

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

SYNTHESIS OF ALKYL QUATERNARY AMINO CELLULOSES  
AND AN INVESTIGATION OF THEIR POTENTIAL AS  
BILE ACID SEQUESTRANTS

A thesis  
presented in partial fulfilment  
of the requirements for the degree of  
Master of Science  
in Chemistry

at

Massey University  
New Zealand

Michael John LILLY

1988

## ABSTRACT

One method of treatment for patients with elevated serum cholesterol levels (hypercholesterolemia) is the oral administration of an anion exchange resin to adsorb bile acids in the gastrointestinal tract. The resins used successfully to date have been mainly synthetic ones, whereas anion exchange derivatives of cellulose have not been shown to be effective.

Alkyl quaternary amino (QA) cellulose ion exchangers were prepared from hydroxypropylated regenerated cellulose (HP Indion®). Their capacities for cholate anions were measured in the presence of a background of chloride ions at physiological concentration. It was shown that butyl QA cellulose was ineffective at binding cholate anions. However, alkyl QA celluloses with alkyl groups longer than butyl (octyl and dodecyl) bound cholate anions in preference to the chloride ions and greatly improved capacities were obtained. The capacities of the octyl and dodecyl QA celluloses were dependent on the cross-linking present in the HP Indion used. If the cross-linking was greater than the 15% used in HP5 Indion, then the capacity for binding cholate dropped off. A significant increase in substitution level and decrease in swollen volume were obtained by reprocessing the alkyl QA cellulose ion exchangers and this improved their cholate binding capacities.

The alkyl QA celluloses were all prepared by first attaching chemically reactive epoxide groups to HP Indion and then reacting those epoxide groups with a tertiary amine (N,N-dimethyl-N-alkylamine) to form the quaternary amino cellulose. These two steps were repeated for the reprocessed products. The optimum conditions for epoxidation of HP5 Indion were shown to be 48 hours at 4-6°C in 6% sodium hydroxide with excess epichlorohydrin. In the reprocessing step, where this reaction was used a second time, 24 hours was sufficient to reach maximum epoxidation levels. When coupling the N,N-

dimethyl-N-alkylamine to the epoxide cellulose it was necessary to use aqueous ethanol as a solvent. The ethanol concentration was dependent on the size of the alkyl group. For N,N-dimethyl-N-dodecylamine a concentration of greater than 60% ethanol was required. For larger amines considerable hydrolysis of the epoxide groups occurred during coupling. This was avoided almost completely in the first coupling step by half-neutralising the amine with hydrochloric acid, to lower the pH and buffer the reaction. The optimum coupling time and temperature were shown to be 10 hours at 70°C.

As a result of optimising these processing steps the substitution level obtained for dodecyl QA cellulose was increased from 0.83 to 1.14 meq/g at the end of the first stage and from 1.18 to 1.50 meq/g with the reprocessed product.

A range of alkyl QA celluloses were prepared using the optimum conditions found for dodecyl QA cellulose. Octyl and dodecyl QA celluloses prepared on HP7 Indion were shown to have higher capacities for cholate than cholestyramine and colestipol (Colestid<sup>®</sup>) at cholate concentrations less than 6 mmol/L. The dodecyl QA cellulose had superior capacities at all cholate concentrations (0-15 mmol/L) when measured on the basis of the volume of the ion exchanger used. In contrast, cholestyramine and colestipol had higher capacities for deoxycholate anions than either of the cellulose products, except when the residual concentration of deoxycholate was very low (less than 0.5 mmol/L). Cholestyramine and colestipol bound deoxycholate efficiently (97-99%) when the residual concentration was between 5-10 mmol/L, but not cholate (29-48%). However the alkyl QA celluloses bound deoxycholate and cholate with very high efficiency (82-138%).

To compare the efficiencies of the ion exchangers at low concentrations a more sensitive test was developed utilising a mixture of conjugated bile salts, similar in composition to that found in the human duodenum. This was equilibrated with

samples of each ion exchanger and then analysed by HPLC. Methyl and butyl QA celluloses were the least effective at reducing the concentrations of the bile salts, however they still performed as well as colestipol. The capacity of octyl QA cellulose was much better than the methyl or butyl QA celluloses, indicating the importance of the hydrophobic alkyl chain. Cholestyramine had a similar capacity to the octyl QA cellulose, except that it was not as effective at binding glycocholate. The most effective product was found to be dodecyl QA cellulose, which reduced the concentration of all of the bile salts to lower levels than any other ion exchanger.

### ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr J.S. Ayers for the help and encouragement he has given to me throughout the course of this study.

I would also like to thank all members of the Chemistry and Biochemistry Department at Massey University for their help, especially: Dave Elgar for his valuable suggestions and technical expertise; Dick Poll for his advice on HPLC; and Satendra Ram for editing my introduction.

Warm thanks is extended to Glenda Shaw for her assistance in preparing the text, and to Jenny Trow for her assistance in preparing the Figures.

