III. RESULTS

PART I

1. Fibrinogen preparation

All of the precipitates of fibrinogen preparation from cryoprecipitaton, repeat cryoprecipitation, saturated ammonium sulfate precipitation, saturated ammonium sulfate precipitation and followed by cryoprecipitation, absolute ethanol precipitation, absolute ethanol precipitation and followed by cryoprecipitation, 10% ethanol precipitation, and 10% ethanol precipitation and followed by cryoprecipitation were dissolved in the supernate that leaved for reconstitution except the precipitate from polyethylene glycol precipitation (2, 4, 10, 15 and 30%) and polyethylene glycol precipitation (2, 4, 10, 15 and 30%) and followed by cryoprecipitation. The result was shown that the stickiness of precipitate was increased when the percentage of polyethylene glycol was increased. The precipitate from 10% ethanol precipitation and 10% ethanol precipitation and followed by cryoprecipitation were also not dissolved after stored at –20 °C which were not be used for further determinations.

2. Determination of protein

In order to evaluate the fibrinogen soluion, the protein in plasma and all of the fibrinogen solutions from precipitations were determined by Biuret's method. Protein in CPD-plasma was 6.6 $\pm$ 0.5 g/dl (n=60). The maximal to minimal represented as g/dl in mean $\pm$ SD were from saturated ammonium sulfate precipitation and followed by cryoprecipitation 8.7 $\pm$ 2.2 (n=16), absolute ethanol precipitation 8.3 $\pm$ 2.9 (n=22), repeat cryoprecipitation 8.0 $\pm$ 2.2 (n=17), absolute ethanol precipitation and followed by cryoprecipitation 7.6 $\pm$ 1.9 (n=14), cryoprecipitaton 7.1 $\pm$ 1.3 (n=17)

and saturated ammonium sulfate precipitation 6.9 ± 1.8 (n=17). There was no statistically different ($p>0.05$) (Fig.17).

3. **Determination of albumin**

In order to evaluate the fibrinogen solution, the albumin in plasma and all of the fibrinogen solutions from precipitations were determined by Bromcresyl green method. Albumin in CPD-plasma was 4.3 ± 0.3 g/dl (n=9). The maximal to minimal represented as g/dl in mean ± SD were from cryoprecipitation 4.5 ± 0.8 (n=12), repeat cryoprecipitation 4.4 ± 0.8 (n=13), saturated ammonium sulfate precipitation and followed by cryoprecipitation 4.4 ± 0.7 (n=13), saturated ammonium sulfate precipitation 4.0 ± 0.6 (n=13), absolute ethanol precipitation and followed by cryoprecipitation 3.6 ± 1.2 (n=8) and absolute ethanol precipitation 3.2 ± 0.6 (n=9). There was statistically different ($p<0.05$) (Fig. 18).

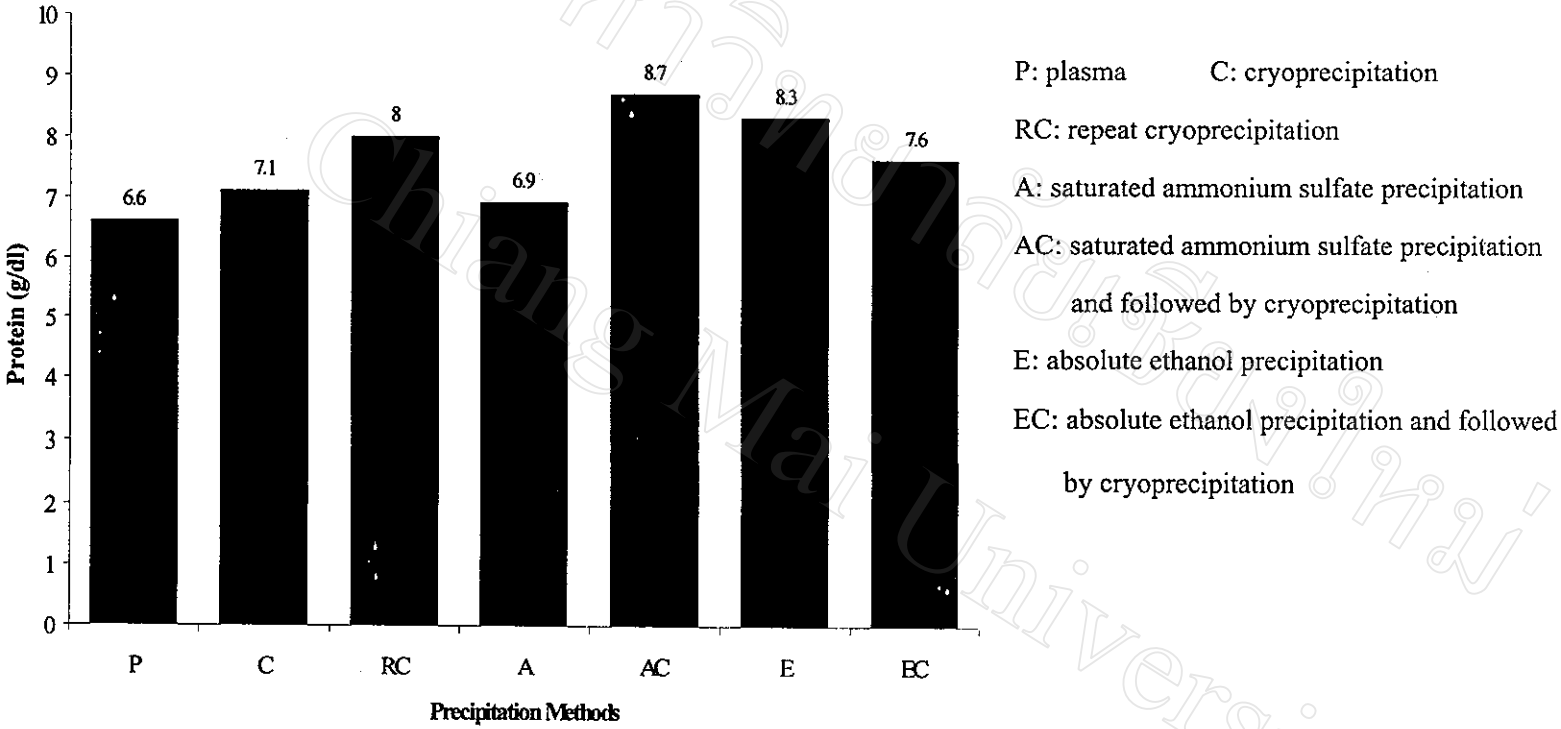
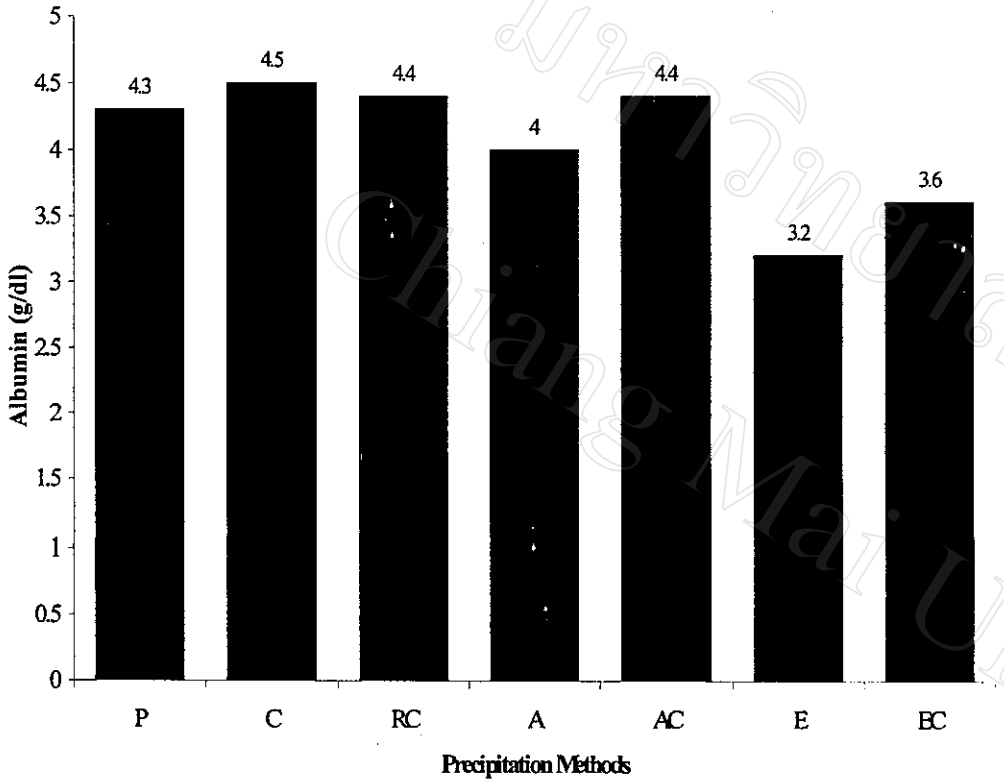


