CHAPTER IV

RESULTS

4.1 Enzyme Purification

4.1.1 Partial purification of Dihydropteroate Synthase

The result of the partial purification of enzyme (the method described in section 3.7) was summarized in table 4. The purification was 417 fold, and the yield was 14%. The elution profiles of the enzyme from sulfonamide-Sepharose column (I) and sulfonamide-Sepharose column (II) were shown in figures 2 and 3 respectively. Because the amount of protein in the fractions eluted from sulfonamide-Sepharose column (II) was so little that the protein concentration could not be detected by Lowry method, hence the more sensitive coomassie brilliant blue G-250 method described in section 3.3 was used instead. However, the optical density at 595 nm was effected by sodium chloride. Figure 4 shows that 1 mol/l of sodium chloride could lower the optical density of 0.315 units. Thereby, the amount of protein in the elution profile shown in figure 3 was corrected for the sodium chloride effect. The method of correction has three steps. In the first step, the conductance values obtained from the experiment were converted to the concentration values of sodium chloride by using the standard curve shown in figure 5. In the second step, the sodium chloride concentration in each fraction obtained from the first step was converted to the OD values by using the standard curve in figure 4. In the third step, the OD_{595} values of each fraction was measured, and the mixture

Table 4 Partial purification of dihydropteroate synthase from E. coli

Purification Step	Total Volume (ml)	Protein Concentration (mg/ml)	Total Protein (mg)	Enzyme Concentration (units/ml)	Specific Activity (units/mg protein)	Total Activity (units)	Yield (%)	Purification fold
1) Crude cell-free extract	31.2	30.0	936.0	5.98	0.199	186.6	100	1
2) Ammonium sulfate precipitation (30-70% saturation fraction)	21.0	21.6	453.6	7.73	0.358	162.3	87	1.8
3) Sulfonamide-Sepharose column (I)	20.8	0.140	2.912	2.96	21.13	61.54	33	106
4) Sulfonamide-Sepharose column (II)	5.3	0.060	0.318	4.99	83.11	26.43	14	417

1 Enzyme unit is defined as the amount of enzyme that catalyzes the formation of 1.0 nmol of DHP per minute under the experimental conditions

Figure 2 Partial purification of dihydropteroate synthase from the crude enzyme by sulfonamide-Sepharose column (I). The gel column was 2.5 cm in diameter and 28 cm in height. The fractions 1-118 were eluted with buffer I with the flow rate of 21 ml/hr and the fraction volume of 11 ml. The fractions 119-240 were eluted with buffer II, and the fraction volume of 7 ml was collected. The experimental details were described in section 3.7.3.