## CONTENTS

ABSTRACT	
ACKNOWLEDGEMENTS	
LIST OF CONTENTS	
LIST OF TABLES	
LIST OF FIGURES	
ABBREVIATIONS	

### CHAPTER 1: INTRODUCTION

1.1	OUTLINE	1
1.2	EFFECTS OF ELEVATED PLASMA CHOLESTEROL	1
1.3	GENERAL APPROACH TO CLINICAL MANAGEMENT OF HYPERCHOLESTEROLEMIA	2
1.4	USE OF BILE ACID SEQUESTRANTS TO REDUCE PLASMA CHOLESTEROL LEVELS	3
1.5	BILE ACID SEQUESTRANTS CURRENTLY AVAILABLE	5
	A. Cholestyramine	
	B. Colestipol Hydrochloride	
	C. Diethylaminoethyl (DEAE) Sephadex	
1.6	SOME PROBLEMS ASSOCIATED WITH THE USE OF BILE ACID SEQUESTRANTS	7
1.7	THE USE OF HP INDION CELLULOSE MATRIX FOR THE PREPARATION OF ION EXCHANGERS	8
1.8	EPOXIDE ACTIVATION OF HP INDION CELLULOSE MATRIX	9
1.9	AIM OF THESIS	11

### CHAPTER 2: MATERIALS AND METHODS

2.1	MATERIALS	12
	2.1.1 Chemicals	12
	2.1.2 Equipment	13
2.2	DRY MATTER ANALYSIS	13
2.3	GENERAL METHOD FOR PREPARING CELLULOSE ION EXCHANGERS	14
	2.3.1 Activation of HP Indion Cellulose	14
	2.3.2 Coupling the Activated Celluloses with Tertiary Amines	14
2.4	REPROCESSING	16
2.5	OPTIMISATION OF THE SYNTHESIS OF DODECYL QA CELLULOSE	16
	2.5.1 Coupling Temperature	16
	2.5.2 Half-Neutralisation of DMDo-Amine	18

2.5.3	Coupling Time at 70°C	18
2.5.3a	Coupling with Trimethylamine in Ethanol	18
2.5.4	Coupling Time at 22°C	19
2.5.5	Optimum Time for Activation	19
2.5.5a	Coupling with Sulphite	20
2.5.6	Reinvestigation of Coupling Time at 70°C	20
2.5.7	Large Scale Preparation of Dodecyl QA Cellulose (DMDo 72)	20
2.5.8	Coupling Conditions for Trimethylamine	21
2.5.9	Conditions for Activating Dodecyl QA Cellulose	21
2.5.9a	Coupling with Trimethylamine in Water	22
2.5.10	Coupling Conditions for Reactivated DMDo 72	22
2.6	DETERMINATION OF SUBSTITUTION LEVEL AND SWOLLEN VOLUME	23
2.6.1	Anion Exchangers	23
2.6.2	Sulphopropyl Celluloses (SP-Celluloses)	24
2.7	TITRATION CURVES	24
2.8	BILE ACID CAPACITY TESTS	25
2.8.1	Capacity for Cholate and Deoxycholate	25
2.8.2	Capacity for Conjugated Bile Acid Anions	26
2.8.2a	HPLC Analysis of Bile Salts	27
2.9	PREPARATION OF N,N-DIMETHYL-N-BUTYLAMINE	28

### CHAPTER 3: PRELIMINARY STUDIES

3.1	INTRODUCTION	29
3.2	PREPARATION OF QUATERNARY AMINO CELLULOSE	29
3.3	CHOLATE CAPACITY TESTS	32
3.3.1	The Effect of the Length of the Alkyl Group	33
3.3.2	The Effect of Cross-Linking	35
3.4	THE EFFECT OF REPROCESSING	37
3.5	CHOLATE CAPACITY TESTS	39
3.5.1	The Effect of Reprocessing on the Cholate Capacity	39
3.5.2	The Effect of Cross-Linking on the Cholate Capacity	40
3.6	CONCLUSION	44

### CHAPTER 4: PREPARATION OF N,N-DIMETHYL-N- DODECYLAMINO CELLULOSE

4.1	INTRODUCTION	45
-----	--------------	----



4.2	THE EFFECT OF TEMPERATURE ON COUPLING	45
4.3	THE EFFECT OF HALF-NEUTRALISATION OF DMDo-AMINE ON COUPLING	46
4.4	INVESTIGATION OF THE COUPLING TIME REQUIRED FOR HALF-NEUTRALISED DMDo-AMINE	49
4.4.1	Introduction	49
4.4.2	Coupling Time at 70°C	49
4.4.3	Coupling Time at Room Temperature	52
4.5	OPTIMUM TIME FOR ACTIVATION	54
4.6	REINVESTIGATION OF COUPLING TIME AT 70°C	55
4.6.1	Preparation of a Large Batch of DMDo-Cellulose (DMDo 72)	56
4.7	QUANTITATIVE COUPLING CONDITIONS FOR TRIMETHYLAMINE	58
4.8	OPTIMUM ACTIVATION CONDITIONS FOR REPROCESSING	60
4.9	COUPLING CONDITIONS FOR REACTIVATED DMDo 72	62
4.10	TITRATION CURVES	64
4.11	SUMMARY	70

#### CHAPTER 5: PREPARATION OF ION EXCHANGERS FOR IN VITRO BILE ACID CAPACITY TESTS

5.1	INTRODUCTION	72
5.2	PREPARATION OF SINGLE AND DOUBLE PROCESSED ION EXCHANGERS	72
5.3	'SPECIAL' ION EXCHANGERS	74
5.3.1	Reprocessing Dodecyl QA Cellulose with Either Trimethylamine or n-Dodecylamine	74
5.3.2	Triple Processing of Octyl QA Cellulose	78