Figure 17 Protein in plasma and all of fibrinogen solutions



P: plasma C: cryoprecipitation

RC: repeat cryoprecipitation

A: saturated ammonium sulfate precipitation

AC: saturated ammonium sulfate precipitation and followed by cryoprecipitation

E: absolute ethanol precipitation

EC: absolute ethanol precipitation and followed by cryoprecipitation

4. Determination of fibrinogen concentration

4.1 Modified thrombin time method

In order to evaluate the fibrinogen, the fibrinogen concentration in plasma and all of the fibrinogen solutions from precipitations were determined by modified thrombin time method. Fibrinogen calibration curve was shown in figure 19. Fibrinogen concentration in plasma was 271.5 ± 57.0 mg/dl (n=23). The maximal to minimal represented as mg/dl in mean ± SD were from saturated ammonium sulfate precipitation and followed by cryoprecipitation 1452.7 ± 515.0 (n=15), saturated ammonium sulfate precipitation 1373.0 ± 468.5 (n=15), repeat cryoprecipitation 1251.3 ± 508.5 (n=15), absolute ethanol precipitation 825.3 ± 209.8 (n=10), cryoprecipitaton 735.2 ± 325.8 (n=15) and absolute ethanol precipitation and followed by cryoprecipitation 554.8 ± 254.2 (n=7). Fibrinogen solutions in cryoprecipitate from Thai Red Cross Society was 892.0 ± 108.4 mg/dl (n=10). Fibrinogen concentration in these fibrinogen solutions determined by modified thrombin time method was statistically different ($p<0.05$) (Fig. 20). Clottable fibrinogen was maximal in fibrinogen solution from saturated ammonium sulfate precipitation and followed by cryoprecipitation, and minimal in fibrinogen solution from absolute ethanol precipitation and followed by cryoprecipitation.