Proteins

Dihydropteroate synthase activity

The point where buffer II was added

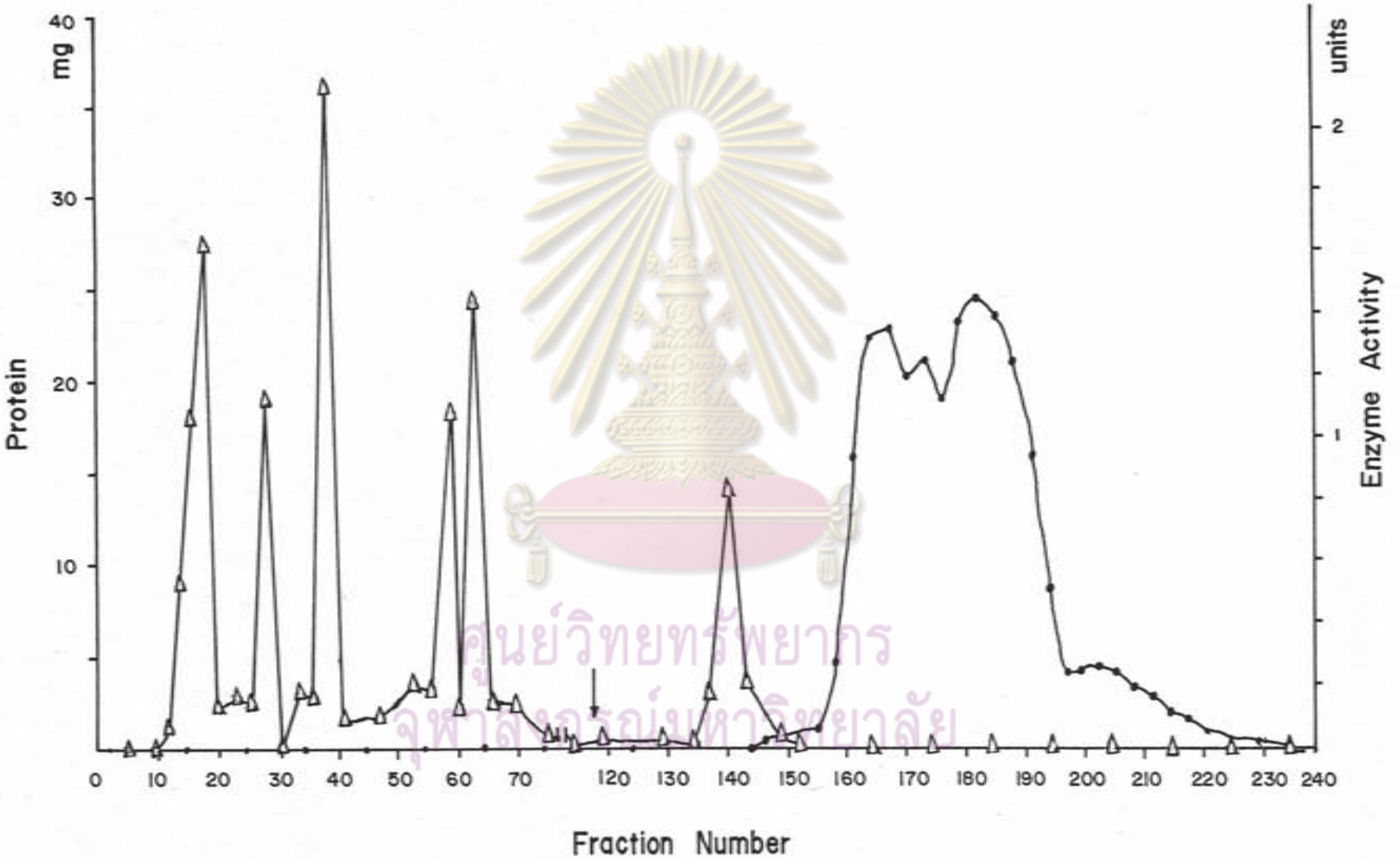


FIGURE 2

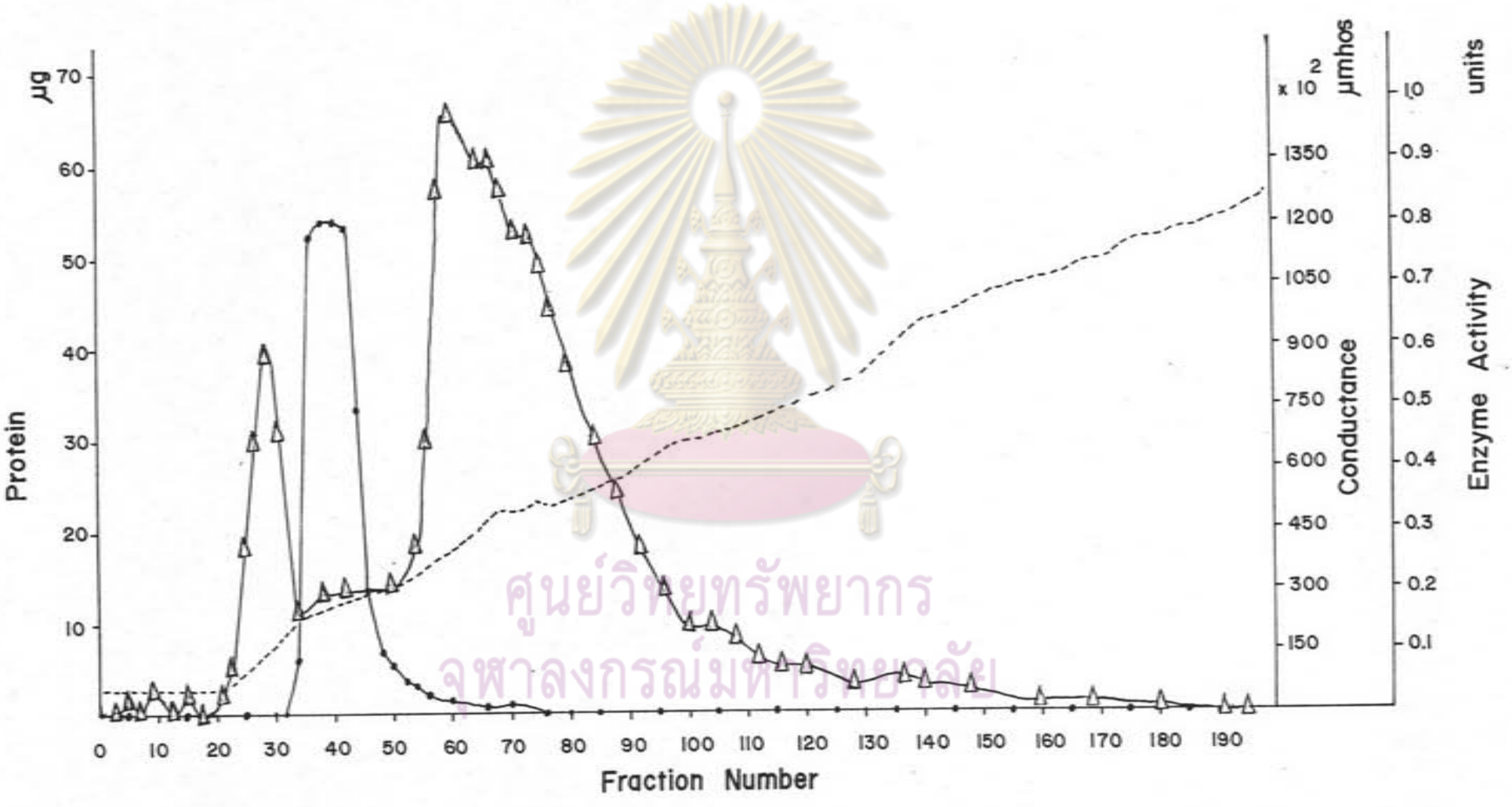


FIGURE 3

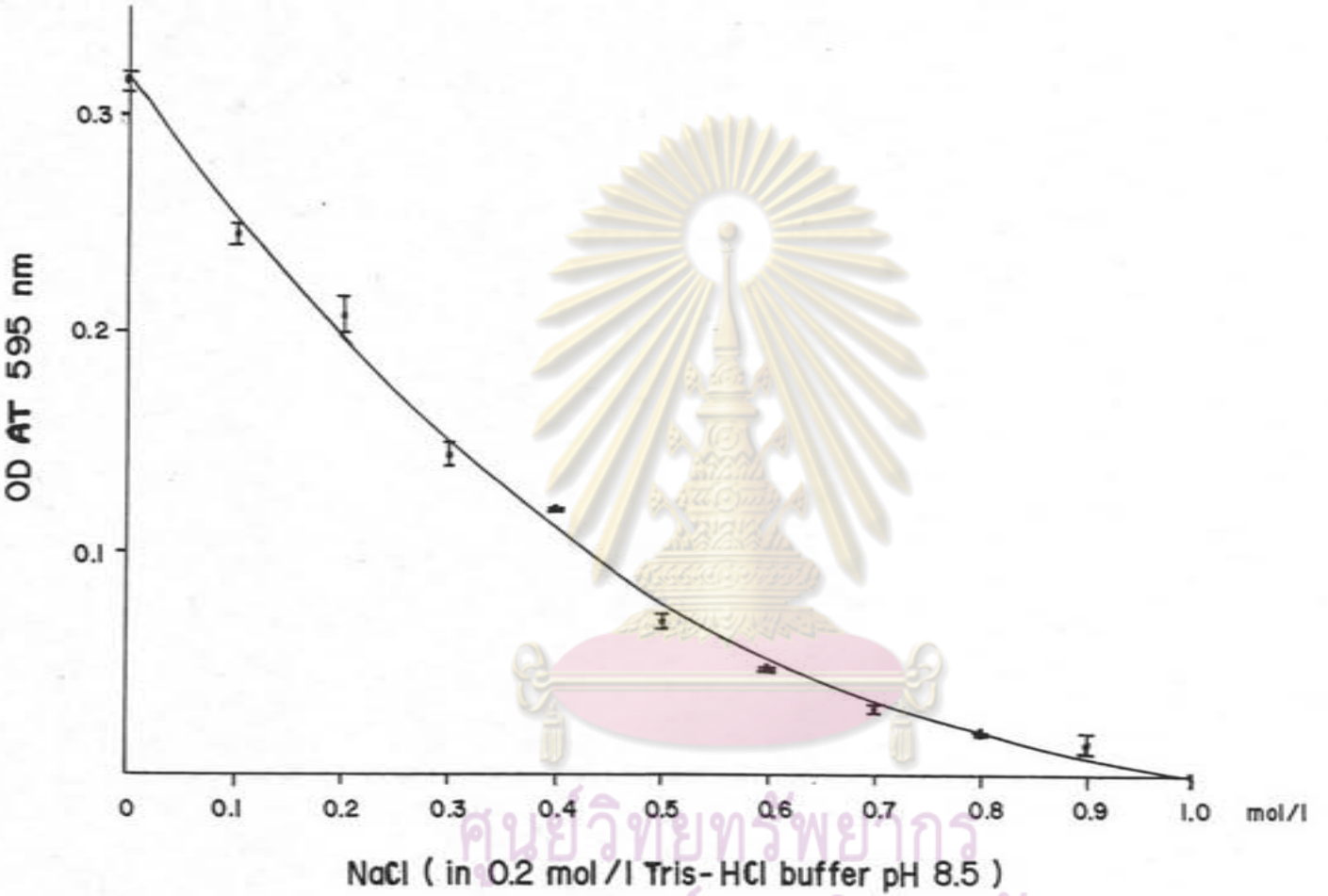


Figure 4 The standard curve obtained from plotting sodium chloride concentration (in 0.2 mol/l Tris-HCl buffer pH 8.5) against OD_{595} obtained from the coomassie brilliant blue method of protein determination. The data shown were obtained from duplicate experiments.

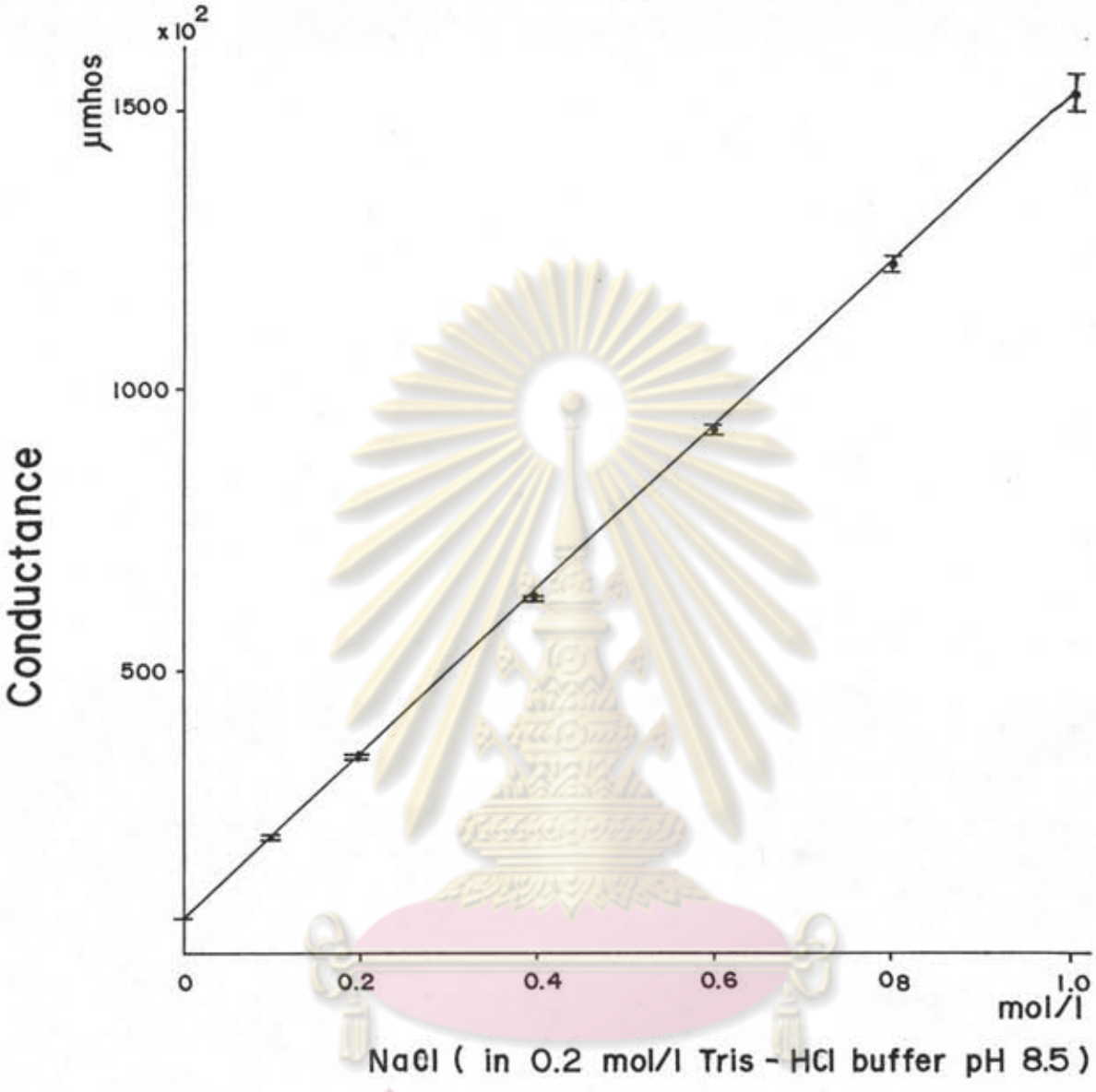


Figure 5 The standard curve obtained by plotting of sodium chloride concentration (in 0.2 mol/l Tris-HCl buffer pH 8.5) against conductance. The data shown were obtained from duplicate experiments.

solution of Tris buffer (0.2 mol/l, pH 8.5) and sodium chloride (1 mol/l) was used as a blank. The OD values obtained from the second step were subtracted from that obtained from the third step. The resultant OD values were then converted to the protein concentrations by using the standard curve of protein in figure 6. The standard curve for protein determination by Lowry method was shown in figure 7.

4.1.2 The Disc Polyacrylamide Gel Electrophoresis

The result of polyacrylamide gel electrophoresis of protein from all purification steps (the method described in section 3.8) were shown in figure 8. It can be observed that many proteins were removed in the last two steps of purification. However, the polyacrylamide gel electrophoresis pattern showed at least 6 bands of protein from the sulfonamide-Sepharose column (II) which was the last steps of purification.

4.2 Modification of Dihydropteroate Synthase by the Modifying Agents

4.2.1 The Enzyme Modification

The results of the enzyme modification (the method described in section 3.10.1) were shown in table 5. The results indicated that all the modifying agents except PMSF could lower the enzyme activity. Thus, the possible amino acids at the active site may be cysteine, arginine, and lysine.

4.2.2 Investigation of the Possible Reaction Between the Modifying Agents and the Substrates

The experimental method was described in section 3.10.2. The result was shown in table 6. From the result, the enzyme activities obtained from the test and the control conditions were likely to be the

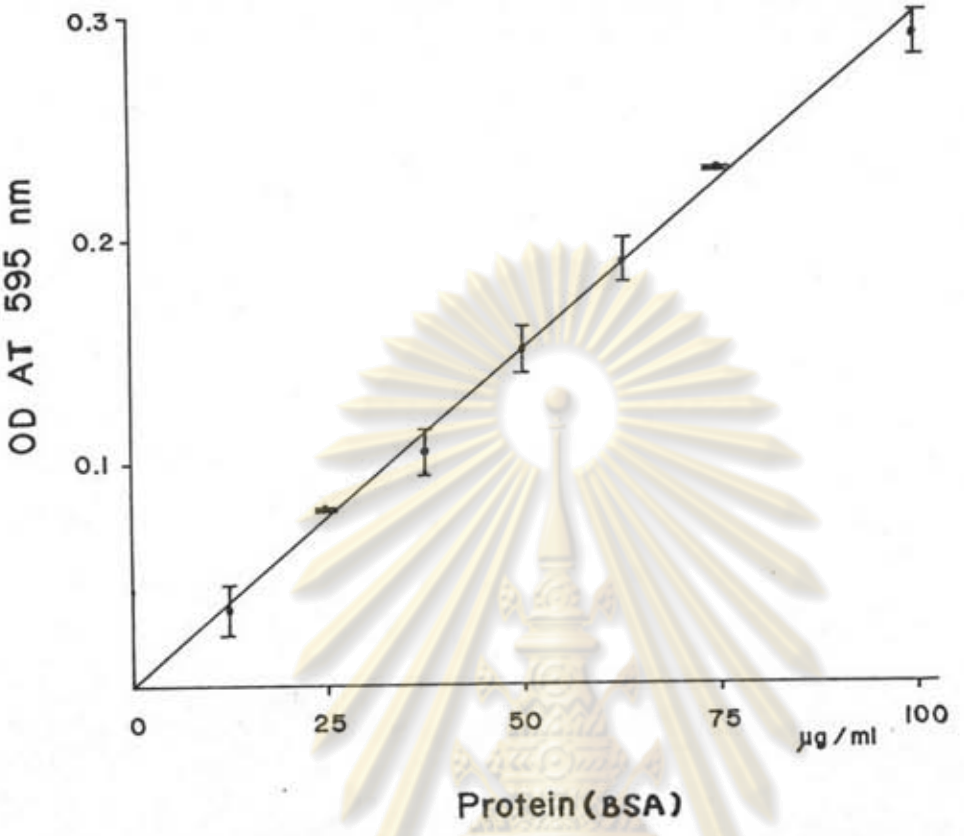


Figure 6 Standard curve of protein by coomassie brilliant blue G-250 method (Bearden, 1978). BSA in Tris buffer (0.2 mol/l, pH 8.5) was used as the standard protein.

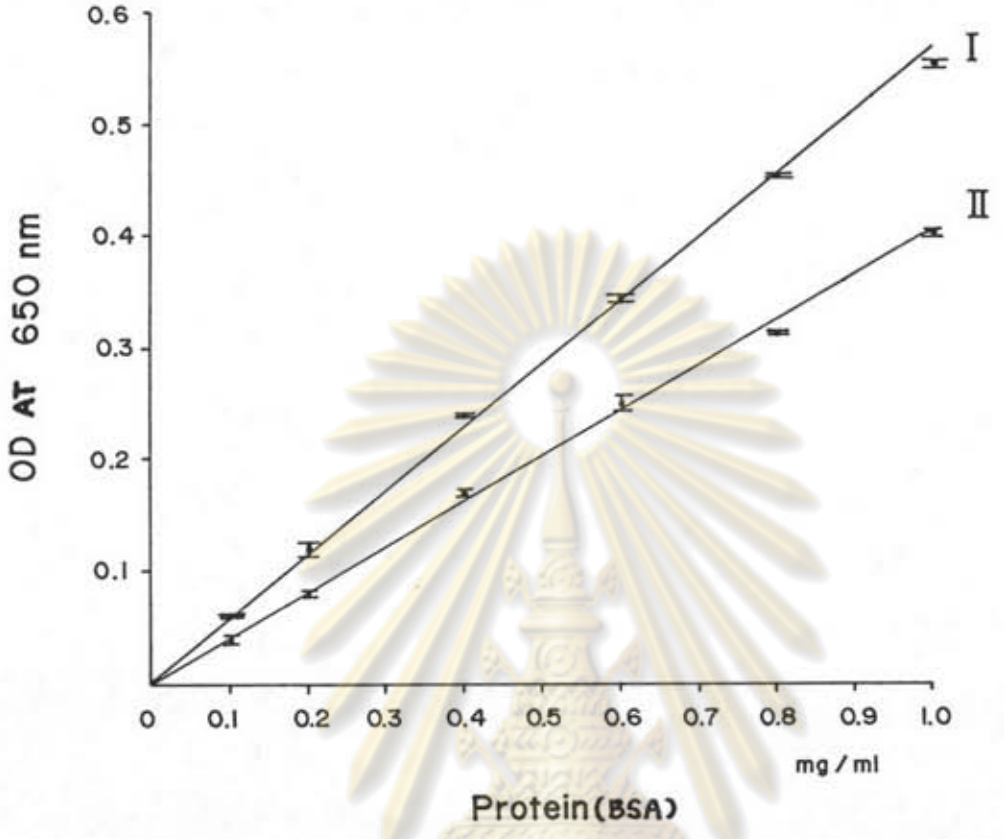


Figure 7 Standard curve of protein by Lowry method (Lowry et al. 1951)

I : BSA in Tris buffer (0.05 mol/l, pH 8.0)

II : BSA in Tris buffer (0.2 mol/l, pH 8.5)

Figure 8 The polyacrylamide gel electrophoresis patterns of the protein solution from each enzyme purification steps :

1) from crude cell-free extract
2) from ammonium sulfate precipitation (30-70% saturation fraction)
3) from the sulfonamide-Sepharose column (I)
4) from the sulfonamide-Sepharose column (II)

Talbe 5 Modification of dihydropteroate synthase by modifying agents. The data shown were obtained from duplicate experiments. The method was described in section 3.10.1.