#### CHAPTER 6: INVESTIGATION OF BILE ACID BINDING CAPACITY IN VITRO

6.1	INTRODUCTION	80
6.2	CHOLATE CAPACITY TESTS	80
6.2.1	The Effect of Reprocessing Dodecyl QA Celluloses With and Without Half-Neutralisation of the Amine	81
6.2.2	Comparison of Octyl and Dodecyl QA Celluloses Prepared on 10% and 15% Cross-Linked HP Indion	82
6.2.3	The Effect of Reactivating Dodecyl QA Cellulose and Coupling Trimethylamine on Cholate Binding	87

6.2.4	The Effect of Triple Processing Octyl QA Celluloses on Cholate Binding	88
6.2.5	Cholate Capacity Curves of Cholestyramine and Colestipol	91
6.3	DEOXYCHOLATE CAPACITY TESTS	96
6.4	BINDING STUDIES WITH CONJUGATE MIXTURES	100
6.4.1	Development of HPLC Method for Determination of Conjugated Bile Acids	101
6.4.2	Measurement of Capacity for Binding Conjugated Bile Acid Anions	105
6.4.3	The Effect on Capacity of Reprocessing Dodecyl QA Cellulose	107
6.4.4	Effect on Capacity of the Alkyl Group in the Alkyl QA Celluloses	108
6.4.5	Cholestyramine and Colestipol	115
6.5	CONCLUSION	119
APPENDIX I	Preparation and Properties of the Ion Exchangers Discussed in Chapters 5 and 6	
REFERENCES		

## LIST OF TABLES

### Table No.

- 1.1 Hypolipidemic Drugs Used in the Management of Plasma Cholesterol
- 1.2 Ion Exchangers Prepared from Epoxide Activated HP Indion
- 2.1 Summary of the Alkyl QA Cellulose Ion Exchangers Prepared in Chapter 3
- 3.1 Properties of the Alkyl QA Celluloses
- 3.2 Number of Epoxide Groups Converted to QA Groups During Coupling
- 3.3 Properties of the Reprocessed Alkyl QA Celluloses
- 4.1 The Effect of Temperature on Coupling
- 4.2 The Effect of Half-Neutralisation on Coupling
- 4.3 Optimum Time for Activation
- 4.4 Preparation of DMDo 72 from 100 g of HP Indion
- 4.5 Quantitative Conversion of Epoxides with Trimethylamine
- 4.6 Optimum Activation Conditions for Reprocessing
- 4.7 Preparation and Properties of Alkyl QA Celluloses Prepared from DMDo 72
- 5.1 Single Processed Ion Exchangers
- 5.2 Double Processed Ion Exchangers
- 5.3 'Special' Ion Exchangers Prepared by Reprocessing DMDo 72
- 5.4 Octyl QA Cellulose Ion Exchangers
- 6.1 Efficiency of Ion Exchangers for Binding Cholate Against a Background of 150 mmol/L Sodium Chloride at pH 8.0
- 6.2 Efficiency of Ion Exchangers for Binding Deoxycholate Against a Background of 150 mmol/L Sodium Chloride at pH 8.0
- 6.3 Concentration of Bile Acids in Human Duodenal Fluid
- 6.4 Identification of Conjugated Bile Acids by HPLC
- 6.5 Stability of Refrigerated Standards Containing 0.05  $\mu$ moles of Each Conjugate per 100  $\mu$ L
- 6.6 Binding Characteristics of Single Processed and Double Processed Dodecyl QA Celluloses
- 6.7 Binding Characteristics of the Alkyl QA Celluloses
- 6.8 Comparison of Methyl and Dodecyl QA Celluloses, Cholestyramine and Colestipol
- 6.9 The Effect of Ion Exchangers on Serum Cholesterol Concentrations in Cockerels

## LIST OF FIGURES

### Figure No.

- 1.1 Major Bile Acids Present in Human Bile
- 1.2 Partial Structures of Bile Acid Sequestrants Used in Therapy
- 3.1 The Effect of Alkyl Chain Length on Cholate Capacity
- 3.2 A Proposed Theory to Account for Binding Efficiencies Greater than 100%
- 3.3 The Effect of Cross-Linking on Cholate Capacity
- 3.4 The Effect of Reprocessing on Cholate Capacity (mg/g)
- 3.5 The Effect of Reprocessing on Cholate Capacity (mg/mL)
- 3.6 The Effect of Cross-Linking on Cholate Capacity
- 4.1 Titration Curve for DMDo-Amine in 66% Aqueous Ethanol
- 4.2 Coupling Time for Half-Neutralised DMDo-Amine at 70°C
- 4.3 Coupling Time for Half-Neutralised DMDo-Amine at 22°C
- 4.4 Coupling Time for Half-Neutralised DMDo-Amine at 70°C
- 4.5 Titration Curves for Alkyl QA Cellulose Ion Exchangers
- 4.6 Titration Curve for Cholestyramine
- 4.7 Titration Curve for Colestipol
- 5.1 Titration Curve for Dodecyl QA Cellulose Reprocessed with n-Dodecylamine (DMDo 112)
- 6.1 Cholate Capacity Curves (mg/g) for Dodecyl QA Celluloses Coupled With and Without Half-Neutralisation of DMDo-amine
- 6.2 Cholate Capacity Curves (mg/mL) for Dodecyl QA Celluloses Coupled With and Without Half-Neutralisation of DMDo-amine
- 6.3 Cholate Capacity Curves (mg/g) for 10% and 15% Cross-Linked Octyl and Dodecyl QA Celluloses
- 6.4 Cholate Capacity Curves (mg/mL) for 10% and 15% Cross-Linked Octyl and Dodecyl QA Celluloses
- 6.5 Cholate Capacity Curves for Dodecyl QA Celluloses Reprocessed with Trimethylamine and DMDo-Amine
- 6.6 Cholate Capacity Curves for Double and Triple Processed Octyl QA Celluloses
- 6.7 Cholate Capacity Curves for Double Processed Dodecyl QA Cellulose and Triple Processed QA Cellulose
- 6.8 Cholate Capacity Curves (mg/g) for Octyl and Dodecyl QA Celluloses, Cholestyramine and Colestipol
- 6.9 Cholate Capacity Curves (mg/mL) for Octyl and Dodecyl QA Celluloses, Cholestyramine and Colestipol
- 6.10 Deoxycholate Capacity Curves (mg/g) for Octyl and Dodecyl QA Celluloses, Cholestyramine and Colestipol
- 6.11 Deoxycholate Capacity Curves (mg/mL) for Octyl and Dodecyl QA Celluloses, Cholestyramine and Colestipol
- 6.12 Detection of Bile Acid Conjugates by UV Absorption
- 6.13 Working Curve for Bile Acid Conjugates
- 6.14 Conjugated Bile Acid Binding to Single Processed Dodecyl QA Cellulose (DMDo 72, 1.08 meq/g)