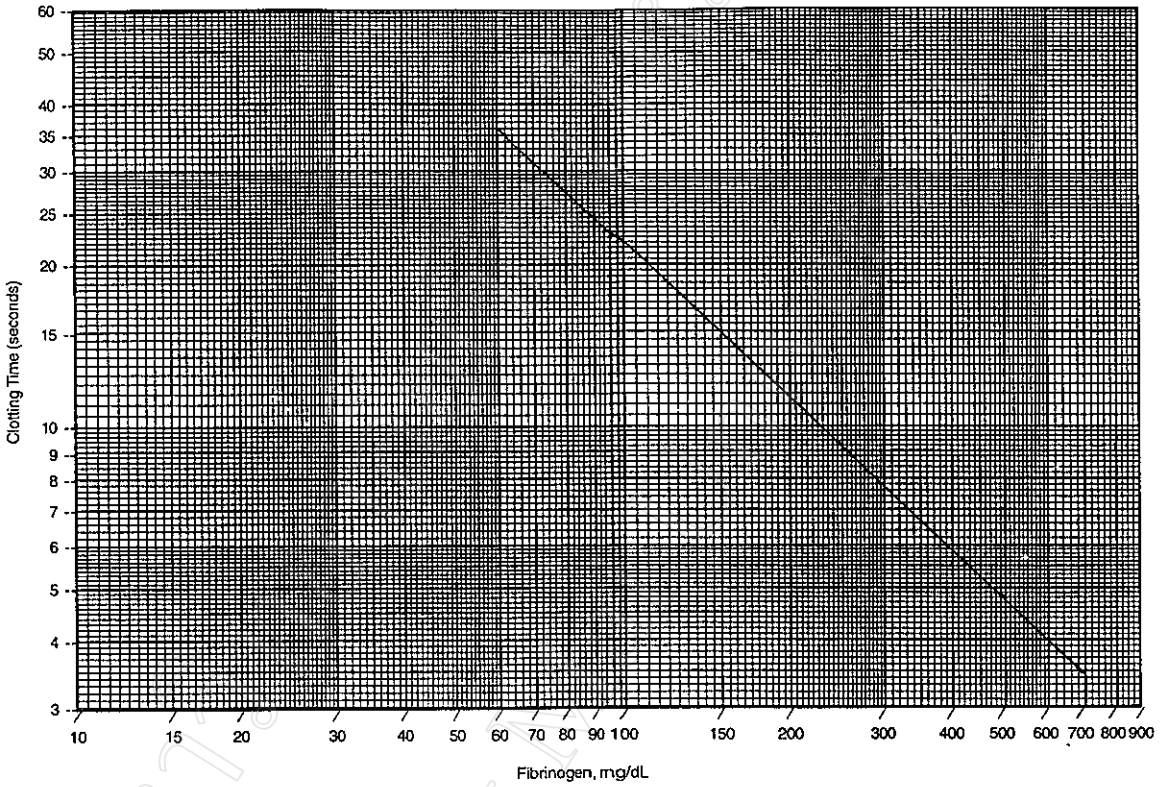
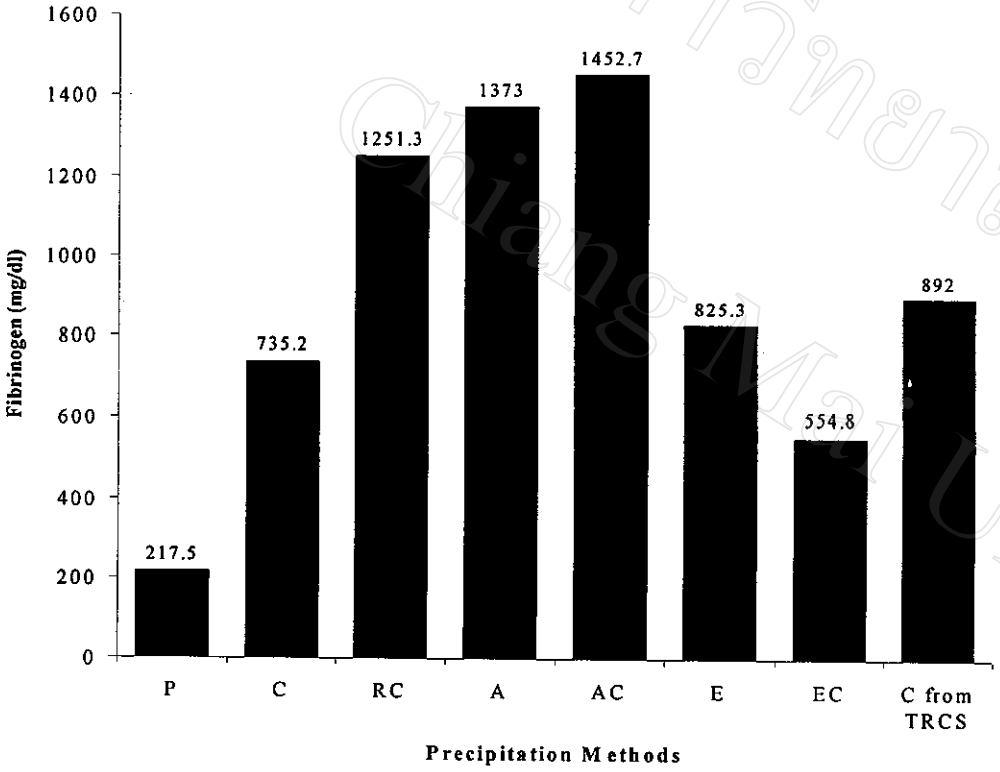


Figure 19 Fibrinogen calibration curve (modified thrombin time method)



P: plasma C: cryoprecipitation

RC: repeat cryoprecipitation

A: saturated ammonium sulfate precipitation

AC: saturated ammonium sulfate precipitation and followed by cryoprecipitation

E: absolute ethanol precipitation

EC: absolute ethanol precipitation and followed by cryoprecipitation

C from TRCS: cryoprecipitate from Thai Red Cross Society

Figure 20 Fibrinogen in plasma and all of fibrinogen solutions by modified thrombin time method

4.2 **Determination of fibrinogen concentration by Ratnoff's method**

In order to evaluate the fibrinogen, the fibrinogen concentration in plasma and all of the fibrinogen solutions from precipitations were determined by Ratnoff's method. Fibrinogen concentration in CPD-plasma was 171.5 $\pm$ 48.1 mg/dl (n=10). The maximal to minimal represented as mg/dl in mean $\pm$ SD were from repeat cryoprecipitation 1,160.3 $\pm$ 456.5 (n=10), saturated ammonium sulfate precipitation and followed by cryoprecipitation 963.6 $\pm$ 218.2 (n=10), cryoprecipitation 856.5 $\pm$ 411.8 (n=10) and saturated ammonium sulfate precipitation 706.6 $\pm$ 259.4 (n=10). Fibrinogen solutions in cryoprecipitate from Thai Red Cross Society was 902.3 $\pm$ 130.0 mg/dl (n=4). Fibrinogen concentration in these fibrinogen solutions determined by Ratnoff's method was statistically different ($p<0.05$) (Fig. 21). Tyrosine-liked activity in fibrinogen solution from repeat cryoprecipitation was maximal. It was minimal in fibrinogen solution from saturated ammonium sulfate precipitation.

