

PREPARATION AND CHARACTERIZATION OF WATER-SOLUBLE CHITOSAN GEL FOR SKIN HYDRATION

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**PREPARATION AND CHARACTERIZATION OF WATER-SOLUBLE CHITOSAN
GEL FOR SKIN HYDRATION**

by

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS.....	ii
TABLE OF CONTENTS.....	iii
LIST OF TABLES.....	viii
LIST OF FIGURES.....	xii
LIST OF PLATES.....	xv
LIST OF EQUATIONS	xvi
LIST OF ABBREVIATION & SYMBOLS.....	xviii
ABSTRAK.....	xx
ABSTRACT.....	xxii
CHAPTER 1: INTRODUCTION	
1.1 Chitosan.....	1
1.2 Production of chitosan.....	4
1.3 History of chitosan.....	6
1.4 Properties of chitosan.....	8
1.4.1 Molecular weight.....	9
1.4.2 Degree of deacetylation.....	10
1.4.3 Solubility of chitosan.....	12
1.5 Applications.....	16
1.5.1 Pharmaceutical and medical.....	17
1.5.2 Biotechnology.....	19
1.5.3 Wastewater treatment.....	21

1.5.4	Cosmetics.....	22
1.5.5	Others.....	25
1.6	Experimental work and scope of the present study.....	26
CHAPTER 2: ENZYMATIC HYDROLYSIS OPTIMIZATION IN		
 THE PREPARATION OF WATER-SOLUBLE		
 CHITOSAN		
2.1	Introduction.....	28
2.2	Materials and Methods.....	30
2.2.1	Materials.....	30
2.2.2	Preparation of water-soluble chitosan by enzymatic hydrolysis.....	30
2.2.3	Preparation of citric acid buffer.....	33
2.2.4	Preparation of enzyme solution.....	33
2.2.5	Determination of solubility of water-soluble chitosan.....	34
2.2.6	Statistical analysis.....	35
2.3	Results and Discussion.....	35
2.4	Conclusions.....	50
CHAPTER 3: CHARACTERIZATION AND EVALUATION OF		
 WATER-SOLUBLE CHITOSAN		
3.1	Introduction.....	51
3.2	Materials and Methods.....	55
3.2.1	Materials.....	55
3.2.2	Preparation of chitosan film for FTIR analysis.....	55

3.2.3	Preparation for NMR analysis.....	56
3.2.4	Determination of viscosity-average molecular mass of chitosan.....	56
3.2.4 (a)	Determination of density and pH.....	56
3.2.4 (b)	Determination of relative viscosity	58
3.2.4 (c)	Estimation of intrinsic viscosity.....	59
3.2.4 (d)	Mark-Houwink equation and viscosity- average molecular mass.....	60
3.2.5	Determination of degree of deacetylation of chitosan.....	60
3.2.5 (a)	Determination of zero crossing point.	60
3.2.5 (b)	Calibration curve.....	61
3.2.5 (c)	Correction of the effect of <i>D</i> -glucosamine on <i>H</i> values.....	61
3.2.5 (d)	Determination of <i>DD</i> of chitosan.....	62
3.2.6	Statistical analysis.....	63
3.3	Results and Discussion.....	63
3.3.1	FTIR analysis.....	63
3.3.2	NMR analysis.....	67
3.3.3	Determination of viscosity-average molecular mass of chitosan.....	71
3.3.4	Determination of degree of deacetylation of chitosan.....	86
3.4	Conclusions.....	92

**CHAPTER 4: OPTIMAZATION OF GEL PREPARATION
CONTAINING WATER-SOLUBLE CHITOSAN
USING RESPONSE SURFACE METHODOLOGY**

4.1	Introduction.....	93
4.2	Materials and Methods.....	96
4.2.1	Materials.....	96
4.2.2	Experimental design optimization.....	97
4.2.3	Preparation of gel formulations.....	98
4.2.4	Determination of pH.....	99
4.2.5	Texture profile analysis.....	99
4.2.5 (a)	Determination of hardness.....	102
4.2.5 (b)	Determination of compressibility.....	102
4.2.5 (c)	Determination of adhesiveness.....	103
4.2.5 (d)	Determination of cohesiveness.....	103
4.2.6	Determination of rheological properties.....	104
4.2.7	Validation of model equation using a selected gel formulation.....	105
4.2.8	Statistical analysis.....	105
4.3	Results and Discussion.....	106
4.3.1	Determination of pH.....	106
4.3.2	Texture profile analysis.....	116
4.3.2 (a)	Determination of hardness.....	116
4.3.2 (b)	Determination of compressibility.....	122
4.3.2 (c)	Determination of adhesiveness.....	129