- 6.15 Conjugated Bile Acid Binding to Double Processed, 10% Cross-Linked, Dodecyl QA Cellulose (DMDo 109, 1.50 meq/g)
- 6.16 Conjugated Bile Acid Binding to Double Processed, 10% Cross-Linked, Octyl QA Cellulose (DMO 14, 1.70 meq/g)
- 6.17 Conjugated Bile Acid Binding to Double Processed, 15% Cross-Linked, Butyl QA Cellulose (DMB 9, 1.68 meq/g)
- 6.18 Conjugated Bile Acid Binding to Double Processed, 15% Cross-Linked, Methyl QA Cellulose (TMA 33, 1.89 meq/g)
- 6.19 Binding Characteristics of Alkyl QA Celluloses
- 6.20 Conjugated Bile Acid Binding to Cholestyramine
- 6.21 Conjugated Bile Acid Binding to Colestipol

## ABBREVIATIONS

BBS	Bicarbonate Buffer System
CHD	Coronary Heart Disease
CL	Cross-Linking
Conc.	Concentration
DEAE	2-Diethylaminoethyl
DMB-Cellulose	Butyl Quaternary Amino Cellulose
DMDo-Amine	N,N-dimethyl-N-dodecylamine
DMDo-Cellulose	Dodecyl Quaternary Amino Cellulose
DMO-Cellulose	Octyl Quaternary Amino Cellulose
ECH	Epichlorohydrin
FH	Familial Hypercholesterolemia
GC	Glycocholate
GDC	Glycodeoxycholate
HDL	High Density Lipoprotein
HP Indion	Hydroxypropylated Regenerated Cellulose
HP3 Indion	25% Cross-Linked HP Indion Cellulose
HP5 Indion	15% Cross-Linked HP Indion Cellulose
HP7 Indion	10% Cross-Linked HP Indion Cellulose
HPLC	High Performance Liquid Chromatography
LDL	Low Density Lipoproteins
meq	Milliequivalent/s
ODS	Octadecyl Silica
% aq	Percentage Aqueous (v/v)
QA	Quaternary Amino
RT	Room Temperature
SD	Standard Deviation
SP-Cellulose	Sulphopropyl Cellulose
TMA-Cellulose	Methyl Quaternary Amino Cellulose
TC	Taurocholate
TDC	Taurodeoxycholate
TPC	Total Plasma Cholesterol
UV	Ultraviolet
VLDL	Very Low Density Lipoproteins

## CHAPTER 1. INTRODUCTION

### 1.1 OUTLINE

The object of this thesis was to produce a quaternary amino (QA) cellulose ion exchanger with potential for use as a bile acid sequestrant in the treatment of hypercholesterolemia.

This introduction backgrounds the debilitating effects of hypercholesterolemic disorders and gives a brief summary of the clinical methods which are used to treat them. Treatments involving bile acid sequestrants, their mode of action and chemistry are reviewed. The procedures previously developed at Massey University for the preparation of cellulose ion exchangers are summarised. A rationale for the type of exchanger to be synthesised is given and the modifications considered necessary for promoting the selective and efficient binding of bile acids are discussed.

### 1.2 EFFECTS OF ELEVATED PLASMA CHOLESTEROL

It is now widely accepted that elevated plasma cholesterol is associated with coronary heart disease (CHD)<sup>1</sup>. In normal caucasians the total plasma cholesterol (TPC) level is approximately 200 mg/dL<sup>2</sup>, but only a small fraction of this circulates as free cholesterol in the blood. Between 50-70% of TPC is transported between tissues as a low density lipoprotein (LDL) fraction, 20-45% as a high density lipoprotein (HDL) fraction, 5-10% as a very low density lipoprotein (VLDL) fraction and an insignificant amount in the chylomicrons<sup>3</sup>. Abnormally high levels of LDL ( $\beta$ -lipoproteins), and therefore cholesterol, can be associated with a number of diseases. This condition is classified as hyper- $\beta$ -lipoproteinemia and there are two recognised subtypes: type IIa and type IIb<sup>4</sup>. Both of these subtypes result in premature CHD<sup>5</sup>.

