TABLE OF CONTENTS

		Page
ACKNOWLEDGE	MENT	iii
ABSTRACT		iv
LIST OF TABLES		xii
LIST OF ILLUST	PRATIONS	xiii
ABBREVIATIONS	S AND SYMBOLS	xvi
CHAPTER I: IN	TRODUCTION	502
I.	Bilirubin metabolism	
II,	Chemistry and structure of bilirubin in circulation	2
111	. Clinical significances of bilirubin	7
IV	. Methods for determination of serum bilirubin	8
V.	The problems of the measurement of bilirubin	
	fractions in serum and urine	10
VI	. Literature reviews	12
VI	I. Objectives	16
CHAPTER II: M	ETERIALS AND METHODS	17
M	aterials	17
	Specimen	17.
II.	Instruments	17
ш	. Chemicals and reagents	17
Me	ethods	L /18 G C
I. S	Separation of Bc in bile	18
II.	Identification of bilirubin fractions separated from	
	bile using HPLC	18

a. Determination of the bilirubin	
fractions retention time	18
b. Post column identification	19
III. Biosynthesis of Bc from different types of liver	20
IV. Preparation of lyophilized Bc product	21
V. Determination of chemical properties of conjugated	
bilirubin	21
a. Absorption spectra	21
b. Diazotization	22
VI. Determination of physical properties of	
conjugated bilirubin	22
a. Effects of photooxidation on Bc product	22
b. Effects of exposing Bc product to oxygen (air)	
oxidation	23
VII. Determination of type of sugar conjugated to bilirubin	23
VIII. Application for using conjugated bilirubin isolated	
from bile	24
a. Molar absorptivity of conjugated bilirubin	
prepared for use as a standard	24
b. Application of conjugated bilirubin for	
preparation of control serum	niv ²⁴ rsity

CHAPTER III: RESULTS	25
I. Separation of Bc in bile	25
II. Identification of bilirubin fractions separated from	
bile using HPLC	29
a. Determination of the bilirubin fractions	
retention time	29
b. Post column identification	37
III. Biosynthesis of Bc from different types of liver	45
IV. Preparation of lyophilized Bc product	49
V. Determination of chemical properties of conjugated	
bilirubin	49
a. Absorption spectra	49
b. Diazotization	49
VI. Determination of physical properties of conjugated	
bilirubin	49
a. Effects of photooxidation on Bc product	49
b. Effects of exposing Bc product to oxygen (air)	
oxidation	50
VII. Determination of type of sugar conjugated to bilirubin	59
VIII. Application for using conjugated bilirubin isolated	
from bile	59
a. Molar absorptivity of conjugated bilirubin	
prepared for use as a standard	59
b. Application of conjugated bilirubin for	
preparation of control serum	61

CHAPTER IV:	DISCUSSION AND CONCLUSION	65
REFERENCES		71
APPENDICES		78
	Appendix A	79
	Appendix B	80
	Appendix C	82
	Appendix D	87
	Appendix E	88
	Appendix F	90
	Appendix G	94
	Appendix H	95
	Appendix I	96
CURRICULUM	VITAE	100

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

LIST OF TABLES

	LIST OF TABLES		
Table			
	Precisions of peak areas and retention times of bile pigments separated	Page	
	from different bile sources	28	
2	2 Concentrations of bilirubin calculated from the absorbance of azopigment		
	formation according to the Malloy-Evelyn diazo reagent reaction	44	
3	The concentrations of direct and total bilirubin in the most prominent		
	peak of bovine, chicken and human bile eluate	45	
4	Molar absorptivity of Bc isolate solubilized in 0.1 mol/L Tris-HCl		
	buffer, pH 7.4 (calculated as Bu equivalence)	60	
5	Molar absorptivity of Bc isolate solubilized in 4 % BSA	51//	٠
	(calculated as Bu equivalence)	60	
6	Analytical concentration of total and direct bilirubin (TBIL and DBIL)		
	in two preparations of control serum by the diazo reaction based on		
	Jendrassik and Grof method	60	
7	The recovery of a commercial Bu standard added to control serum		
	analyzed by the diazo reaction based on Jendrassik and Grof method	62	
8	The recovery of Bc isolates added to control serum analyzed by the		
	diazo reaction based on Jendrassik and Grof method	62	
9	The OCV and RCV precision of direct and total bilirubin in the prepared	nivers	
	control serum and commercial control serum analyzed by Jendrassik and		ė d
	Grof method	63	