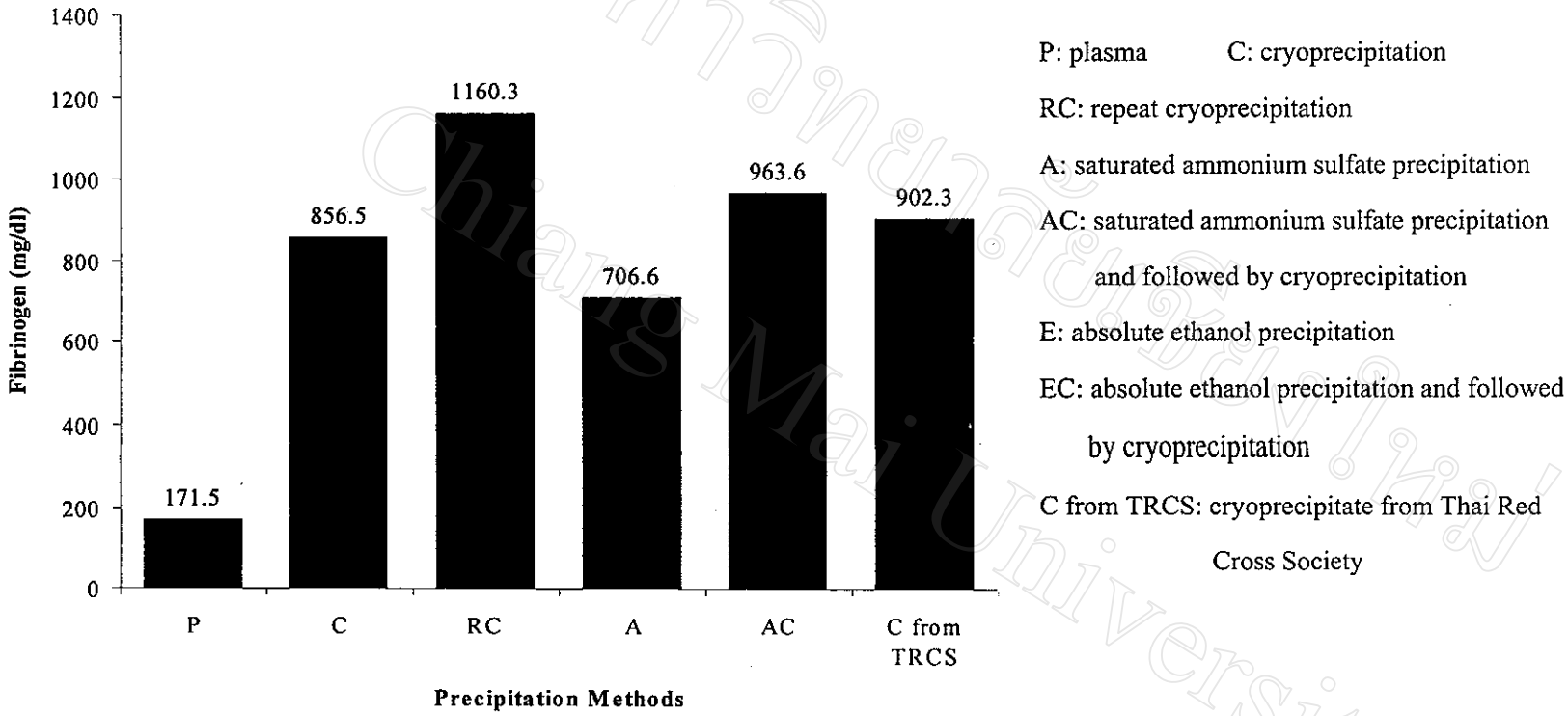
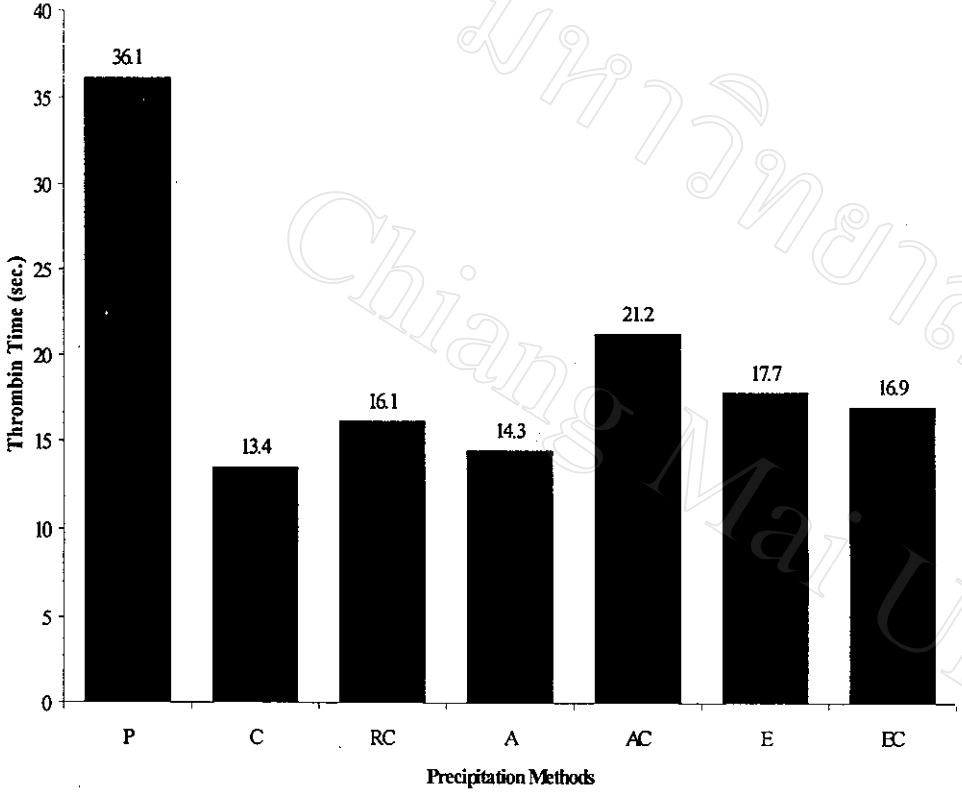