Familial hypercholesterolemia (FH) is a relatively common inherited form of hyper- $\beta$ -lipoproteinemia, which can be identified at birth. FH individuals have significantly elevated TPC and LDL levels (2-3 times greater than normal in heterozygotes and 4-6 times greater than normal in homozygotes). The clinical consequences of unmanaged FH are well documented and include arcus coroneae, xanthomas, marked atherosclerosis and premature CHD<sup>3</sup>.

### 1.3 GENERAL APPROACH TO CLINICAL MANAGEMENT OF HYPERCHOLESTEROLEMIA

Substantial reductions, sometimes resulting in normalisation of TPC and LDL concentrations, can now be made in all forms of hyper- $\beta$ -lipoproteinemia except for homozygous FH<sup>6</sup>. Initial treatment involves adherence to a strictly controlled diet, followed by administration of hypolipidemic drugs where necessary (Table 1.1). Altered diets (which limit cholesterol intake to less than 300 mg per day and are low in saturated fat) can be expected to reduce TPC and LDL levels by up to 10%<sup>7</sup>, but normalisation is still not achieved in some cases. Recent studies<sup>6,8,9</sup> indicate that the most promising treatment of severe hyper- $\beta$ -lipoproteinemia probably lies with combined treatment regimens, incorporating a bile acid sequestrant with complimentary drugs.



TABLE 1.1 Hypolipidemic Drugs Used in the Management of Plasma Cholesterol<sup>6</sup>

<u>Drug</u>	<u>Mode of Action</u>
Nicotinic acid	Inhibits secretion of VLDL (a precursor for LDL synthesis)
Compactin Mevinolin	Cause competitive inhibition of the major rate limiting step of cholesterol biosynthesis
Clofibrate Gemfibrozil	Cause hydrolysis of VLDL triglycerides by lipoprotein lipase
Neomycin	Inhibits reabsorption of bile acids (used where bile acid sequestrants are poorly tolerated)

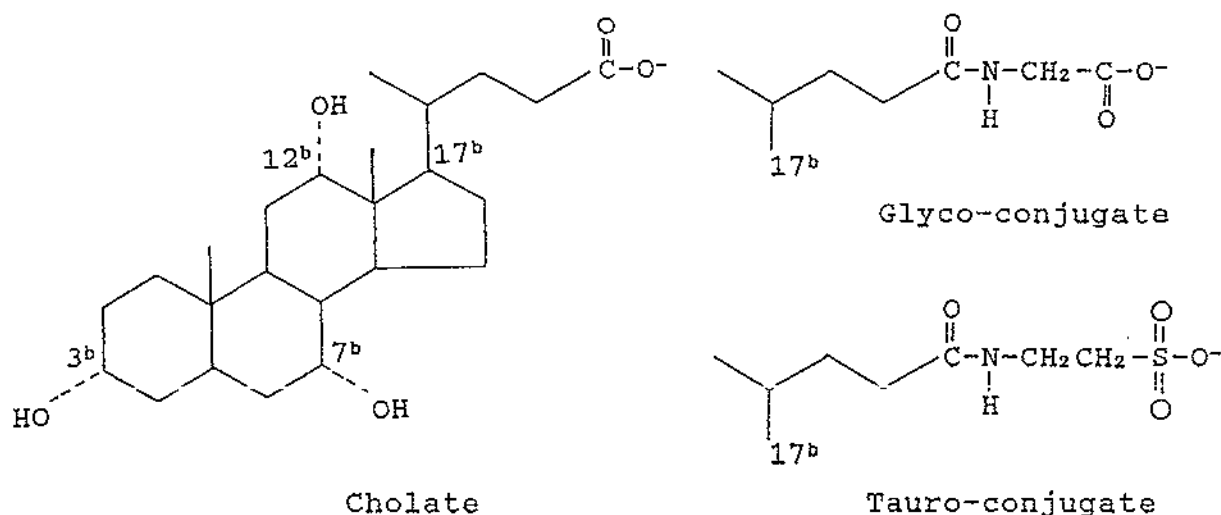
#### 1.4 USE OF BILE ACID SEQUESTRANTS TO REDUCE PLASMA CHOLESTEROL LEVELS

One well established method of treating hypercholesterolemia involves oral administration of non-absorbable anion exchangers which function as bile acid sequestrants. These materials have basic functional groups attached to a semi-rigid polymer backbone and they operate by exchanging chloride ions for bile acid anions in the intestinal lumen<sup>10,11</sup>.

Bile acids are secreted into the duodenum in response to food intake, where they promote the absorption of dietary fat. The structure of the major bile acids and their distribution in human bile is given in Figure 1.1<sup>12</sup>. Ingestion of bile acid sequestrants increases the faecal excretion of bile acids and lowers their concentration in the intestine. This results in decreased absorption of dietary cholesterol and other lipids<sup>13</sup>. It also causes an indirect decrease in plasma cholesterol, resulting from disruption of the enterohepatic circulation by which bile acids are reabsorbed. In healthy individuals approximately 95% of bile acids are actively reabsorbed from

the distal ileum and the remaining 5% are compensated for by new synthesis<sup>12</sup>. However, under the conditions of bile acid sequestrant therapy fewer bile acids are reabsorbed, so an increased rate of synthesis must occur. Plasma cholesterol (from LDL) is the precursor for all new liver synthesis of bile acids<sup>14</sup>, therefore when bile acid sequestrants are used there is increased removal of plasma LDL and a corresponding decrease in TPC<sup>15</sup>.