Modifying Agents	dpm (1)	Modifying Agents	dpm (1)
Iodoacetamide		2,4-Pentanedione	
68.5 mmol/l	187 ± 2	77.70 mmol/l	0 ± 0
6.85 mmol/l	557 ± 2	38.85 mmol/l	74 ± 21
0.137 mmol/l	1608 ± 21	7.77 mmol/l	670 ± 54
Control	1698 ± 69	Control	719 ± 57
PMB		PMSF	
426.24 µmol/l	211 ± 7	720 µmol/l	1746 ± 76
42.62 µmol/l	1059 ± 20	72 µmol/l	1684 ± 8
0.852 µmol/l	1631 ± 4	1.44 µmol/l	1783 ± 38
Control	1808 ± 18	Control	1746 ± 8
2,3-Butanedione		Phenylglyoxal	
86.4 mmol/l	801 ± 1	29.59 mmol/l	632 ± 3
Control	1405 ± 17	Control	1825 ± 64

(1) Mean ± the deviation from the mean

Table 6 Investigation of the possible reaction between the modifying agent and the substrates. The data shown were obtained from duplicate experiments. The experimental details were described in section 3.10.2.

Modifying Agent	dpm (1)	% control
Iodoacetamide	2572 ± 1	109.2
Control	2356 ± 12	100
PMB	2492 ± 10	103.0
Control	2419 ± 36	100
2,4-Pentanedione	1730 ± 26	102.5
Control	1687 ± 17	100
2,3-Butanedione	2050 ± 39	98.6
Control	2080 ± 54	100
Phenylglyoxal	1872 ± 5	94.3
Control	1985 ± 48	100

(1) Mean ± the deviation from the mean

same. Thus, it might be interpreted that the reaction between the modifying agent and the substrates was not occurred within 15 minutes.

4.2.3 Investigation of the Possible Reaction Between the Modifying Agents and Sulfanilamide

The experimental method was described in section 3.10.3. The result was shown in table 7. From the result, the enzyme activities obtained from the test and the control conditions were likely to be the same. Thus, it might be interpreted that the reaction between the modifying agent and sulfanilamide was not occurred within the incubation time.

4.2.4 Investigation of the Possible Reaction Between the Modifying Agents and DHPP

The experimental method was described in section 3.10.4. The result was shown in table 8. From the result, the enzyme activities obtained from the test and the control conditions were likely to be the same. Thus, it might be interpreted that the reaction between the modifying agent and DHPP was not occurred within the incubation time.

4.2.5 Investigation of the Possible Reaction Among the Modifying Agents, 2-Mercaptoethanol and Sodium Dithionite

The experimental method was described in section 3.10.5. The result was shown in table 9. The result indicated that 2-mercaptoethanol and sodium dithionite might react with iodoacetamide and PMB. However, 2-mercaptoethanol and sodium dithionite was unlikely to react with 2,3-butanedione, 2,4-pentanedione, and phenylglyoxal.

Table 7 Investigation of the possible reaction between the modifying agent and sulfanilamide. The data shown were obtained from duplicate experiments. The experimental details were described in section 3.10.3.

Modifying Agent	dpm (1)	% Control
Iodoacetamide	1609 ± 49	95.5
Control	1685 ± 0	100
PMB	1768 ± 7	104.4
Control	1694 ± 10	100
2,4-Pentanedione	1280 ± 16	101.9
Control	1256 ± 13	100
2,3-Butanedione	1532 ± 36	104.9
Control	1461 ± 37	100
Phenylglyoxal	1324 ± 6	111.6
Control	1186 ± 5	100

(1) Mean ± the deviation from the mean

Table 8 Investigation of the possible reaction between the modifying agent and DHPP. The data shown were obtained from duplicate experiments. The experimental details were described in section 3.10.4.

Modifying Agent	dpm (1)	% Control
Iodoacetamide	2288 ± 81	100.4
Control	2278 ± 45	100
PMB	2212 ± 42	109.5
Control	2020 ± 31	100
2,4-Pentanedione	1296 ± 25	88.0
Control	1472 ± 18	100
2,3-Butanedione	1820 ± 44	111.9
Control	1626 ± 25	100
Phenylglyoxal	1672 ± 4	113.4
Control	1474 ± 46	100

(1) Mean ± the deviation from the mean

Table 9 Investigation of the possible reaction among the modifying agent, 2-mercaptoethanol and sodium dithionite. The data shown were obtained from duplicate experiments. The experimental details were described in section 3.10.5.
R = 2-mercaptoethanol and sodium dithionite, E = enzyme, M = modifying agent.

Modifying Agent	Tube No.	dpm (1)
Iodoacetamide	1 E+M+R	476 ± 3
	2 E+M	186 ± 7
	3 E	1587 ± 19
PMB	1 E+M+R	1387 ± 11
	2 E+M	464 ± 15
	3 E	1598 ± 27
2,4-Pentanedione	1 E+M+R	50 ± 16
	2 E+M	91 ± 17
	3 E	614 ± 26
2,3-Butanedione	1 E+M+R	485 ± 20
	2 E+M	461 ± 19
	3 E	713 ± 24
Phenylglyoxal	1 E+M+R	296 ± 5
	2 E+M	338 ± 10
	3 E	962 ± 5

(1) Mean ± the deviation from the mean

4.2.6 Protection of the Enzyme from the Modifying Agents

4.2.6.1 Protection of the Enzyme by DHPP, and by Sulfanilamide in the Presence of DHPP

The method was described in section 3.10.6.1. The results were shown in table 10 and table 11. Both DHPP and sulfanilamide could protect the enzyme from all five modifying agents, for example, for the enzyme modification by iodoacetamide (table 10), DHPP could increase the enzyme activity from 4.5% to 76.3% and sulfanilamide (in the presence of DHPP) could increase the enzyme activity from 4.5% to 80.0%. The results of the protection of the enzyme also indicated that the amino acid residues of cysteine, arginine, and lysine may be present at the enzyme active site.

4.2.6.2 Protection of the Enzyme by DHPP and by Sulfanilamide in the Absence of DHPP

The method was described in section 3.10.6.2. The result was shown in table 12. The result indicated that sulfanilamide itself (in the absence of DHPP) could not protect the enzyme from PMB.

4.3 Study of the General Properties of Dihydropteroate Synthase and Determination of the Inhibitor Constant (K_I) for p-Aminobenzenesulfonamidoalkanoic Acids

4.3.1 Study of the General Properties of Dihydropteroate Synthase

Table 10 Protection of dihydropteroate synthase from the modifying agents (iodoacetamide and PMB) by DHPP and by sulfanilamide in the presence of DHPP. The data shown were obtained from duplicate experiment. The experimental details were described in section 3.10.6.1.

E = enzyme, M = modifying agent, R = 2-mercaptoethanol and sodium dithionite, I = sulfanilamide

Tube No.	Iodoacetamide		PMB	
	dpm(1)	Enzyme Activity (%Control)	dpm(1)	Enzyme Activity (%Control)
1) E+M	6±20	0.4	148±13	10.6
2) E+M+R	68±14	4.5	366±3	26.1
3) E+R (control)	1499±17	100	1400±2	100
4) E+M+R+DHPP	1381±7	76.3	1551±16	96.8
5) E+R+DHPP (control)	1810±5	100	1603±25	100
6) E+M+R+DHPP+I	854±30	80.0	952±28	97.9
7) E+R+DHPP+I (control)	1068±14	100	972±19	100

(1) Mean ± the deviation from the mean

Table 11 Protection of dihydropteroate synthase from the modifying agents (2,4-pentanedione, 2,3-butanedione, and phenylglyoxal) by DHPP and by sulfanilamide in the presence of DHPP. The data shown were obtained from duplicate experiment. The experimental details were described in section 3.10.6.1. E = enzyme, M = modifying agent, R = 2-mercaptoethanol and sodium dithionite, I = sulfanilamide

Tube No.	2,4-Pentanedione		2,3-Butanedione		Phenylglyoxal	
	dpm(1)	Enzyme Activity (% Control)	dpm(1)	Enzyme Activity (% Control)	dpm(1)	Enzyme Activity (% Control)
1) E+M	56±4	10.1	815±4	59.8	210±10	19.7
2) E(control)	555±29	100	1362±21	100	1066±34	100
3) E+M+R+DHPP	1216±13	99.7	1672±17	104.4	975±30	83.8
4) E+R+DHPP(control)	1220±6	100	1601±44	100	1163±37	100
5) E+M+R+DHPP+I	849±59	107.2	1297±39	106.1	588±7	80.9
6) E+R+DHPP+I(control)	792±17	100	1222±1	100	727±33	100

(1) Mean ± the deviation from the mean

Table 12 Protection of dihydropteroate synthase from the modifying agent (PMB) by DHPP and by sulfanilamide in the absence of DHPP. The data shown were obtained from duplicate experiment. The experimental details were described in section 3.10.6.2.

E = enzyme, M = modifying agent, R = 2-mercaptoethanol and sodium dithionite, I = sulfanilamide

Tube No.	dpm (1)	Enzyme Activity (%Control)
1) E+M	164 ± 2	11.9
2) E+M+R	368 ± 22	26.7
3) E+R (control)	1380 ± 10	100
4) E+M+R+DHPP	1350 ± 0	83.3
5) E+R+DHPP (control)	1620 ± 17	100
6) E+M+R+I	156 ± 7	15.2
7) E+R+I (control)	1029 ± 17	100

(1) Mean ± the deviation from the mean

4.3.1.1 Study of the Effect of the Incubation Time on the Enzyme Activity

The method was described in section 3.11.1.1. The result was indicated in figure 9 which shows the linearity between the enzyme activity and the incubation time.

4.3.1.2 Study of the Effect of the Enzyme Concentration on the Enzyme Activity

The method was described in section 3.11.1.2. The result was indicated in figure 10 which shows the linearity between the enzyme activity and the amount of enzyme.

4.3.1.3 Determination of the Optimum pH

The method was described in section 3.11.1.3. The result was indicated in figure 11a which shows the optimum pH at 8.55.

4.3.1.4 Determination of the Optimum Temperature

The method was described in section 3.11.1.4. The result was indicated in figure 11b which shows the optimum temperature at 42^{o} C.

4.3.1.5 Determination of the Michaelis Constant (K_m) for PABA

The method was described in section 3.11.1.5. The result was shown in figure 12. The K_m value for PABA was 1.30×10^{-6} mol/l. This Km value was determined by regression analysis with the correlation coefficient (r) value of 0.9995.