Figure 21 Fibrinogen in plasma and all of fibrinogen solutions by Ratnoff's method

5. **Determination of the quality of fibrinogen**

In order to evaluate the fibrinogen, the quality of fibrinogen in plasma and all of the fibrinogen solution from precipitations were determined by thrombin time method. Thrombin time from CPD-plasma was 36.1 ± 6.3 seconds (n=37). The shortest to longest thrombin time from fibrinogen solutions represented as seconds in mean ± SD were from cryoprecipitation 13.4 ± 6.3 (n=17), saturated ammonium sulfate precipitation 14.3 ± 5.9 (n=16), repeat cryoprecipitation 16.1 ± 17.0 (n=17), absolute ethanol precipitation and followed by cryoprecipitation 16.9 ± 15.4 (n=11), absolute ethanol precipitation 17.7 ± 15.3 (n=22), and saturated ammonium sulfate precipitation and followed by cryoprecipitation 21.2 ± 13.1 (n=16). Thrombin time from these fibrinogen solutions was no statistically different ($p>0.05$) (Fig. 22). The quality of fibrinogen in these fibrinogen solutions was not differed.



P: plasma C: cryoprecipitation

RC: repeat cryoprecipitation

A: saturated ammonium sulfate precipitation

AC: saturated ammonium sulfate precipitation and followed by cryoprecipitation

E: absolute ethanol precipitation

EC: absolute ethanol precipitation and followed by cryoprecipitation

Figure 22 Thrombin time of fibrinogen in plasma and all of fibrinogen solutions

6. **Determination of the quantity and quality of factor XIII**

In order to evaluate the fibrinogen solution, the quantity and quality of factor XIII in plasma and all of the fibrinogen solution from precipitations were as followed.

Thirty minutes after adding 0.025 M $CaCl_2$, plasma and all of the fibrinogen solutions were formed fibrin clot except the fibrinogen solutions from saturated ammonium sulfate precipitation, and saturated ammonium sulfate precipitation and followed by cryoprecipitation. Thirty minutes after adding 5 M urea solution, all of the fibrin clot that formed was not dissolved. Twenty-four hours after adding 5 M urea solution, the fibrin clot from plasma, and the fibrinogen solutions from cryoprecipitation and repeat cryoprecipitation were not dissolved but from absolute ethanol precipitation and absolute ethanol precipitation followed by cryoprecipitation were dissolved (Table 4).

Thirty minutes after adding 5 NIH units/ml thrombin, plasma and all of the fibrinogen solution were formed fibrin clot. Thirty minutes after adding 5 M urea solution, all of the fibrin clot that formed was not dissolved. Twenty-four hours after adding 5 M urea, the fibrin clot from plasma, and the fibrinogen solution from cryoprecipitation and repeat cryoprecipitation were not dissolved but from saturated ammonium sulfate precipitation, saturated ammonium sulfate precipitation and followed by cryoprecipitation, absolute ethanol precipitation, and absolute ethanol precipitation and followed by cryoprecipitation were dissolved (Table 5).

The fibrinogen solutions from cryoprecipitation and repeat cryoprecipitation followed by adding $CaCl_2$ or thrombin were contained sufficient factor XIII to stabilize fibrin clot. But fibrinogen solution from saturated ammonium sulfate precipitation, saturated ammonium sulfate precipitation and followed by cryoprecipitation, absolute ethanol precipitation, and absolute ethanol precipitation and followed by cryoprecipitation

were contained factor XIII less than 2%. It was insufficient factor XIII to stabilize fibrin.

7. **Thrombin preparation**

In order to prepare thrombin for clinical used, absolute ethanol, saturated ammonium sulfate and polyethylene glycol were used as precipitating agents including acetone as the original method. Thrombin solutions from acetone, absolute ethanol and 50% PEG 8000 had thrombin-liked activity. They could convert fibrinogen to fibrin. Thrombin-liked activity in these thrombin solutions was compared with 10 NIH units/ml commercial thrombin (Sigma Co.). Thrombin time from commercial thrombin (10 NIH units/ml), thrombin solution from acetone, absolute ethanol and 50% PEG 8000 precipitation which not longer than 40 seconds were in dilution 1:8, 1:32, 1:32, and 1:16, respectively. So the approximately thrombin-liked activity in thrombin solution from acetone, absolute ethanol and 50% PEG 8000 precipitation were 40, 40 and 20 NIH units/ml, respectively. While as thrombin solution from saturated ammonium sulfate precipitation had not thrombin-liked activity, they could not converted fibrinogen to fibrin. All dilutions of thrombin solution from saturated ammonium sulfate precipitation were longer than 200 seconds. Therefore, thrombin solution from absolute ethanol precipitation was suitable to use in this study (Table 6).

Table 4 Urea solubility test (by adding 0.025M $CaCl_2$)

Preparation methods	**Fibrin clot**		
	After adding $CaCl_2$	**After adding 5M urea**	
		30 min.	**24 hrs.**
Plasma	C	-	-
Cryoprecipitation	C	-	-
Repeat cryoprecipitation	C	-	-
Absolute ethanol precipitation	C	-	+
Absolute ethanol precipitation and followed by cryoprecipitation	C	-	+

C: clot +: clot dissolved -: clot not dissolved

Remark: Saturated ammonium sulfate precipitation, saturated ammonium sulfate precipitation and followed by cryoprecipitation were not formed clot after adding 0.025 M $CaCl_2$.