Figure 1.1 Major Bile Acids Present in Human Bile<sup>a</sup>



<u>Bile Acid</u>	<u>3<sup>b</sup></u>	<u>7<sup>b</sup></u>	<u>12<sup>b</sup></u>	<u>Proportion (%)</u>
Cholate	OH	OH	OH	50
Chenodeoxycholate	OH	OH	H	30
Deoxycholate	OH	H	OH	15
Lithocholate	OH	H	H	5

<sup>a</sup> Bile acids are usually present in human bile as sodium or potassium salts, and they are conjugated with either glycine or taurine

<sup>b</sup> Numbers refer to positions in the sterol ring

## 1.5 BILE ACID SEQUESTRANTS CURRENTLY AVAILABLE

The ion exchangers which have been used for therapy are:

### A. Cholestyramine

Cholestyramine, the most commonly used bile acid sequestrant, is or has been available as Cuemid (83% cholestyramine<sup>16</sup>), Questran (45% cholestyramine<sup>16</sup>) and Quantalan<sup>17</sup>. It is a copolymer of styrene and 2% divinylbenzene, with quaternary amino groups attached using trimethylamine and balanced with chloride counterions (Figure 1.2A).

### B. Colestipol Hydrochloride

Colestipol hydrochloride is a copolymer of diethylenetriamine and epichlorohydrin with approximately 20% of the available nitrogens titrated with hydrochloric acid (Figure 1.2B). It is sold under the proprietary name Colestid<sup>17</sup>.

### C. Diethylaminoethyl (DEAE) Sephadex

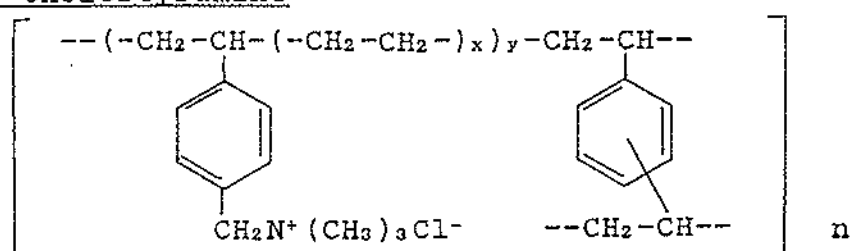
Polydextide or PDX chloride (formerly Secholex) is 79% DEAE-Sephadex<sup>16</sup>. It is a dextran cross-linked with epichlorohydrin and orthosubstituted with 2-diethylaminoethyl (DEAE) groups, some of which are quaternised with 2-diethylaminoethyl chloride<sup>17</sup> (Figure 1.2C).

Cholestyramine based bile acid sequestrants (Questran and Cuemid) are usually preferred over other products (Colestid and Secholex) because they have superior capacity and binding kinetics for bile acids in vitro<sup>16,20</sup>. Laboratory studies have demonstrated that Questran binds 40% more bile acids than Cuemid after mixing with bile for 2 hours at 37°C<sup>16</sup>. In contrast, clinical studies show that the different bile acid sequestrants produce similar hypolipidemic effects in vivo<sup>21,22,23</sup>. Typically, the TPC in patients can be expected to drop by 20-25% more than that achieved with dietary control alone. This drop occurs in a very short time after administration of

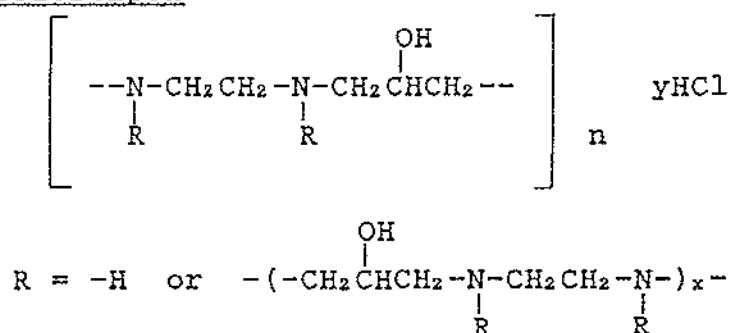
the sequestrant and can be sustained indefinitely with continued use.