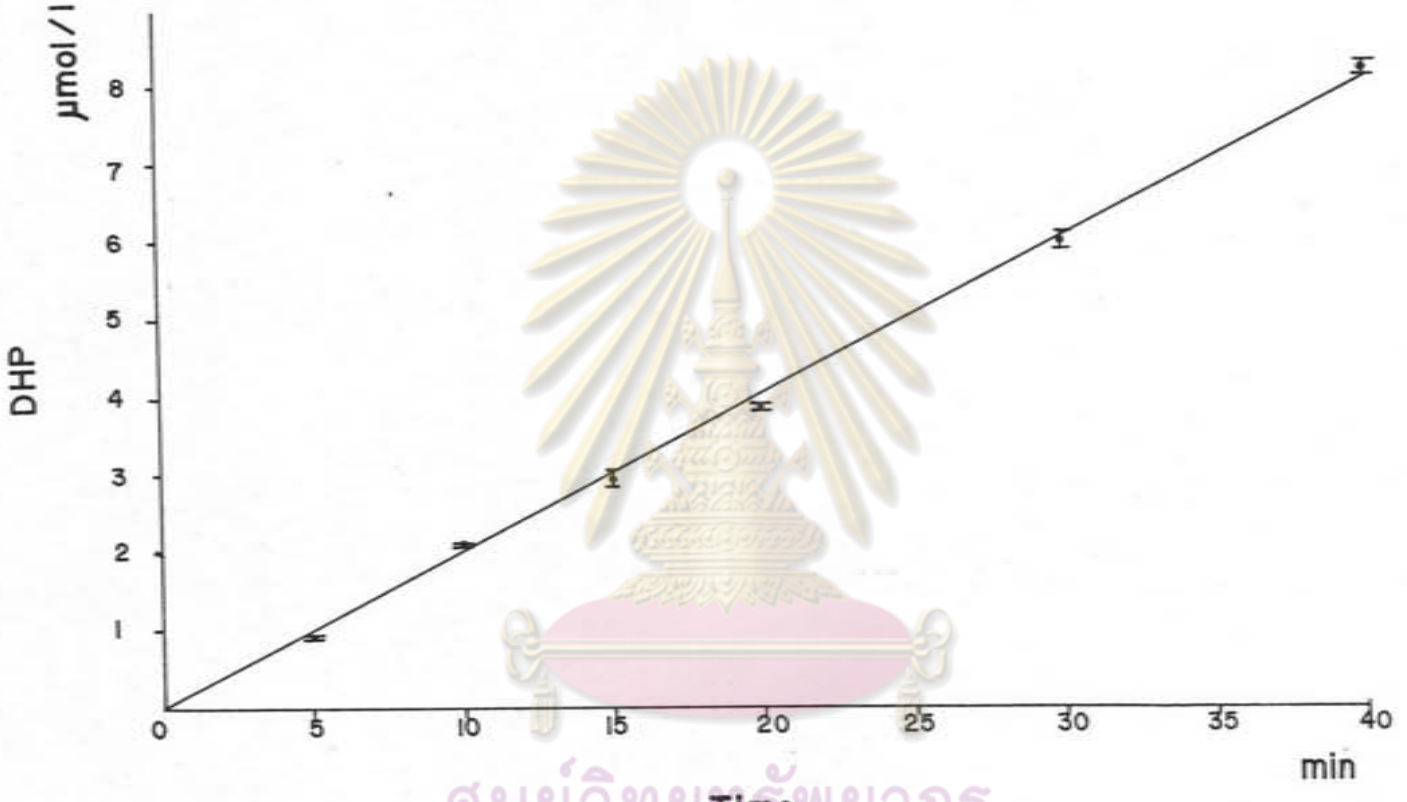


Figure 9 Plot of dihydropteroate synthase activity (µmol DHP occurred/l) and the incubation time. The data shown were obtained from duplicate experiments. The experimental details were described in section 3.12.1.

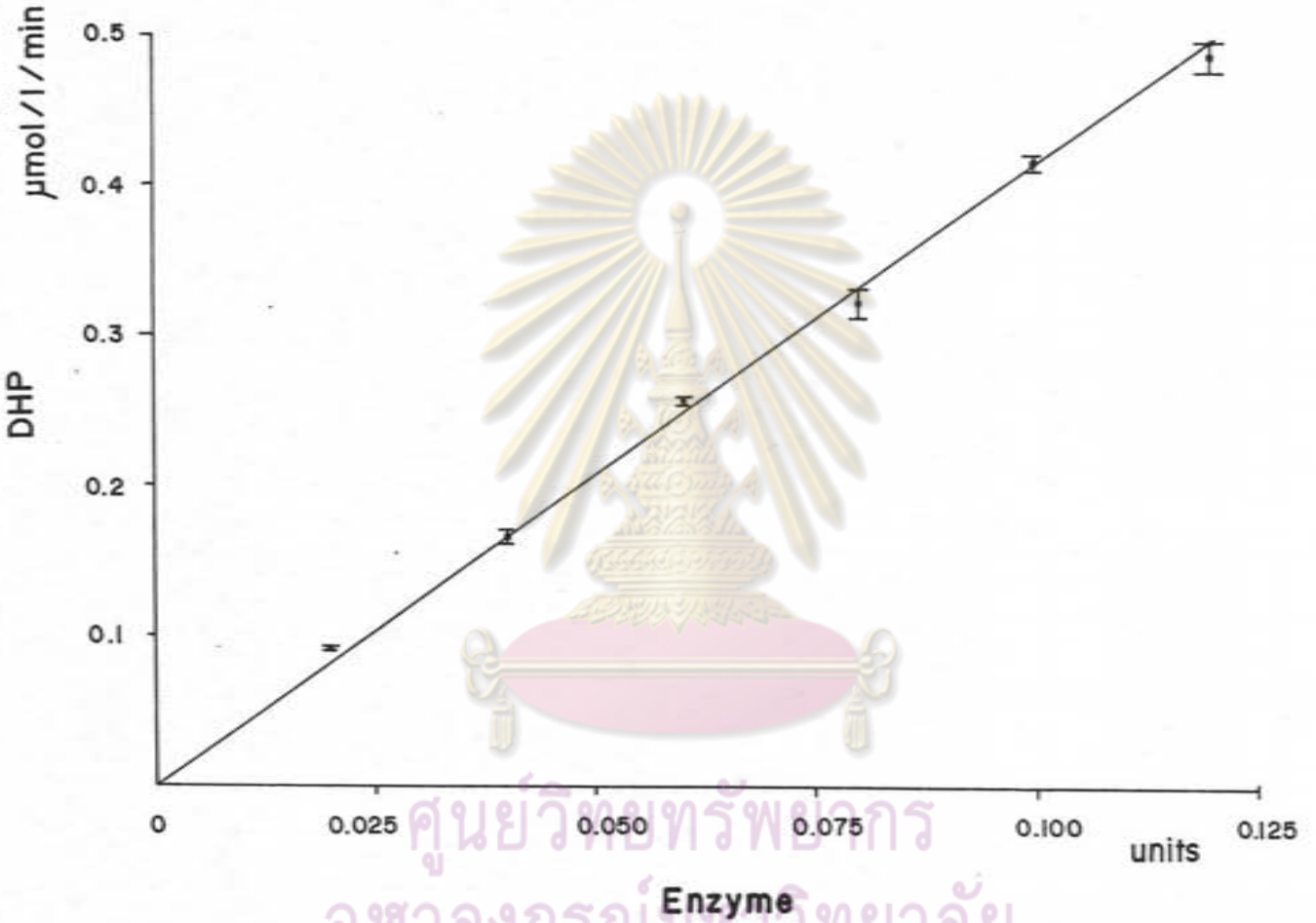


Figure 10 Plot of dihydropteroate synthase velocity (µmol DHP occurred/l/min) and the amount of enzyme. The data were obtained from duplicate experiments. The experimental details were described in section 3.12.2.

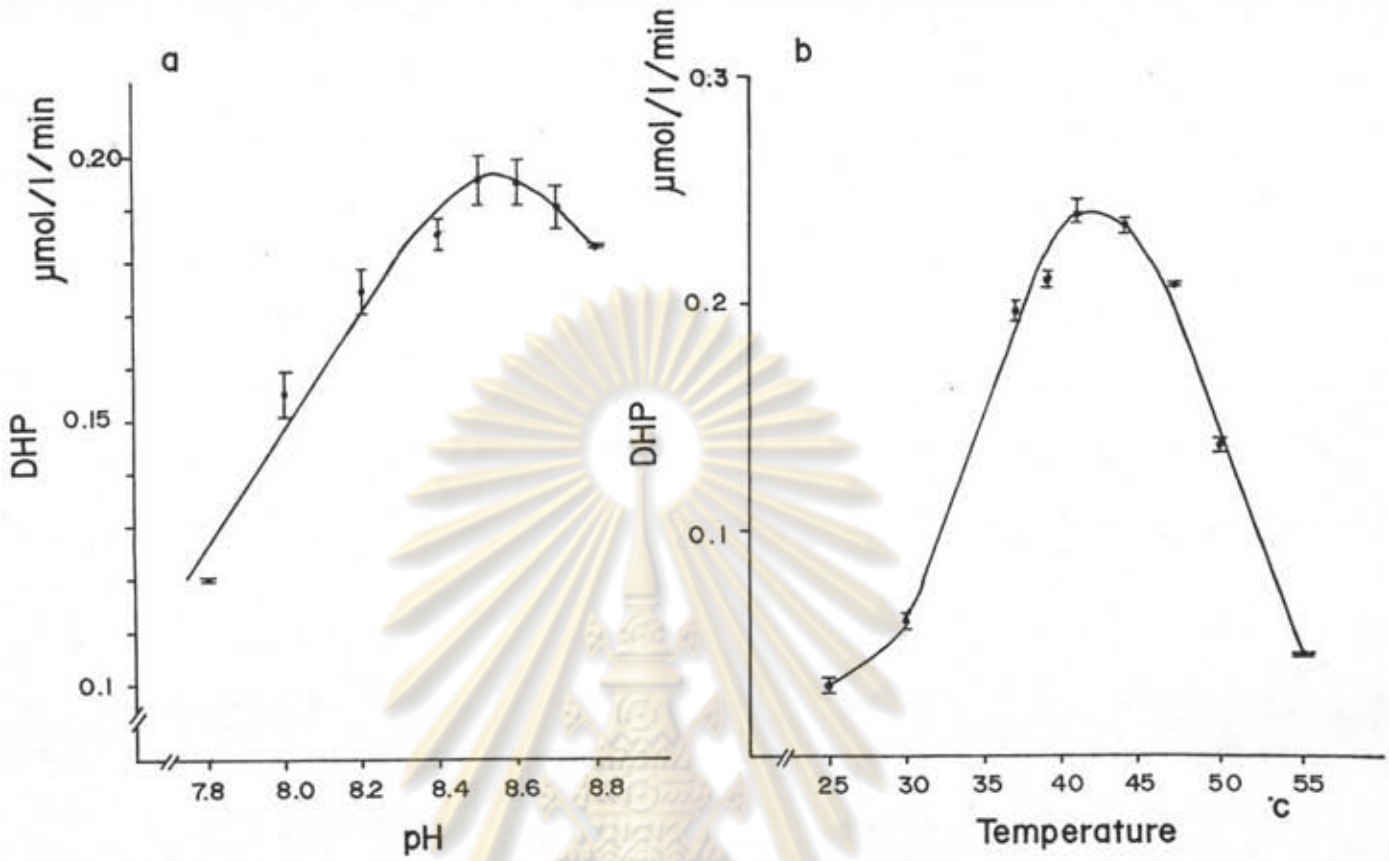


FIGURE II

Figure 11 a; The plot of dihydropteroate synthase velocity (µmol DHP occurred/l/min) and pH, b; the plot of dihydropteroate synthase velocity and incubation temperature. The data were obtained from duplicate experiments. The experimental details were described in section 3.12.3 and 3.12.4.

