be concluded that Span 20 formulation represents the inconsistent release characteristic.

Brij 97 and Tween 80 formulations show some release only at 6 hour, thus these surfactants should not be used as emulsifier in tea tree oil emulgel, which gel base is hydrogel.

In topical formulations, dissolving of active ingredient in base is very important since dissolvable ingredient can stay in formulation and can be released to skin. In this study, formulation base is hydrogel, which is very hydrophilic. Even though terpinen-4-ol is partly dissolve in water, because of OH group in structure, whole tea tree oil is oil and composed of many components that are lipophilic, therefore surfactant system is necessary to be used for formulating the emulgel of tea tree oil.

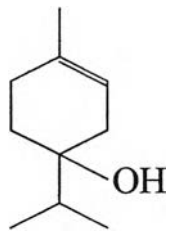

Figure 21 Structure of terpinen-4-ol, the main component of tea tree oil

Figure 21 illustrates structure of terpinen-4-ol, the main component of tea tree oil, which has alcohol group and terpine ring. Alcohol is a hydrophilic group, which requires high HLB value emulsifier to dissolve it in water, while terpine ring is a lipophilic group, which requires low HLB emulsifier to dissolve it in water. HLB value of Tween 20 is just under 17, which is very high hydrophilic, thus it can interact very well with alcohol group in terpinen-4-ol structure and dissolve very well in hydrogel. Therefore, the release of Tween 20 formulations is consistent in both 1% and 2% formulations. On the contrary, 0.5% Tween 20 formulation represents no release in every hour due to less emulsifier in formulation.

Span 20 has HLB value around 9, which is lipophilic, thus it can interact with terpine ring and dissolve in hydrogel. However, at high Span 20 concentration, which is 2%, the emulsifier is too much for hydrophilic gel system, thus terpinen-4-ol can not dissolve in hydrogel and represents no release at all.

Brij 97 (HLB 12.4) and Tween 80 (HLB 15) have moderate HLB value that cannot dissolve terpinen-4-ol very well in hydrogel, therefore it can release only at 6 hour.

Increasing of emulsifier concentration should increase the release of terpinen-4-ol from formulations since the increase of emulsifier will increase solubility of terpinen-4-ol in gel base. However, the release characteristic of terpinen-4-ol from all formulations seems to be haphazard, for example, 1% Tween 20 formulation shows higher release than 2% Tween 20 formulation. When increase emulsifier concentration, the reduction in release characteristic of terpinen-4-ol might be because of the reduction of the concentration of terpinen-4-ol in the continuous phase. (Ktistis and Niopas 1998:413-418) Therefore, emulsifiers should be used in the optimum concentration in every formulation.

In order to make this study more complete, further experiment could be conducted to determine the antiviral activity of these gel formulations, especially determine the susceptibility of herpes simplex virus type 1 and 2 (HSV-1 and HSV-2) to tea tree oil gel formulations.

This experiment has some limitation due to volatile component, terpinen-4-ol, which is the main component of tea tree oil. Volatility may happen between the experiment periods and cause some error. Beside, more carbomer have been used in the formulations. Crosslink of gelling agent may occur and affect the release characteristic of terpinen-4-ol, main component of tea tree oil, from the formulations.