Figure 1.2 Partial Structures of Bile Acid Sequestrants Used in Therapy

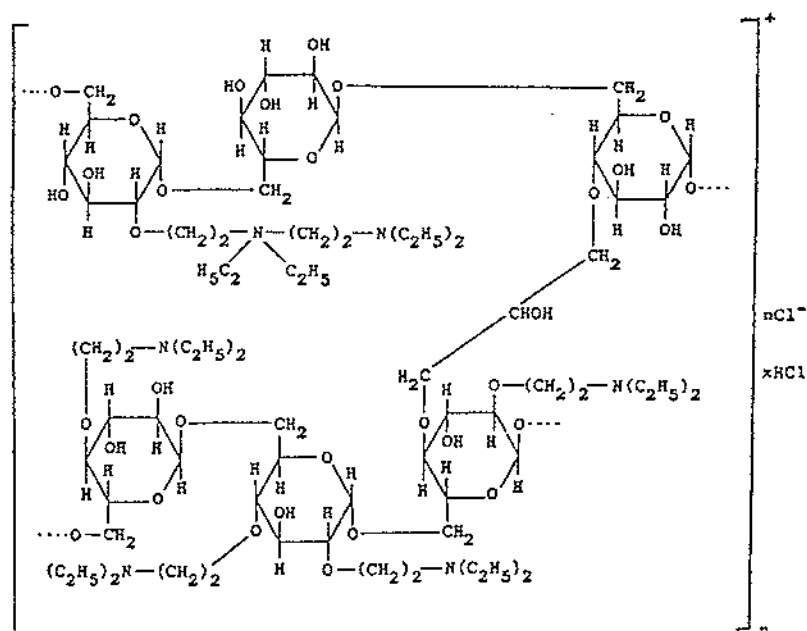
A. Cholestyramine<sup>18</sup>



B. Colestipol<sup>18</sup>



C. DEAE Sephadex<sup>19</sup>



## 1.6 SOME PROBLEMS ASSOCIATED WITH THE USE OF BILE ACID SEQUESTRANTS

Bile acid sequestrants are nonspecific and, as a result, a number of anions are able to compete with the bile acid anions for the available binding sites. The in vitro efficiency for binding bile acids in duodenal fluid is low for all of the sequestrants mentioned in Section 1.5, with only about 50% of the available ion exchange capacity being used under physiological conditions<sup>16</sup>. The efficiency may be even less in vivo. Also small reductions of plasma cholesterol are compensated for by an increase in cholesterol biosynthesis<sup>15</sup>. Thus for effective therapy prolonged and/or large dose rates are required<sup>17</sup>.

Since the ion exchangers are not absorbed from the gastrointestinal tract no systemic ill effects can be expected. Nevertheless, prolonged therapeutic treatment (e.g. 16-20 g cholestyramine per 24 hours) commonly results in constipation (10-50% incidence) and can sometimes lead to nausea, vomiting and other gastrointestinal discomfort. Because normal fat absorption is impaired, malabsorption of fat soluble vitamins may also occur, leading to side-effects including hypocalcemia, hypothyronemia and/or folic acid deficiency<sup>10,11</sup>. Considerable work has therefore focused on making bile acid sequestrants more effective.

The bile acid binding capacity of cholestyramine is enhanced by the addition of taurine and its 'pharmaceutically accepted' salts<sup>25</sup>, ascorbic acid<sup>26</sup> and some essential fatty acids<sup>27</sup>. Cholestyramine resins with different mesh sizes have been tested, however while finer particle sizes were less gritty and more palatable, their in vivo efficiency in binding bile acids was not improved over the standard grade<sup>28</sup>. More recent studies have attempted to make cholestyramine more palatable by the addition of CM-cellulose, Primogel, lactose and flavours<sup>29</sup>. Also, formulations incorporating dietary fiber and

citric acid have been shown to eliminate the mouthfeel and taste of ion exchange drugs<sup>30</sup>. These studies point to the need for the development of new sequestrants which have superior ion exchange capacity and higher selectivity for binding bile acids.

#### 1.7 THE USE OF HP INDION CELLULOSE MATRIX FOR THE PREPARATION OF ION EXCHANGERS

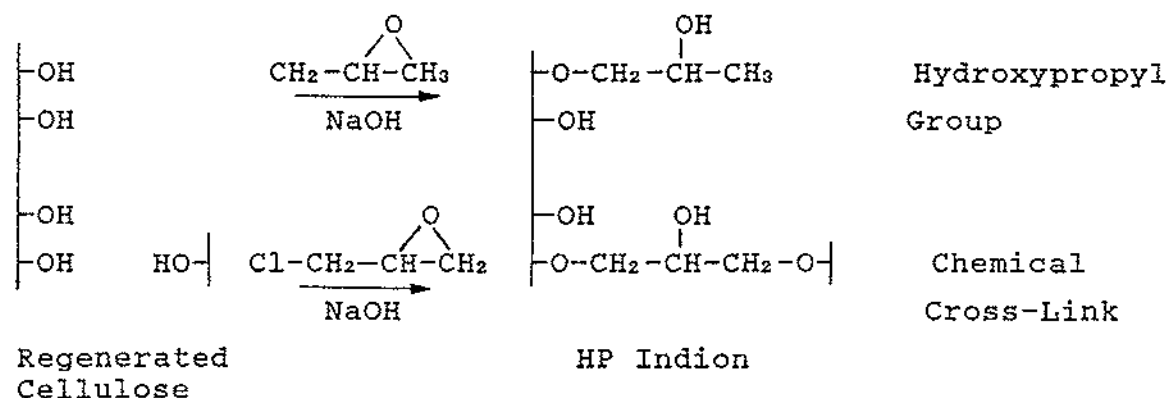
Earlier work by Dr J.S. Ayers at Massey University has demonstrated that the chemical modification of cellulose can produce materials suitable for derivatisation and subsequent use as chromatography media. These studies demonstrated that hydroxypropylated regenerated cellulose (now available as HP Indion<sup>a</sup>) was an acceptable starting matrix for the preparation of ion exchangers<sup>31</sup>. Some of the HP Indion ion exchangers produced locally have proved to be ideal for large scale protein separation<sup>32</sup>, because they are resilient to both chemical and physical attrition, and have high capacities for binding macromolecules<sup>31</sup>.

HP Indion is prepared from regenerated cellulose, which is first ground and then graded into different particle sizes. Next it is swollen with propylene oxide and cross-linked with epichlorohydrin in the presence of sodium hydroxide, according to the method first described by Sheerin<sup>33</sup>. Typical groups present in the HP Indion cellulose matrix are shown in Equation 1.1. HP Indion is available in a range of particle sizes and degrees of cross-linking<sup>34</sup>.