Table 5 Urea solubility test (by adding 5 NIH units/ml thrombin)

Preparation methods	**Fibrin clot**		
	After adding thrombin	**After adding 5M urea**	
		30 min.	**24 hrs.**
Plasma	C	-	-
Cryoprecipitation	C	-	-
Repeat cryoprecipitation	C	-	-
Saturated ammonium sulfate precipitation	C	-	+
Saturated ammonium sulfate precipitation and followed by cryoprecipitation	C	-	+
Absolute ethanol precipitation	C	-	+
Absolute ethanol precipitation and followed by cryoprecipitation	C	-	+

C: clot +: clot dissolved -: clot not dissolved

Table 6 Thrombin time derived from thrombin solution prepared by various precipitating agents in several dilutions.

Thrombin from	Thrombin time (sec)						
	Thrombin dilution						
	Undiluted	**1:2**	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**
Commercial Thrombin (10 NIH units/ml)	62.6	34.7	26.2	28.7	83.5	>200	>200
Acetone	32.5	19.5	18.5	22.1	32.1	39.9	>200
Absolute ethanol	20.5	17.3	16.5	19.1	29.9	33.4	>200
Saturated Ammonium Sulfate	>200	>200	>200	>200	>200	>200	>200
50% polyethylene glycol 8000	15.9	16.7	18.1	25.4	39.6	>200	>200

8. **Selection of an appropriate method for fibrinogen and thrombin preparation**

From the results in determination of protein by Biuret's method, albumin by Bromcresol Green method, quantity of fibrinogen by modified thrombin time and Ratnoff's method, quality of fibrinogen by thrombin time method, and quality and quantity of factor XIII by urea solubility test, it was concluded that fibrinogen solution from repeat cryoprecipitation and cryoprecipitation were appropriate to use as a source of fibrinogen for fibrin glue rather than others. Fibrinogen solutions from cryoprecipitation and repeat cryoprecipitation were contained adequate clottable fibrinogen concentration, sufficient factor XIII, less interference and simple preparative method. Although both methods of absolute ethanol and saturated ammonium sulfate precipitation were contained adequate clottable fibrinogen concentration, respectively, they were contained insufficient factor XIII to stabilize fibrin clot. Therefore fibrinogen from cryoprecipitation and repeat cryoprecipitation were prepared in lyophilized form for further study.

An appropriate method for thrombin preparation was selected by its activity, which determined by modified thrombin time. It was concluded that thrombin solution from absolute ethanol was appropriate to use in this study. It had high thrombin-liked activity and simple preparative method. Therefore thrombin solution from absolute ethanol precipitation was prepared in lyophilized form for further study.

9. **Fibrinogen and thrombin preparation in lyophilized form**

The lyophilized fibrinogen and thrombin was dried to yellow and white precipitate, respectively.

PART II

1. Composition of prepared fibrin glue

All of 3 methods of prepared fibrin glue could solubilized in 37 °C waterbath for 10 minutes.

2. Determination of the stability of fibrin glue

In order to evaluate a stability of prepared fibrin glue, fibrinogen from cryoprecipitation and repeat cryoprecipitation were mixed with three concentration of EACA, an antifibrinolytic agent, (7.5, 10.0, 12.5 mg/ml). They were applied with slow-freeze thrombin or quick-freeze thrombin solution by two ratios. In both ratios, the results were demonstrated that all concentration of EACA could prevent autofibrinolysis. All of prepared fibrin glue was stabilize fibrin clot more than 7 days (Table 7 and 8).

In order to evaluate the stability of fibrin glue prepared by Thai Red Cross Society, which used 12.5 mg/ml transamine as antifibrinolytic agent, the result was shown that it could also prevent autofibrinolysis. Fibrin glue was stabilized more than 7 days (Table 9).

Table 7 The stability of fibrin glue from fibrinogen prepared by cryoprecipitation

Day	Tims (hrs.)	Clot dissolution					
		Concentration of EACA (mg/ml) in fibrinogen solution from cryoprecipitation					
		7.5 (Method 1)		10.0 (Method 2)		12.5 (Method 3)	
		SFT	QFT	SFT	QFT	SFT	QFT
1	6	-	-	-	-	-	-
	12	-	-	-	-	-	-
	18	-	-	-	-	-	-
	24	-	-	-	-	-	-
2	30	-	-	-	-	-	-
	36	-	-	-	-	-	-
	42	-	-	-	-	-	-
	48	-	-	-	-	-	-
3	54	-	-	-	-	-	-
	60	-	-	-	-	-	-
	66	-	-	-	-	-	-
	72	-	-	-	-	-	-
4	78	-	-	-	-	-	-
	84	-	-	-	-	-	-
	90	-	-	-	-	-	-
	96	-	-	-	-	-	-
5	102	-	-	-	-	-	-
	108	-	-	-	-	-	-
	114	-	-	-	-	-	-
	120	-	-	-	-	-	-
6	126	-	-	-	-	-	-
	132	-	-	-	-	-	-
	138	-	-	-	-	-	-
	144	-	-	-	-	-	-
7	150	-	-	-	-	-	-
	156	-	-	-	-	-	-
	162	-	-	-	-	-	-
	168	-	-	-	-	-	-

SFT: slow-freeze thrombin QFT: quick-freeze thrombin +: clot dissolved -: clot not dissolved

Table 8 The stability of fibrin glue from fibrinogen prepared by repeat cryoprecipitation

Day	Times (hrs.)	Clot dissolution					
		Concentration of EACA (mg/ml) in fibrinogen solution from cryoprecipitation					
		7.5 (Method 1)		10.0 (Method 2)		12.5 (Method 3)	
		SFT	QFT	SFT	QFT	SFT	QFT
1	6	-	-	-	-	-	-
	12	-	-	-	-	-	-
	18	-	-	-	-	-	-
	24	-	-	-	-	-	-
2	30	-	-	-	-	-	-
	36	-	-	-	-	-	-
	42	-	-	-	-	-	-
	48	-	-	-	-	-	-
3	54	-	-	-	-	-	-
	60	-	-	-	-	-	-
	66	-	-	-	-	-	-
	72	-	-	-	-	-	-
4	78	-	-	-	-	-	-
	84	-	-	-	-	-	-
	90	-	-	-	-	-	-
	96	-	-	-	-	-	-
5	102	-	-	-	-	-	-
	108	-	-	-	-	-	-
	114	-	-	-	-	-	-
	120	-	-	-	-	-	-
6	126	-	-	-	-	-	-
	132	-	-	-	-	-	-
	138	-	-	-	-	-	-
	144	-	-	-	-	-	-
7	150	-	-	-	-	-	-
	156	-	-	-	-	-	-
	162	-	-	-	-	-	-
	168	-	-	-	-	-	-