LIST OF ILLUSTRATIONS

	LIST OF ILLUSTRATIONS	
	Figure	Page
	1 Bilirubin subfractions and photoisomers during phototherapy for	lage
	unconjugated bilirubinaemia	4
;	2 Structure of bilirubin glucuronides	5
	Retention time of 20 mg/dL Bu standard in 4 % BSA	26
4	4 HPLC separation profiles of bilirubin species in different types of bile	27
5		39
	untreated and treated with bilirubin oxidase in 0.1 mol/L Glycine-NaOH	
	buffer, pH 10.0	31
6	Comparison of retention time of fractionated human obstractive jaundice	
	patient serum untreated and treated with bilirubin oxidase in 0.1mol/L	
	Glycine-NaOH buffer, pH 10.0	32
7	Comparison of Bu retention time of Bu standard untreated and treated	
	with bilirubin oxidase in 0.1 mol/L Glycine-NaOH buffer, pH 10.0	33
8	Comparison of Bc and Bu retention times of fractionated chicken bile	
	untreated and treated with bilirubin oxidase in 0.1 mol/L Tris-SDS	
	buffer, pH 8.0	34
9	Comparison of Bc and Bu retention times of fractionated human patient	
	serum untreated and treated with bilirubin oxidase in 0.1 mol/L Tris-SDS	
	buffer, pH 8.0	35/ 👝
10	Comparison of retention time of Bu standard dissolved in 4 % BSA	
	untreated and treated with bilirubin oxidase in 0.1mol/L Tris-SDS buffer,	
	pH 8.0	36

Figure	Page
11 The absorption spectra of Bc eluate from HPLC separation	39
12 The absorption spectra of azobilirubin formed by the reaction of bile	
pigment in bovine eluate with diazo reagent (M-E method)	40
13 The absorption spectra of azobilirubin formed by the reaction of bile	
pigment in chicken eluate with the diazo reagent (M-E method)	41
14 The absorption spectra of azobilirubin formed by reaction of bile pigment	
in human eluate with diazo reagent (M-E method)	42
15 The absorption spectra of azobilirubin formed by the reaction of Bu	
standard with diazo reagent (M-E method)	43
16 The kinetic of liver biosynthesis from different types of liver homogenate	46
17 The comparison of Bc absorption spectra from different types of liver	
biosynthesis	47
18 The comparison of Bu absorption spectra from different types of liver	
biosynthesis	48
19 The relationship of the maximum peak of absorbance and irradiation time	
of Bc in 0.1mol/L Tris-HCl buffer, pH 7.4	51
20 The photodegradation rate of Bc in 0.1 mol/L Tris-HCl buffer, pH 7.4	
at 412 nm	52
21 The relationship of the maximum peak of absorbance and irradiation	
time of Bc in 4 % BSA	53
22 The photodegradation rate of Bc in 4 % BSA at 412 nm	54
23 The relationship between the maximum peak of absorption spectra and	
time of exposure to oxygen of Bc in 0.1 mol/L Tris-HCl buffer, pH 7.4	55
24 The rate of oxidation by oxygen of Bc in 0.1 mol/L Tris-HCl buffer,	
pH 7.4 at 412 nm	56

Figure	Page
25 The relationship between absorption spectra and oxidation time of Bc	
in 4 % BSA	57
26 The rate of oxidation by oxygen of Bc in 4 % BSA at 412 nm	58

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

ABBREVIATIONS AND SYMBOLS

AU Absorbance unit

BSA Bovine serum albumin

cm Centimeter

CV Coefficient of variation

Bc Conjugated bilirubin

°C Degree Celsius

B_δ Delta bilirubin

DMSO Dimethyl sulfoxide

DTB Ditaurobilirubin

Fig. Figure

g Gram

g/L Gram per liter

hr

HSA Human serum albumin

L.mol⁻¹.cm⁻¹ Liter per mole per centimeter

μL Microliter

mbar Millibarr

mg Milligram

mg/dL Milligram per deciliter

mg/L Milligram per liter

mL Milliliter

min Minute

mol/L Mole per liter

nm Nanometer N Normality **NMR** Nuclear magnetic resonance No Number **OCV** Optimal condition variation rpm Revolution per minute **RCV** Routine condition variation Sec Second SDS Sodium dodecyl sulfate SD Standard deviation Bu Unconjugated bilirubin U Unit UDP Uridine 5'- diphosphate Alpha β Beta δ Delta ε Molar absorptivity % Percentage

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