---

<sup>a</sup> Waitaki International Biosciences, Christchurch

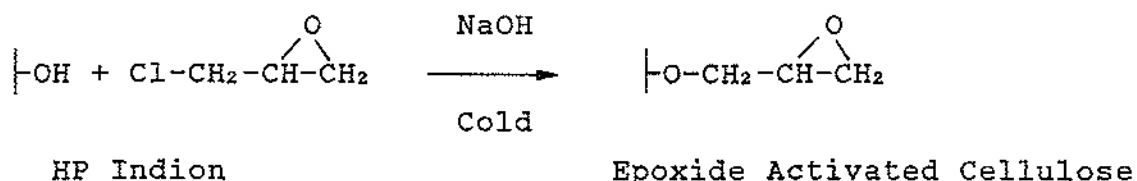
Equation 1.1 Preparation of HP Indion from Regenerated Cellulose



1.8 EPOXIDE ACTIVATION OF HP INDION CELLULOSE MATRIX

One very effective method of preparing ion exchangers from the cellulosic HP Indion starting matrix involves epoxide activation (Equation 1.2) and coupling (Equation 1.5).

Equation 1.2 Epoxide Activation



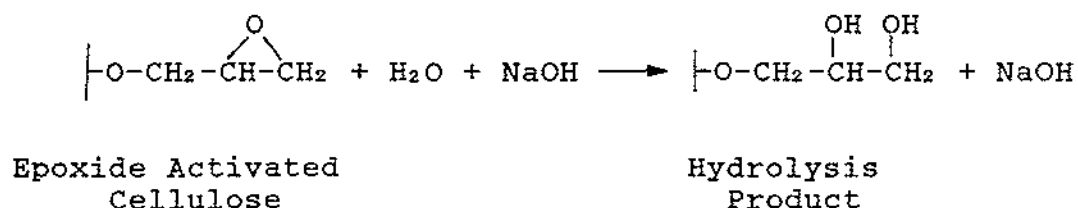
The conditions needed to substitute the maximum number of epoxide groups onto the cellulose matrix have been thoroughly investigated<sup>35</sup>. The critical factor affecting the level of epoxidation relates to the ratio of sodium hydroxide and epichlorohydrin used. Sodium hydroxide is consumed by epichlorohydrin in a 1:1 mole ratio in order to react with the cellulose matrix, and also through hydrolysis (Equation 1.3).

Equation 1.3 Hydrolysis of Epichlorohydrin



Once the sodium hydroxide has been consumed further activation is not possible no matter how much epichlorohydrin is used. However, if excess sodium hydroxide is used hydrolysis of the active epoxide groups occurs (Equation 1.4).

Equation 1.4 Hydrolysis of Activated Cellulose by Excess Sodium Hydroxide



In order to produce the maximum number of active epoxides a 25% molar excess of epichlorohydrin over sodium hydroxide must be used. A 6-8% w/v solution of sodium hydroxide is also essential: above this concentration extra weight is added to the cellulose without a significant increase in the number of epoxides produced (indicating that hydrolysis had occurred); but when less than 6% sodium hydroxide is used limited epoxidation results. The actual volume of sodium hydroxide solution, and the number of active epoxides produced, ultimately depends on the physical properties of the HP Indion used. Other factors influencing the number of epoxides produced are the temperature and time of the reaction. Maximum epoxidation (up to 1.4 meq/g) is obtained by activating at low temperatures, however the time required for the reaction to reach completion at 4°C is in excess of 30 hours.

It is possible to couple a wide variety of functional groups to epoxide activated HP Indion (Table 1.2). Previous work demonstrated that amino cellulose ion exchangers were moderately effective at binding bile acid anions *in vitro*<sup>36</sup>. However, this work was limited and synthetic methods for optimising the ion exchange function were not investigated.



Table 1.2 Ion Exchangers Prepared from Epoxide Activated HP Indion

<u>Ion Exchanger</u>	<u>Structure</u>	<u>Function</u>
Sulphopropyl (SP) cellulose	$-\text{SO}_3^-\text{Na}^+$ )	Cation
Phosphate (PT) cellulose	$-\text{PO}_4^-\text{Na}^+$ )	Exchange
Quaternary Amino (QA) cellulose	$-\text{NR}_3^+\text{Cl}^-$ )	Anion
Tertiary Amino (DE) cellulose	$-\text{NHR}_2^+\text{Cl}^-$ )	Exchange
Primary Amino (PA) cellulose	$-\text{NH}_3^+\text{Cl}^-$ )	

### 1.9 AIM OF THESIS

Bile acid sequestrants operate by exchanging negatively charged counterions (usually chloride ions) for bile acid anions, hence it was anticipated that quaternary amino (QA) cellulose ion exchangers would be effective because they remain charged at all pH levels. Therefore it was decided to prepare a range of dimethylalkyl QA celluloses by coupling N,N-dimethyl-N-alkylamines to epoxide activated HP Indion as shown in Equation 1.5.

The aim was to identify the most effective alkyl group, and then optimise the synthesis of the cellulose ion exchanger. It was hoped that the capacity of the resultant product to bind bile acids in vitro would match or exceed that of cholestyramine. Ultimately it was hoped that a cellulose based drug would alleviate some of the side-effects which occur with the synthetic polystyrene matrix of cholestyramine.

Equation 1.5 Preparation of Quaternary Amino (QA) Cellulose (Coupling)