SFT: slow-freeze thrombin QFT: quick-freeze thrombin +: clot dissolved -: clot not dissolved

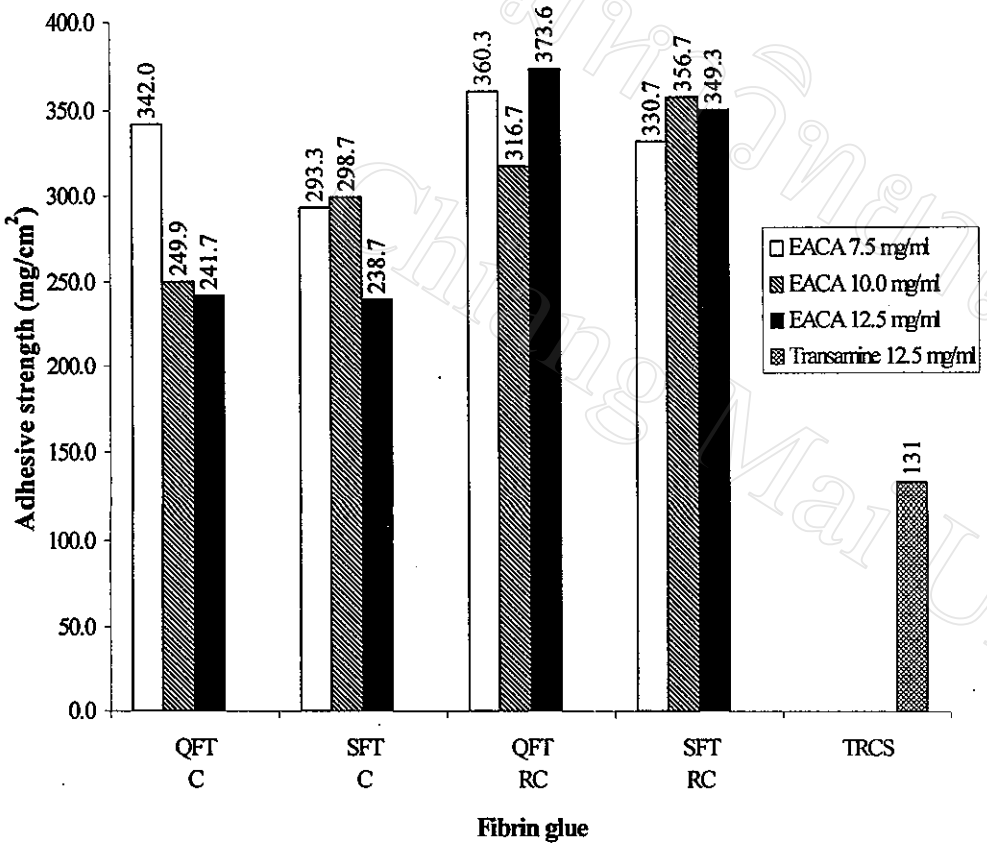
Table 9 The stability of fibrin glue prepared by Thai Red Cross Society

Day	Times (Hrs.)	Clot dissolution
1	6	-
	12	-
	18	-
	24	-
2	30	-
	36	-
	42	-
	48	-
3	54	-
	60	-
	66	-
	72	-
4	78	-
	84	-
	90	-
	96	-
5	102	-
	108	-
	114	-
	120	-
6	126	-
	132	-
	138	-
	144	-
7	150	-
	156	-
	162	-
	168	-

+: clot dissolved -: clot not dissolved

3. **Determination of adhesive strength of fibrin glue**

Adhesive strength of three sets of prepared fibrin glue from both ratios that determined 10 minutes after application were less than 5 g/cm^2. Adhesive strength of three sets of prepared fibrin glue from the first ratio that determined 30 minutes after application was less than 5 g/cm^2. The results showed that BOTTOM was immediately separated from TOP after it was placed on the stand. Adhesive strength of three sets of prepared fibrin glue from the second ratio that determined 30 minutes after application was determined. Adhesive strengths of fibrin glue derived from (1) fibrinogen solution from cryoprecipitation and quick-freeze thrombin solution, (2) fibrinogen solution from cryoprecipitation and slow-freeze thrombin solution, (3) fibrinogen solution from repeat cryoprecipitation and quick-freeze thrombin solution, and (4) fibrinogen from cryoprecipitation and slow-freeze thrombin solution were 342.0 $\pm$ 40.6, 293.3 $\pm$ 32.1, 360.3 $\pm$ 72.9 and 330.7 $\pm$ 69.3 g/cm^2, of the first set respectively; 249.9 $\pm$ 131.7, 298.7 $\pm$ 92.7, 316.7 $\pm$ 30.3 and 356.7 $\pm$ 52.6 g/cm^2, of the second set respectively; and 241.7 $\pm$ 87.8, 238.7 $\pm$ 49.8, 373.6 $\pm$ 142.8 and 349 $\pm$ 52.0 g/cm^2, of the third set respectively; while its 131.0 $\pm$ 29.9 g/cm^2 of Thai Red Cross Society. Adhesive strength from all of fibrin glue were greater than 200 g/cm^2 while as that of fibrin glue from Thai Red Cross Society was less than 200 g/cm^2 (Fig. 23).