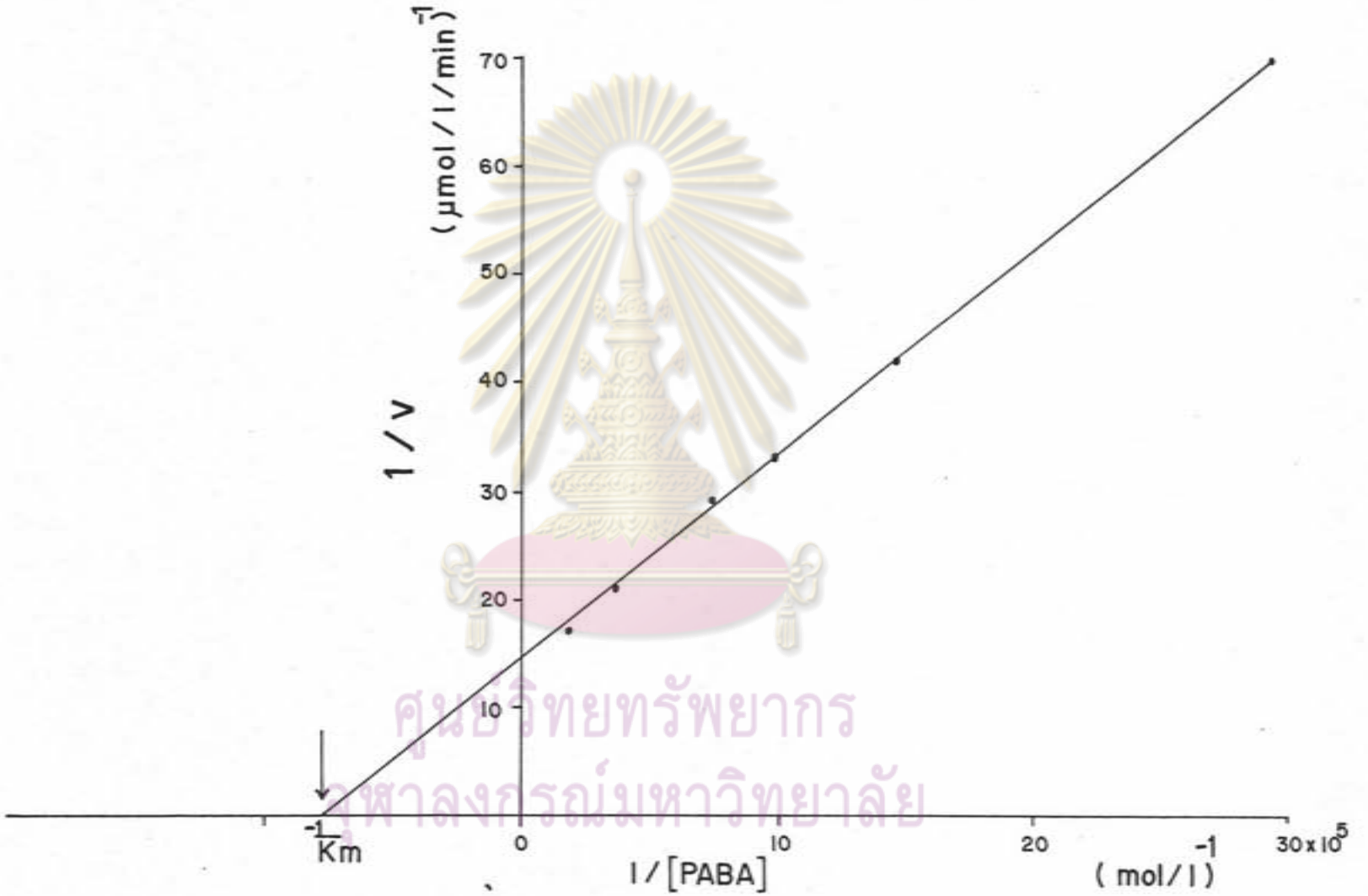


FIGURE 12

4.3.2 Determination of the Inhibitor Constant (K_i) for p-Aminobenzenesulfonamidoalkanoic Acids and Sulfanilamide

The method was described in section 3.11.2. The results were shown in figures 13-20 and table 13. In the case of sulfanilamide, N-(p-aminobenzenesulfonyl) glycine, N-(p-aminobenzenesulfonyl) alanine, N-(p-aminobenzenesulfonyl) phenylalanine and N-(p-aminobenzenesulfonyl) valine, the plots of 1/v versus [I] showed that the intersection of the three lines was at the same point. The r values for the lines were between 0.9814 and 0.9995. In the case of N-(p-aminobenzenesulfonyl)tyrosine, N-(p-aminobenzenesulfonyl)methionine and N-(p-aminobenzenesulfonyl) leucine, the plots of 1/v versus [I] showed that the intersection of the three lines was nearly at the same point and the K_i values reported in the results were the mean values obtained from the three intersection points. The r values for the lines were between 0.9907 and 0.9990. All p-aminobenzenesulfonamidoalkanoic acids and sulfanilamide exhibited competitive inhibitors. N-(p-aminobenzenesulfonyl) glycine was the most potent inhibitor because its K_i value was the lowest (40 µmol/l). N-(p-Aminobenzenesulfonyl) glycine was the only compound that showed more inhibitory activity than sulfanilamide. N-(p-aminobenzenesulfonyl) valine was the least potent inhibitor because its Ki value was the highest (1 mmol/l). The plots of $1/K_i$ versus Δft or π were shown in figure 21 and figure 22 respectively. The $1/K_i$ value for N-(p-aminobenzenesulfonyl) glycine in which the Δft and the π values for the amino acid side chain of this compound equal to zero was the highest value in the curve. It was found that the $1/K_i$ values were decreased when the Δft values were between 0-1500 cal/mol or the π values were between 0-1.5 units, however, the $1/K_i$ values were slightly increased when the Δft values were between 1500-2500 cal/mol or the π values were between 1.5-2.63 units.

Figure 13 Dixon plot of 1/v against [sulfanilamide]. The data shown were obtained from duplicate experiments. The concentrations of sulfanilamide were 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mmol/l. The concentrations of PABA were 11.36 µmol/l (I), 19.89 µmol/l (II) and 27.84 µmol/l (III). The experimental details were described in section 3.11.2. K_i for sulfanilamide obtained from the graph was 4.8×10^{-5} mol/l.

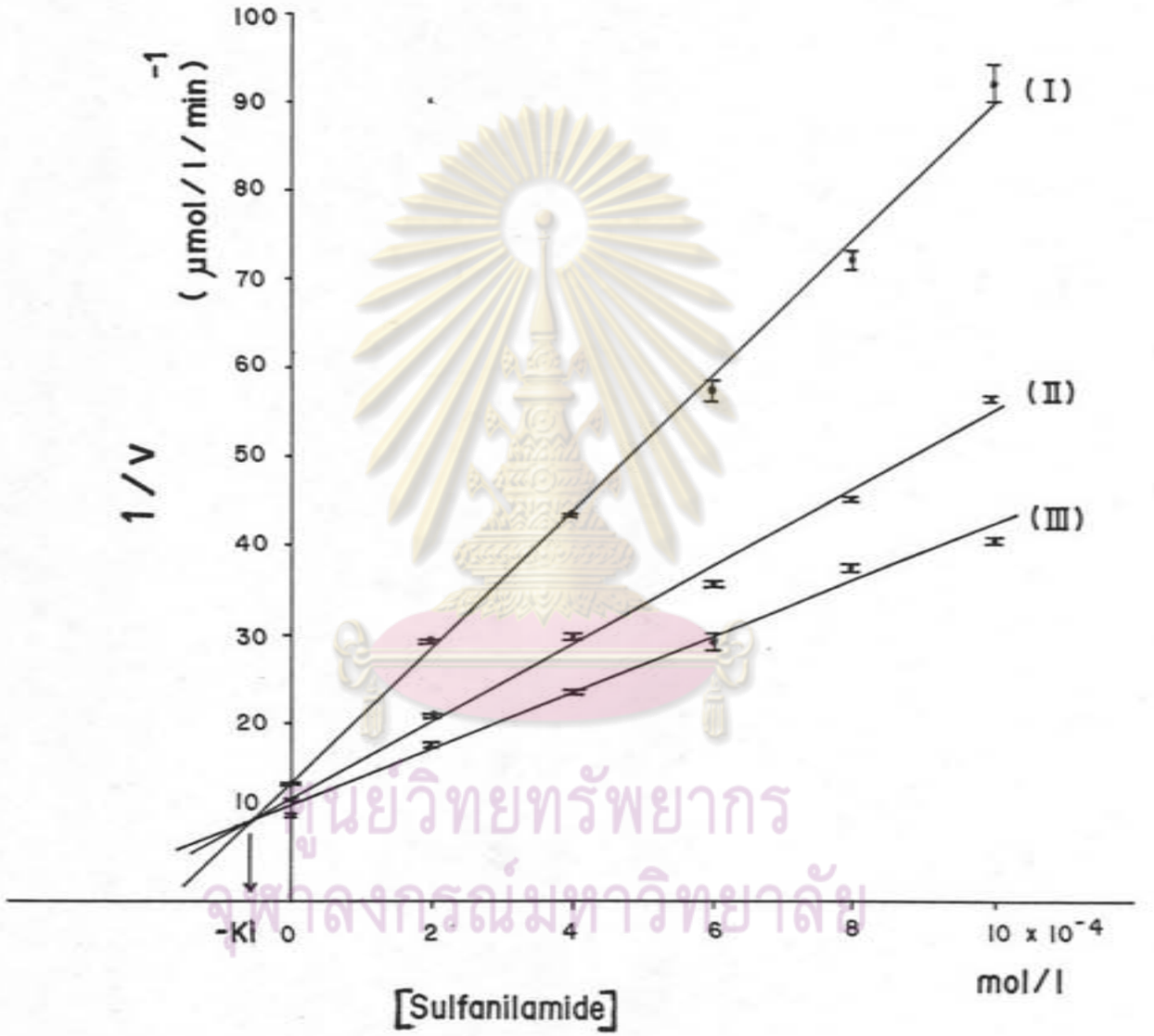
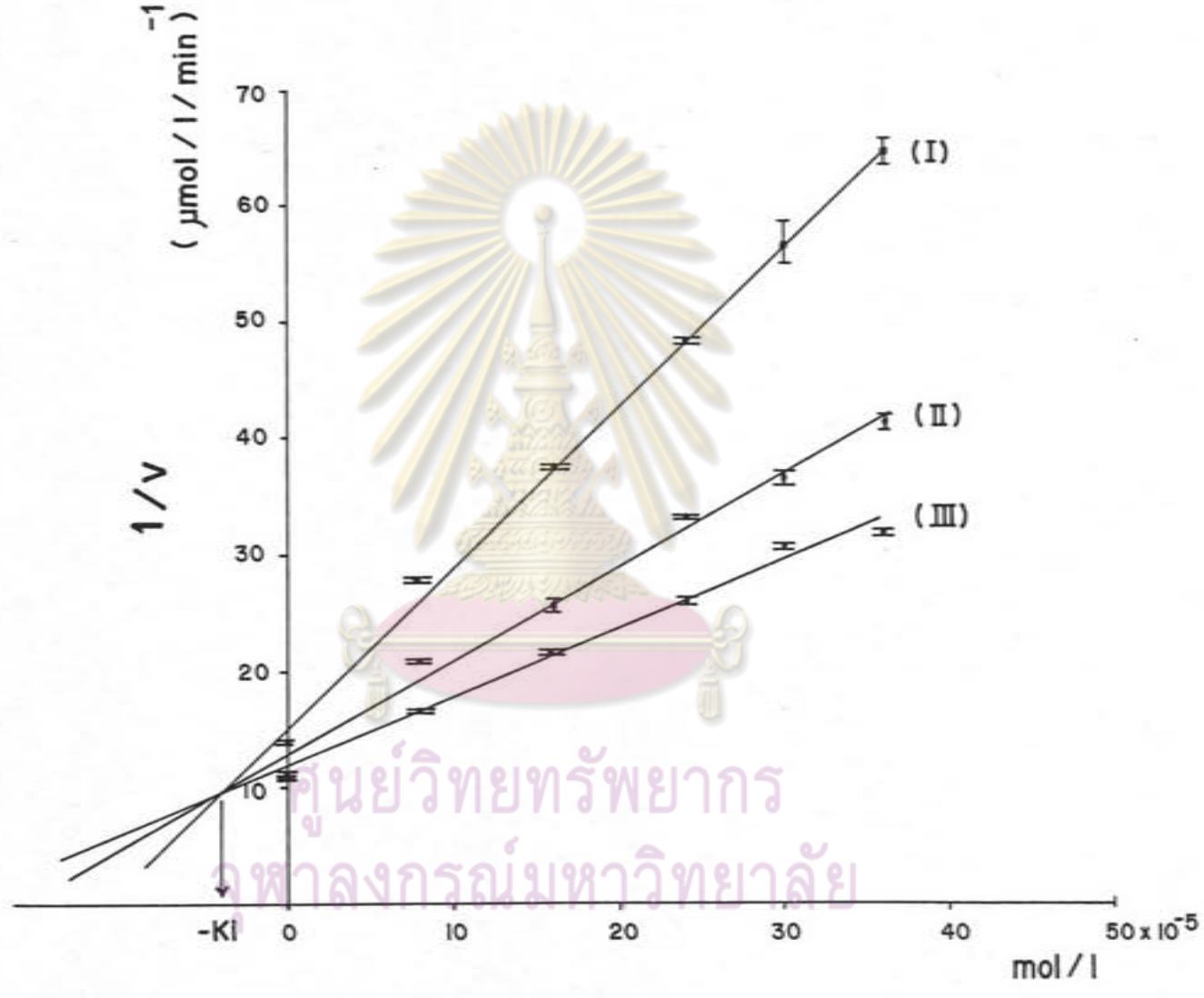


