TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii .
ABSTRACT (ENGLISH)	iv
ABSTRACT (THAI)	v
LIST OF TABLES	ix
LIST OF ILLUSTRATIIONS	xi
CHAPTER I INTRODUCTION	75
1.1 Overview	1
1.2 Hemoglobin and thalassemia	2
1.2.1 Hemoglobin	2
1.2.2 Thalassemia	5
1.2.3 Classification of thalassemia	6
1.2.3.1 Alpha-thalassemia	8
1.2.3.2 Beta-thalassemia	11 9
1.2.4 Methodology for detection and characterization	8 13 M
1.2.4.1 Screening method	ni ¹³ ersity
1.2.4.1.1 Complete Blood Count (CBC)	16
1.2.4.1.2 Osmotic Fragility Test (OFT)	20

1.2.4.1.3 Microcolumn Chromatography	22
1.2.4.1.4 High Performance Liquid Chromatography (HPLC)	23
1.2.4.1.5 Electrophoresis	26
1.2.4.2 Characterization	29
1.2.4.2.1 Polymerase Chain Reaction (PCR)	31
1.3 Flow Injection Analysis (FIA)	34
1.4 Diethylaminoethyl (DEAE)-Sephadex A50	35
1.5 Flow-based Technique for Thalassemia Screening	38
CHAPTER 2 EXPERIMENT	40
2.1 Materials and apparatus	40
2.2 Reagent	40
2.3 Preparation of buffer solution	41
2.4 Preparation of blood sample	41
2.5 Packing of DEAE-Sephadex A-50 beads	41
2.6 Manifold and operation step	42
2.7 Optimization	44
2.8 Evaluation	44
CHAPTER 3 RESULTS AND DISCUSSION	45
3.1 Elution profiles of hemoglobin	45
3.2 Optimization	

3.2.1 Suitable pH	48
3.2.2 Flow rate	49
3.3.3 Amount of resin	51
3.3 Evaluation of the proposed FI-system	52
3.4 Advantages and disadvantages	62
CHAPTER 4 CONCLUSION	63
4.1 Conclusion	63
4.2 Further works	64
REFERENCES	65
APPENDIX A	70
APPENDIX B	72
APPENDIX C	73
APPENDIX D	75
APPENDIX E	78
APPENDIX F	80
CURRICULUM VITAE	89

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

LIST OF TABLES

Table	101015	Page
1.1	Human hemoglobin.	4
1.2	Classification of thalassemia.	7
1.3	Hemoglobin patterns in α-thalassemia.	10
1.4	Hemoglobin patterns in β-thalassemia.	13
1.5	Comparison of various chromatographic resins for Hb A2 quantification.	23
1.6	Types of matrix and functional group.	37
3.1	Optimization of the pH of buffer solutions.	49
3.2	Optimization of the elution flow rate.	50
3.3	Optimization of the amount of resin.	51
3.4	Summarization of the test results with the diagnosis of Hb type.	54
3.5	Comparison of calculated percentages of Hb A ₂ + Hb E peak areas from	55
	the proposed FI-reduced volume column system and from the	
	conventional column system. The results from E-screen, PCR and Hb type	
	diagnosis were done by the Thalassemia Research Laboratories, Maharaj	
	Nakorn Chaing Mai Hospital, Chiang Mai University.	
3.6	Comparison of Hb A ₂ + Hb E percentages obtained from the FI-reduced	57ersity
	volume column and from the conventional larger column. The Hb type	

	diagnosis was done by the Thalassemia Research Laboratories, Maharaj	
	Nakorn Chaing Mai Hospital, Chiang Mai University.	
3.7	Reproducibility of the system that was tested by running 7 replicates of	58
	normal sample.	
3.8	Reproducibility of the system that was tested by running 7 replicates of β-	59
	trait sample.	

Reproducibility of the system that was tested by running 7 replicates of E-

3.9

trait sample.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

LIST OF ILLUSTRATIONS

Figur	03181346	page
1.1	Hemoglobin molecule.	3
1.2	Globin chain production and development. Fetal red blood cells contain	3
	primarily hemoglobin F (two α and two γ globin). Soon after birth, γ chain	
	synthsis is suppressed and β and δ chain production increase, which results	
	in the production of hemoglobins A and A ₂ .	80%
1.3	The geographic of thalassemia distribution	6
1.4	Stepwise approach to the diagnosis of thalassemia.	15
1.5	Distribution of erythrocyte counts (RBC), hemoglobin concentration and	18
	MCV in patients with iron deficiency (solid circles) and thalassemia trait	
	(open circles). This study includes only patients with microcytosis. The	
	thalassemia patients have relatively higher erythrocyte counts and are less	
	anemic than iron-deficient patients.	
1.6	Hemoglobin concentrations, MCV and MCHC in uncomplicated iron	19
	deficiency (A) and uncomplicated thalassemia minor (B). Solid symbols	
	represent males and open symbols represent females. Dashed lines represent	
	limits used in defining the category of microcytosis without marked anemia.	
	The reference values for healthy adults are indicated by the shaded areas.	

1.7	OFT stand showing positive and negative samples in different tubes. Tubes	21
	from L to R: Tubes 1-3 and 6-8 are positive samples where black line is not	
	visible through the solution. Tubes 4-5 and 9-10 are negative samples where	
	black line is visible through the solution.	
1.8	The reactive functional group of the DEAE anion exchanger. R is the fourth	22
	carbon of glucose in the cellulose chain. The COO of hemoglobin binds to	
	the N ⁺ of the DEAE.	
1.9	Separation of normal Hb types by cation exchange HPLC.	25
1.10	Separation of normal polypeptide chains by reversed phase HPLC.	25
1.11	Hemoglobin electrophoresis of the most prevalent hemoglobinopathies	28
	A) Cellulose acetate electrophoresis at alkaline pH causes hemoglobin to	
	move from the cathode toward the anode. AA, normal sample; AS, sickle	
	cell trait; FASC, control containing Hb A, F, S, C; Hb S- $^+$ Th, HbS, β^+ -	
	thalassemia; SC, HbSC disease; SS, sickle cell anemia.	
	B) Citrate agar electrophoresis, at acidic pH. Note that the hemoglobins	
	now travel from anode to cathode.	
1.12	Protein binding to an ion exchange bead.	36
	a) Positively-charged bead associates with negatively-charged counter ions	
	from buffer solution. The negatively-charged protein associates with	
	positively charged counter ions.	
	All rights reser	

	b) When the protein binds to the bead, some of the counter ions are	
	displaced from both the bead and the protein.	
2.1	A reduced volume microcolumn.	42
2.2	FI-reduce volume column manifold.	43
3.1	(a) elution profiles of normal blood sample obtained from the proposed	47
	system.	
	(b) elution profiles of thalassemia patient blood sample obtained from the	
	proposed system.	
3.2	The Correlation plot between the results obtained from the FI-microcolumn	61
	system and those obtained from the conventional microcolumn	
ΑI	A Modified microcolumn.	70
B 1	Hb E screening test; C1 is Normal (AA), C2 is Hb E trait (AE), C3 is Hb	72
	E/β thalassemia (EF) and C4 is Hb E homozygous (EE).	
C1	OF test results (a) Negative OF test result (b) Positive OF test result.	74

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University -All rights reserved