C: cryoprecipitate

RC: repeat cryoprecipitate

QFT: quick-freeze thrombin

SFT: slow-freeze thrombin

TRCS: Thai Red Cross Society

Figure 23 Adhesive strength of fibrin glue

4. **Determination of the elasticity of fibrin glue**

The elasticity of fibrin glue derived from three set of fibrin glue in 1:1 of fibrinogen and thrombin solution that used 50 µl of fibrinogen solution and 50 µl of thrombin solution from cryoprecipitation and quick-freeze thrombin, cryoprecipitation and slow-freeze thrombin, repeat cryoprecipitation and quick-freeze thrombin, and repeat cryoprecipitation and slow-freeze thrombin was shown poor elasticity. They were not resisted to pulling.

The elasticity of fibrin glue derived from three sets of fibrin glue in 2:1 of fibrinogen and thrombin solution that used 66 µl of fibrinogen solution and 33 µl of thrombin solution from cryoprecipitation and quick-freeze thrombin, cryoprecipitation and slow-freeze thrombin, repeat cryoprecipitation and quick-freeze thrombin, and repeat cryoprecipitation and slow-freeze thrombin was shown good elasticity. They were resisted to pulling. The elasticity of all sets of fibrin glue obtained from 30 minutes after application was more than 5 mm.

The elasticity of fibrin glue prepared by Thai Red Cross Society was more than 5 mm after 30 minutes of application.

5. **Comparisons of fibrin glue of the present study with Thai Red Cross Society preparation**

Fibrinogen concentration in cryoprecipitate that prepared in non-lyophilized form and lyophilized form, and prepared by Thai Red Cross Society determined by modified thrombin time method and Ratnoff's method were not statistically difference ($p>0.05$). But fibrinogen concentration in repeat cryoprecipitate was higher. The stability and elasticity of fibrin glue from cryoprecipitate and quick-freeze thrombin, cryoprecipitate and slow-freeze thrombin, repeat cryoprecipitate and quick-freeze

thrombin, repeat cryoprecipitate and slow-freeze thrombin, and fibrin glue prepared by Thai Red Cross Society were not differed. The adhesive strength of fibrin glue from cryoprecipitate and quick-freeze thrombin, cryoprecipitate and slow-freeze thrombin, repeat cryoprecipitate and quick-freeze thrombin, repeat cryoprecipitate and slow-freeze thrombin, and fibrin glue prepared by Thai Red Cross Society were statistically difference ($p<0.05$). The adhesive strength of fibrin glue from cryoprecipitate and quick-freeze thrombin, cryoprecipitate and slow-freeze thrombin, repeat cryoprecipitate and quick-freeze thrombin, and repeat cryoprecipitate and slow-freeze thrombin were higher than fibrin glue prepared by Thai Red Cross Society. The results were shown in table 10 and 11.

Table 10 Comparison of fibrinogen concentration in prepared cryoprecipitate and repeat cryoprecipitate by the present study with cryoprecipitate prepared by Thai Red Cross Society.

	Fibrinogen Concentration (mg/dl)*	
	Modified Thrombin time (n)	**Rattnoff's (n)**
Prepared cryoprecipitate		
Non-lyophilized	735.2 ± 325.8 (15)	856.5 ± 411.8 (10)
Lyophilized	620.5 ± 64.2 (3)	958.8 ± 80.5 (3)
Prepared repeat cryoprecipitate		
Non-lyophilized	1251.3 ± 508.5 (15)	1160.3 ± 456.5 (10)
Lyophilized	688.3 ± 71.9 (3)	1104.8 ± 172.3 (3)
Cryoprecipitate prepared by Thai Red Cross Society	892.0 ± 108.4 (10)	902.3 ± 130.2 (4)

Remark: The results represent as mean ± SD

* $p>0.05$

Table 11 Comparison of stability, elasticity and adhesive strength of fibrin glue preparation of the present study with Thai Red Cross Society.

Fibrin glue from (n)	**Stability**	**Elasticity after 30 min. of application**	**Adhesive strength** (g/cm^2)**
Prepared*			
First set			
- C&QFT (3)	> 7 days	> 5 mm	342.0
- C&SFT (3)	> 7 days	> 5 mm	293.3
- RC&QFT (3)	> 7 days	> 5 mm	360.3
- RC&SFT (3)	> 7 days	> 5 mm	330.7
Second set			
- C&QFT (3)	> 7 days	> 5 mm	249.9
- C&SFT (3)	> 7 days	> 5 mm	298.7
- RC&QFT (3)	> 7 days	> 5 mm	316.7
- RC&SFT (3)	> 7 days	> 5 mm	356.7
Third set			
- C&QFT (3)	> 7 days	> 5 mm	241.7
- C&SFT (3)	> 7 days	> 5 mm	238.7
- RC&QFT (3)	> 7 days	> 5 mm	373.6
- RC&SFT (3)	> 7 days	> 5 mm	349.3
Thai Red Cross Society (10)	> 7 days	> 5 mm	131.0

* Concentration of EACA in fibrinogen solution in first, second and third set were 7.5, 10.0 and 12.5 mg/ml, respectively.

** $p<0.05$

C: cryoprecipitation RC: repeat cryoprecipitation

QFT: quick-freeze thrombin SFT: slow-freeze thrombin

Comparison of cost efficacy of fibrin glue in this present study and fibrin glue from Thai Red Cross Society.

The cost of 1 unit of fibrin glue in this present study was depended on the expense in 1 unit of CPD-blood for fibrinogen and thrombin solution preparation, 1 ml of 2% acetic acid, 1 ml of Na_2CO_3, 50 ml of 0.85% NaCl, 3 ml of 0.25 M $CaCl_2$, 25 ml of absolute ethanol, 0.025 M $CaCl_2$, gentamicin, antifibrinolytic agent and other devices. It was approximate 1,000 bahts. The cost of 1 unit of fibrin glue from Thai Red Cross Society, which contains 250 IU/ml human thrombin that used in this study, was 2,300 bahts. It was shown that the cost of fibrin glue in this present study was less than fibrin glue from Thai Red Cross Society about 2 times.