FIGURE 13

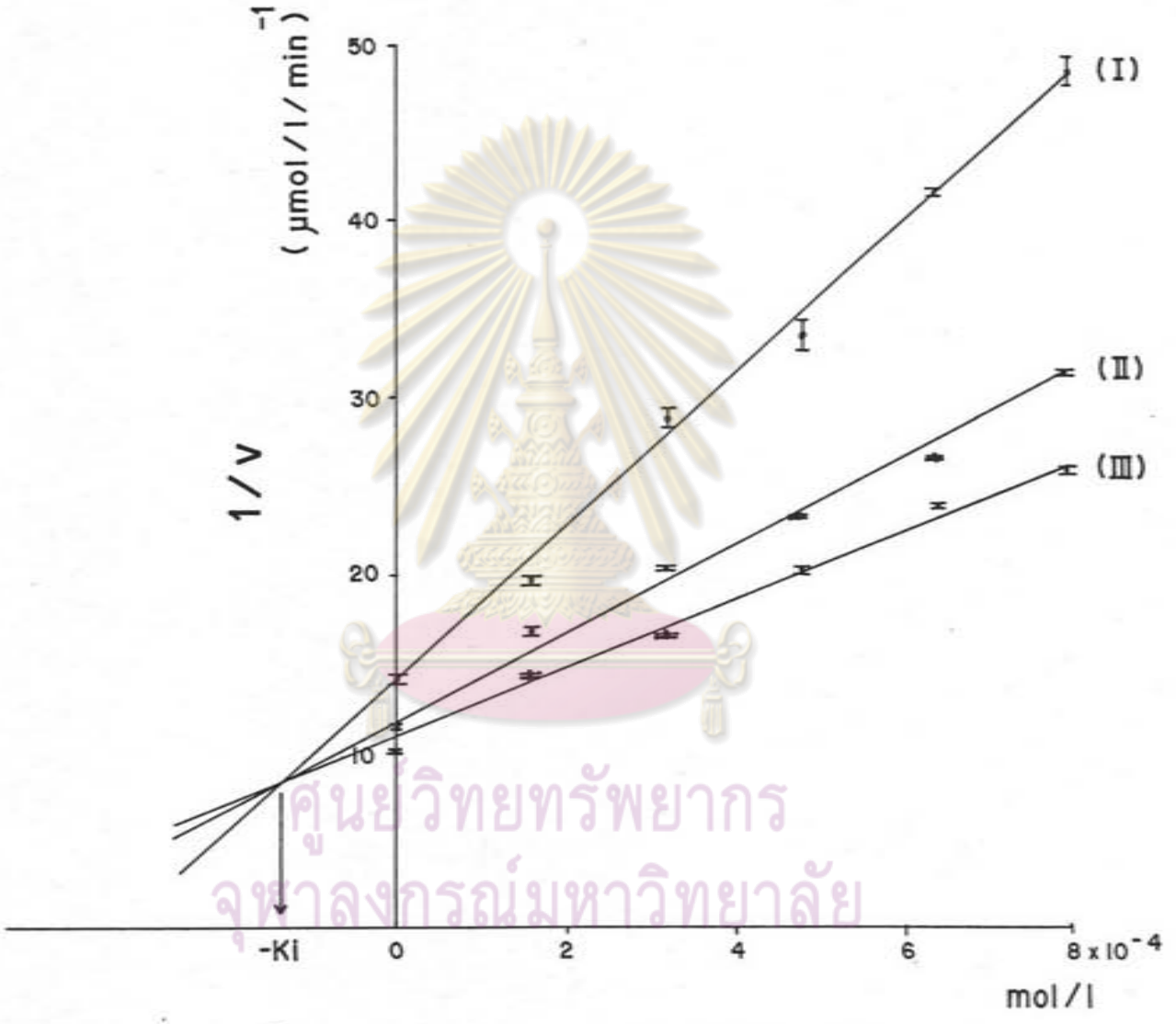
Figure 14 Dixon plot of 1/v against [N-(p-aminobenzenesulfonyl) glycine]. The data shown were obtained from duplicate experiments. The concentrations of N-(p-aminobenzenesulfonyl) glycine were 0, 80, 160, 240, 300 and 360 μmol/l. The concentrations of PABA were 11.36 μmol/l (I), 19.89 μmol/l (II) and 27.84 μmol/l (III). The experimental details were described in section 3.11.2. K_i for N-(p-aminobenzenesulfonyl) glycine obtained from the graph was 4.0×10^{-5} mol/l.



[N - (p - Aminobenzenesulfony) glycine]

FIGURE 14

Figure 15 Dixon plot of 1/v against [N-(p-aminobenzenesulfonyl) tyrosine]. The data shown were obtained from duplicate experiments. The concentrations of N-(p-aminobenzenesulfonyl) tyrosine were 0, 160, 320, 480, 640 and 800 μmol/l. The concentrations of PABA were 11.36 μmol/l (I), 19.89 μmol/l (II) and 27.84 μmol/l (III). The experimental details were described in section 3.11.2. K_i for N-(p-aminobenzenesulfonyl) tyrosine obtained from the graph was 13.8×10^{-5} mol/l.



[N-(p - Aminobenzenesulfonyl) Tyrosine]

FIGURE 15

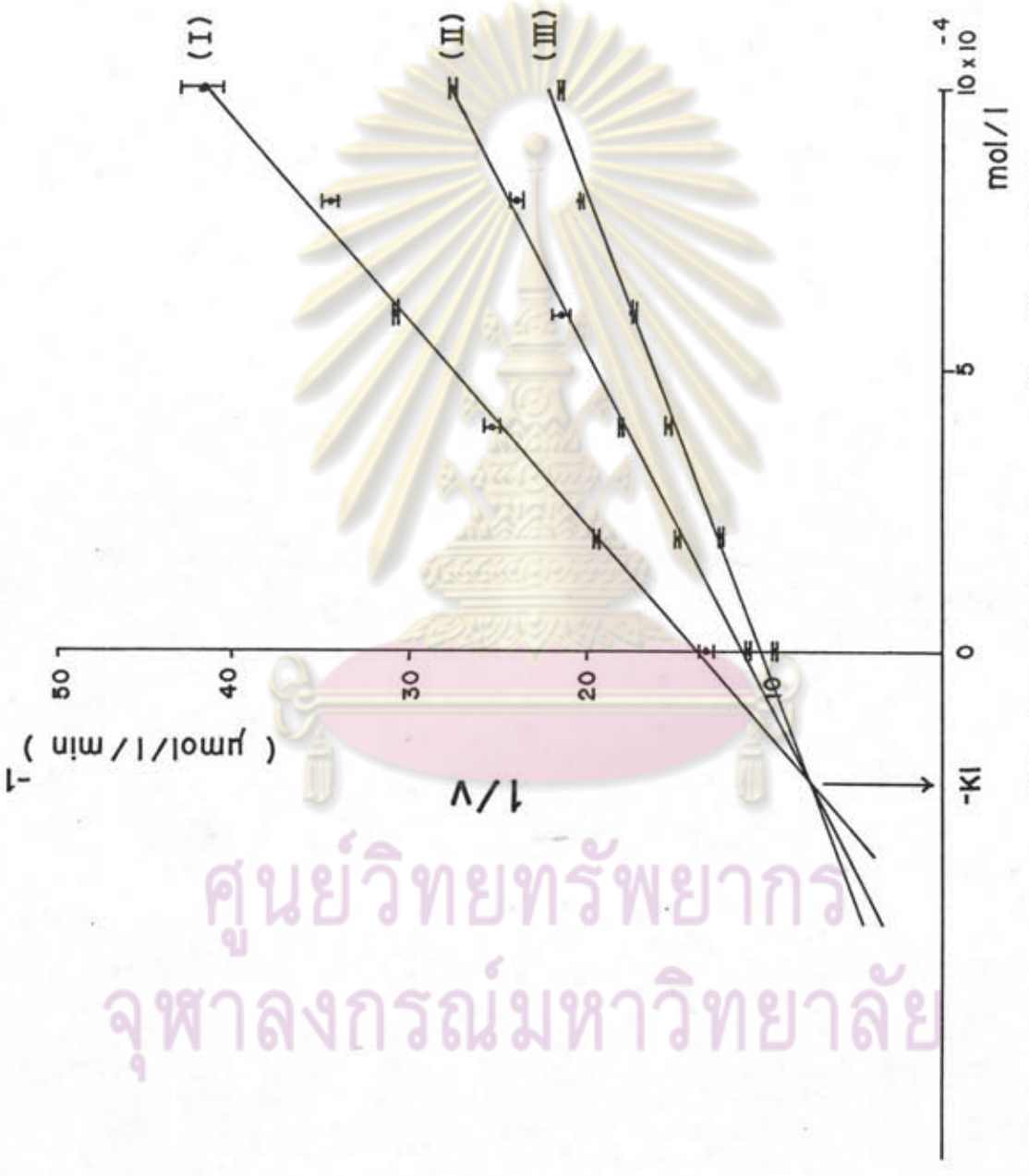
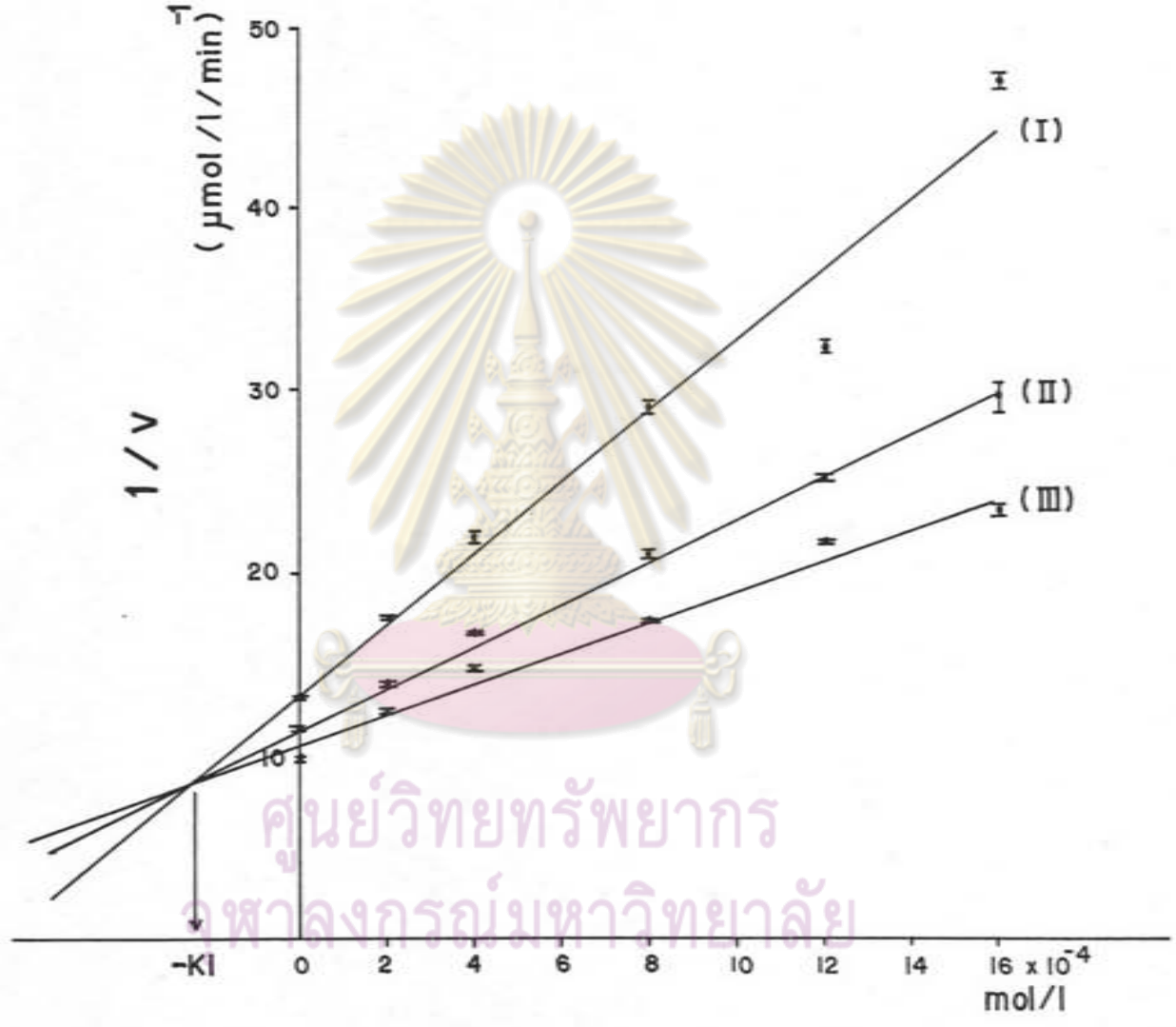


FIGURE 16

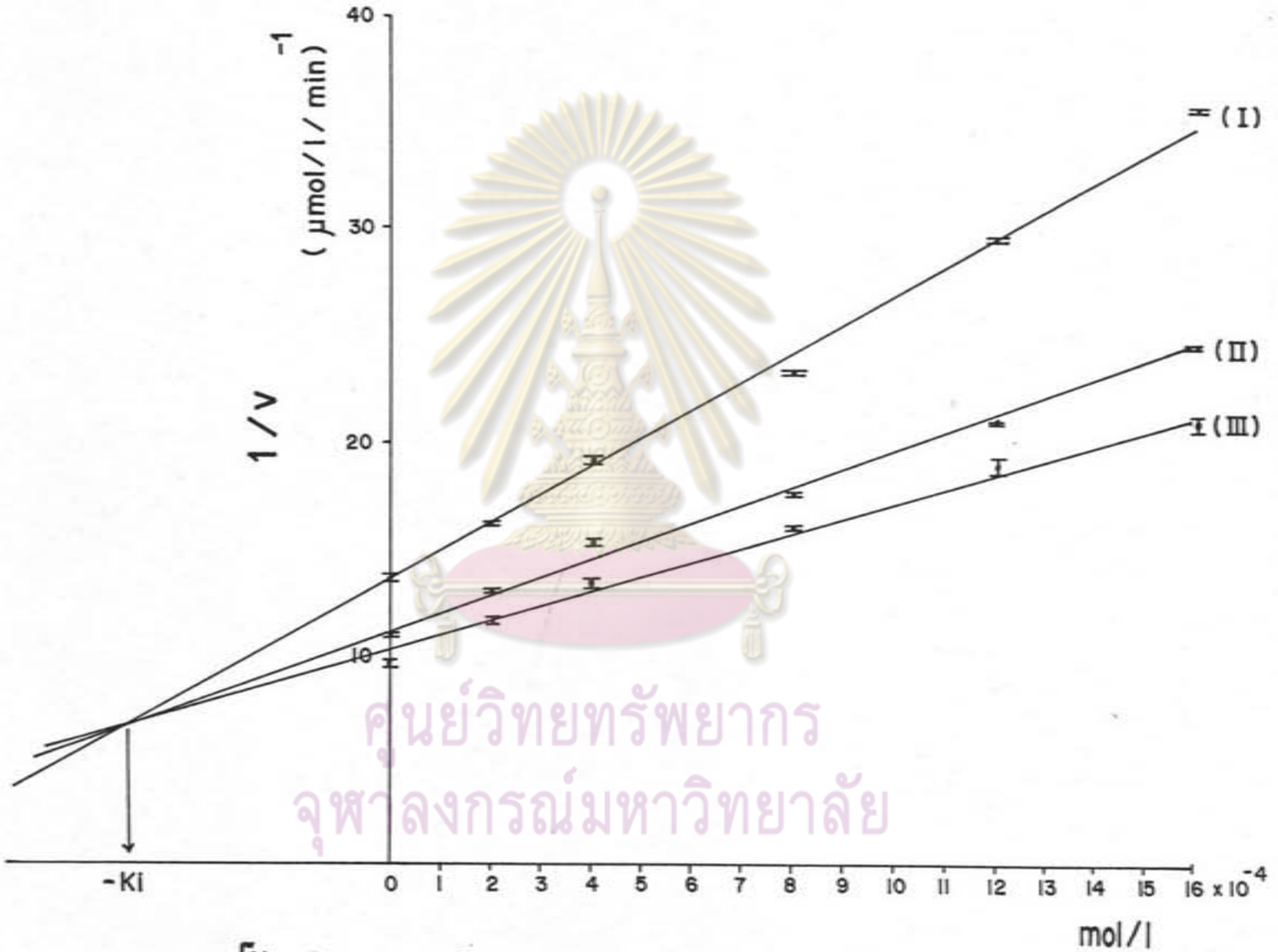
Figure 17 Dixon plot of 1/v against [N-(p-aminobenzenesulfonyl) phenylalanine]. The data shown were obtained from duplicate experiments. The concentrations of N-(p-aminobenzenesulfonyl) phenylalanine were 0, 0.2, 0.4, 0.8, 1.2 and 1.6 mmol/l. The concentrations of PABA were 11.36 μmol/l (I), 19.89 μmol/l (II) and 27.84 μmol/l (III). The experimental details were described in section 3.11.2. K_i for N-(p-aminobenzenesulfonyl) phenylalanine obtained from the graph was 24.0×10^{-5} mol/l.



[N -(p - Aminobenzenesulfonyl) phenylalanine]

FIGURE 17

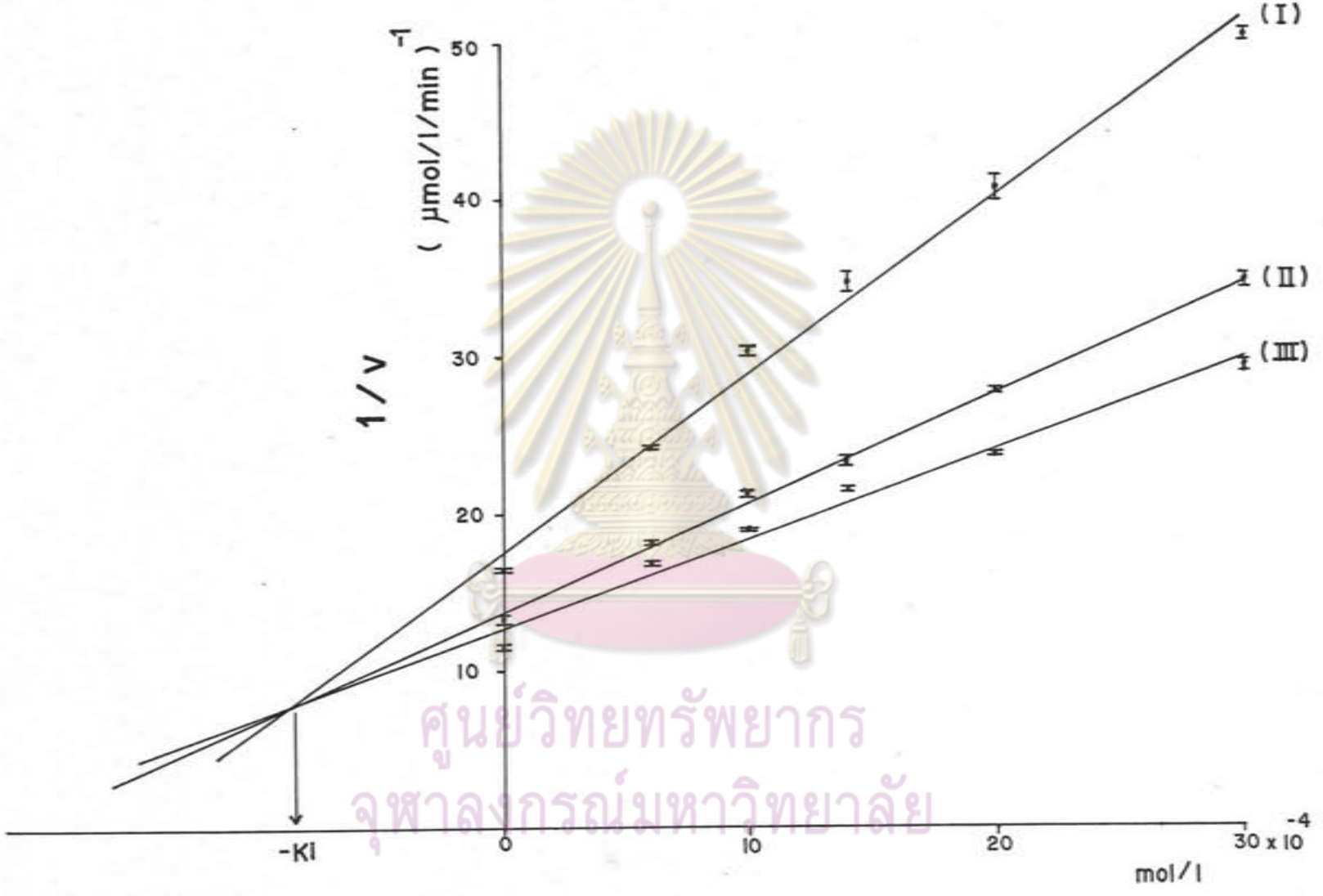
Figure 18 Dixon plot of 1/v against [N-(p-aminobenzenesulfonyl) methionine]. The data shown were obtained from duplicate experiments. The concentrations of N-(p-aminobenzenesulfonyl) methionine were 0, 0.2, 0.4, 0.8, 1.2 and 1.6 mmol/l. The concentrations of PABA were 11.36 µmol/l (I), 19.89 µmol/l (II), and 27.84 µmol/l (III). The experimental details were described in section 3.11.2. K_i for N-(p-aminobenzenesulfonyl) methionine obtained from the graph was 49.3×10^{-5} mol/l.



[N - (p - Aminobenzenesulfonyl) methionine]

FIGURE 18

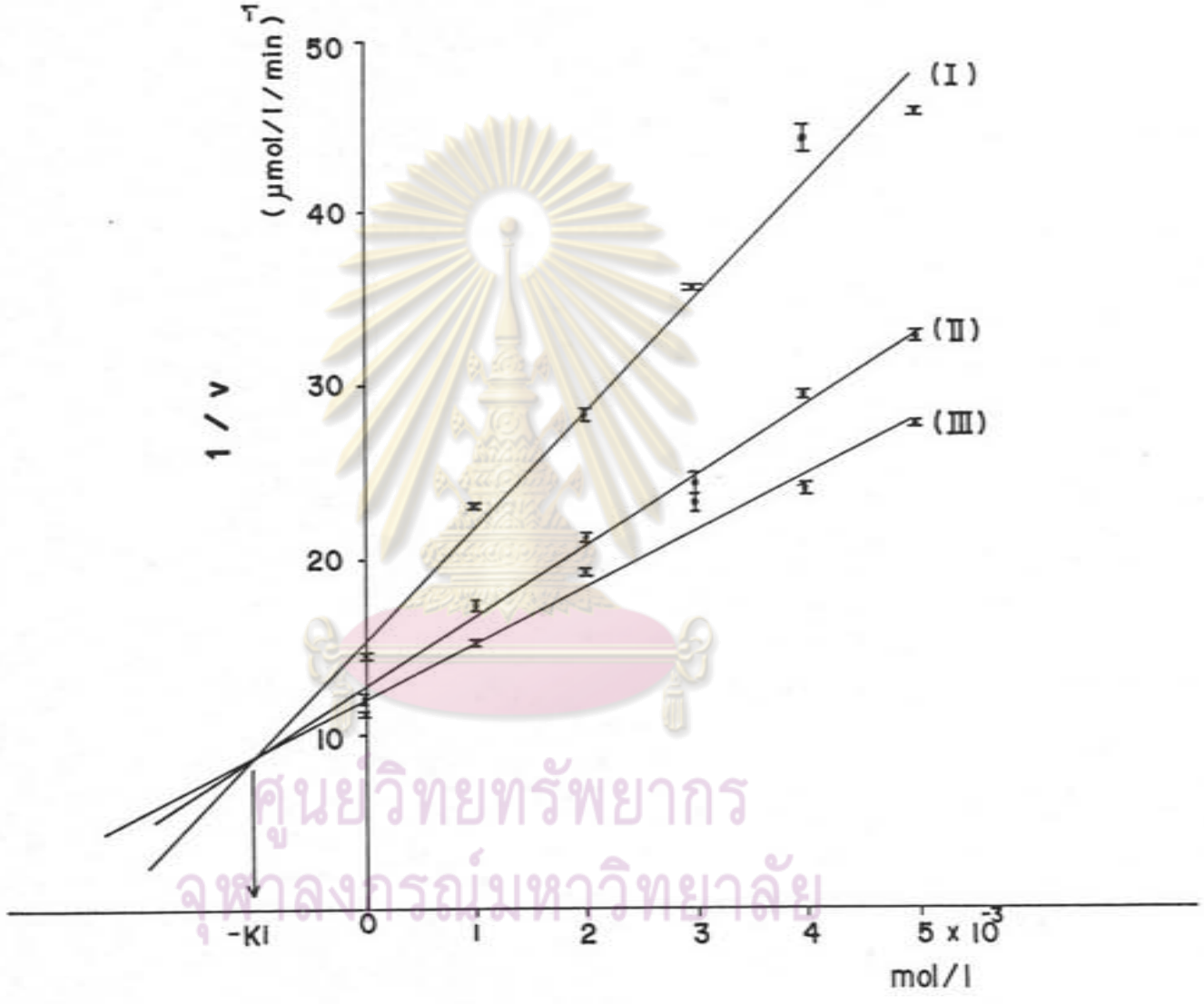
Figure 19 Dixon plot of 1/v against [N-(p-aminobenzenesulfonyl) leucine]. The data shown were obtained from duplicate experiments. The concentrations of N-(p-aminobenzenesulfonyl) leucine were 0, 0.6, 1.0, 1.4, 2.0 and 3.0 mmol/l. The concentrations of PABA were 11.36 µmol/l (I), 19.89 µmol/l (II) and 27.84 µmol/l (III). The experimental details were described in section 3.11.2. K_i for N-(p-aminobenzenesulfonyl) leucine obtained from the graph was 84.0×10^{-5} mol/l.



[N-(p - Aminobenzenesulfonyl) leucine]

FIGURE 19

Figure 20 Dixon plot of 1/v against [N-(p-aminobenzenesulfonyl) valine]. The data shown were obtained from duplicate experiments. The concentrations of N-(p-aminobenzenesulfonyl) valine were 0, 1, 2, 3, 4 and 5 mmol/l. The concentrations of PABA were 11.36 µmol/l (I), 19.89 µmol/l (II) and 27.84 µmol/l (III). The experimental details were described in section 3.11.2. K_i for N-(p-aminobenzenesulfonyl) valine obtained from the graph was 100.0×10^{-5} mol/l.



[N - (p - Aminobenzenesulfonyl) valine]

FIGURE 20

Table 13 Summary of the K_i valeus for p-aminobenezenesulfonamidoalkanoic acids and sulfanilamide.

p-Aminobenzenesulfonamidoalkanoic Acids	K_i (mol/l)
N-(p-Aminobenzenesulfonyl) glycine	4.0×10^{-5}
N-(p-Aminobenzenesulfonyl) tyrosine	13.8×10^{-5}
N-(p-Aminobenzenesulfonyl) alanine	23.5×10^{-5}
N-(p-Aminobenzenesulfonyl) phenylalanine	24.0×10^{-5}
N-(p-Aminobenzenesulfonyl) methionine	49.3×10^{-5}
N-(p-Aminobenzenesulfonyl) leucine	84.0×10^{-5}
N-(p-Aminobenzenesulfonyl) valine	100.0×10^{-5}
Sulfanilamide	4.8×10^{-5}

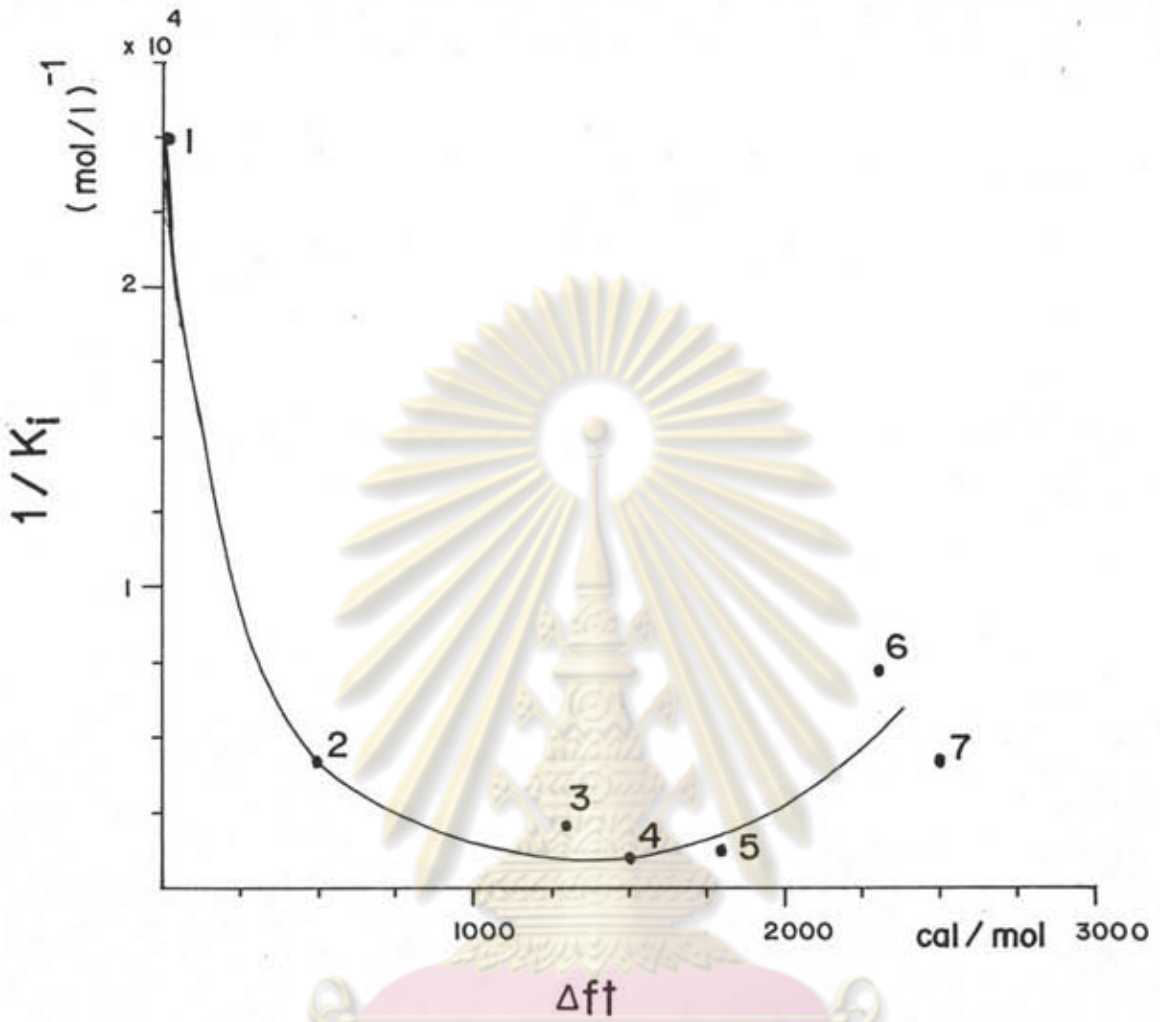


Figure 21 Plot of $1/K_i$ for p-aminobenzenesulfonamidoalkanoic acids versus Δft values of the side chains of p-aminobenzenesulfonamidoalkanoic acids.

1 = N-(p-Aminobenzenesulfonyl) glycine

2 = N-(p-Aminobenzenesulfonyl) alanine

3 = N-(p-Aminobenzenesulfonyl) methionine

4 = N-(p-Aminobenzenesulfonyl) valine

5 = N-(p-Aminobenzenesulfonyl) leucine

6 = N-(p-Aminobenzenesulfonyl) tyrosine

7 = N-(p-Aminobenzenesulfonyl) phenylalanine

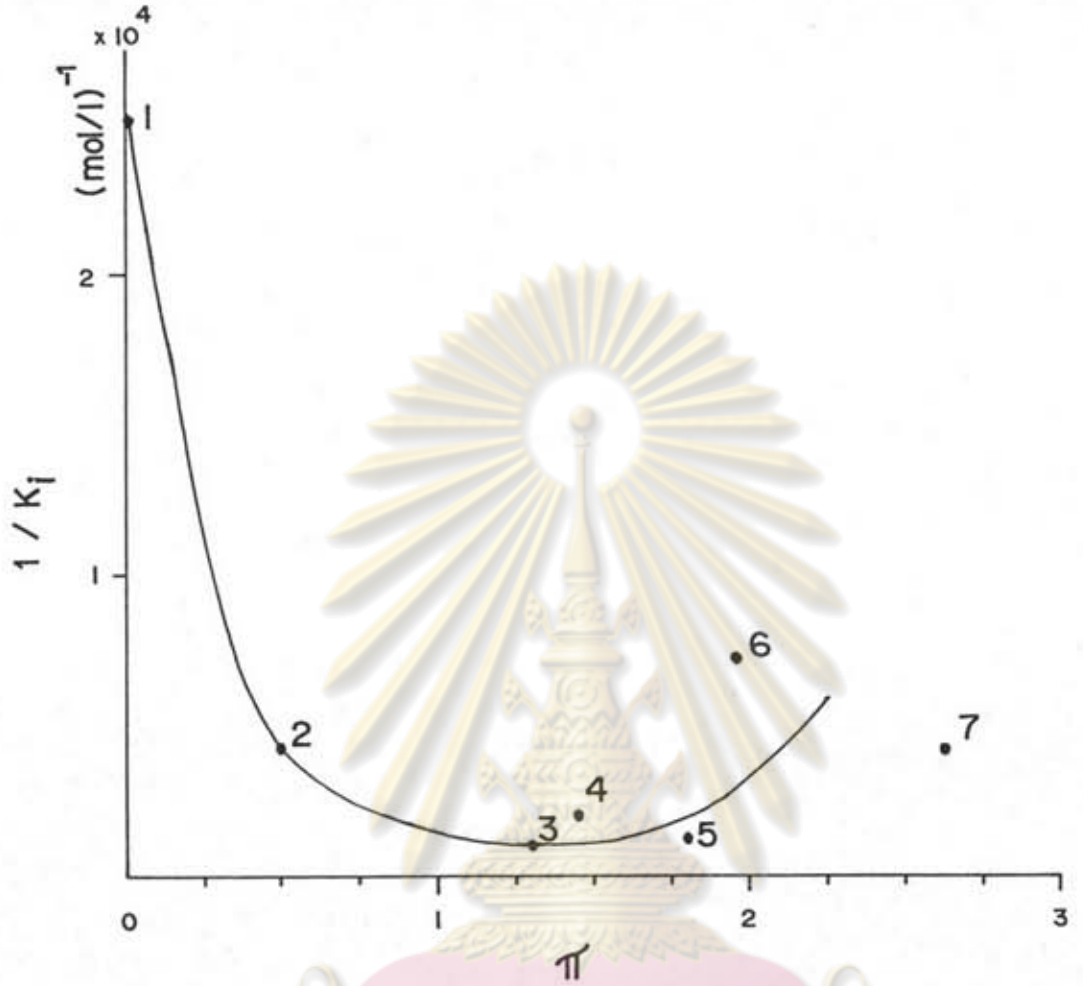


Figure 22 Plot of $1/K_i$ for p-aminobenzenesulfonamidoalkanoic acids versus π values of the side chains of p-aminobenzenesulfonamidoalkanoic acids.

1 = N-(p-Aminobenzenesulfonyl) glycine

2 = N-(p-Aminobenzenesulfonyl) alanine

3 = N-(p-Aminobenzenesulfonyl) valine

4 = N-(p-Aminobenzenesulfonyl) methionine

5 = N-(p-Aminobenzenesulfonyl) leucine

6 = N-(p-Aminobenzenesulfonyl) tyrosine

7 = N-(p-Aminobenzenesulfonyl) phenylalanine