4.3.2 (d)	Determination of cohesiveness.....	136
4.3.3	Determination of rheological properties.....	142
4.3.4	Validation of corresponding model equations.....	148
4.4	Conclusions.....	151
CHAPTER 5: EVALUATION OF EFFICACY OF GEL		
CONTAINING WATER-SOLUBLE CHITOSAN ON		
SKIN HYDRATION		
5.1	Introduction.....	152
5.2	Materials and Methods.....	156
5.2.1	Materials.....	156
5.2.2	Preparation of gel formulations.....	156
5.2.3	Determination of skin hydration.....	157
5.2.4	Statistical analysis.....	160
5.3	Results and Discussion.....	160
5.4	Conclusions.....	178
CHAPTER 6: SUMMARY AND CONCLUSION.....		179
CHAPTER 7: SUGGESTION FOR FURTHER STUDY.....		181
REFERENCES.....		183
APPENDICES		
PUBLICATIONS		

LIST OF TABLES

	Page	
2.1	Calculation of enzyme concentration used and its activity.	34
2.2	Effect of enzyme concentration in stock solution on the solubility of chitosan. Mean \pm S.D, N=3.	39
2.3	Effect of reaction time on the solubility of chitosan. Mean \pm S.D, N=3.	42
2.4	Effect of reaction temperature on the solubility of chitosan. Mean \pm S.D, N=3.	45
2.5	Effect of pH on the solubility of chitosan. Mean \pm S.D, N=3.	48
3.1	IR spectral data for chitosan, water-soluble chitosan and references peak.	65
3.2	¹ H NMR spectral data for chitosan, water-soluble chitosan and references peak.	69
3.3	Determination of density, relative, specific, inherent, and reduced viscosities of chitosan. Mean \pm S.D, N=3.	71
3.4	Determination of density, relative, specific, inherent, and reduced viscosities of water-soluble chitosan. Mean \pm S.D, N=3.	72
3.5	Results of Pearson Correlation test for chitosan concentration, pH, density and efflux time.	79
3.6	Results of Pearson Correlation test for water-soluble chitosan concentration, pH, density and efflux time.	79
3.7	Results of Pearson Correlation test for chitosan concentration, relative, specific, reduced and inherent viscosities.	81
3.8	Results of Pearson Correlation test for water-soluble chitosan concentration, relative, specific, reduced and inherent viscosities.	82

3.9	Intrinsic viscosity and average molecular mass of different chitosan samples. Mean \pm S.D, N=3.	85
3.10	Concentration of GlcNAc and degree of deacetylation of different chitosan samples. Mean \pm S.D, N=5.	90
4.1	Central composite design matrix, percentage and concentration of HEC and chitosan.	98
4.2	The central composite design matrix, percentage of hydroxyethylcellulose(HEC) and chitosan (CHI), and pH values of gel. Mean \pm S.D, N=5.	107
4.3	Analysis of variance and regression parameters of central composite design for all effects.	110
4.4	Results of the <i>t</i> -test for Equation 4.6 coefficients.	110
4.5	Analysis of variance and regression parameters of central composite design for all significant effects.	112
4.6	Results of the <i>t</i> -test for Equation 4.7 coefficients.	112
4.7	Actual and predicted values of pH by using Equation 4.7.	113
4.8	The central composite design matrix, percentage of HEC and chitosan (CHI), and hardness results. Mean \pm S.D, N=3.	117
4.9	Analysis of variance and regression parameters of central composite design for all effects.	118
4.10	Results of the <i>t</i> -test for Equation 4.8 coefficients.	118
4.11	Actual and predicted values of hardness using Equation 4.8.	119
4.12	The central composite design matrix, percentage of HEC and chitosan (CHI), and compressibility values. Mean \pm S.D, N=3.	123
4.13	Analysis of variance and regression parameters of central composite design for all effects.	125
4.14	Results of the <i>t</i> -test for Equation 4.9 coefficients.	125

4.15	Actual and predicted values of compressibility using Equation 4.9.	126
4.16	The central composite design matrix, percentage of HEC and chitosan (CHI), and adhesiveness values. Mean \pm S.D, N=3.	130
4.17	Analysis of variance and regression parameters of central composite design for all effects.	132
4.18	Results of the <i>t</i> -test for Equation 4.10 coefficients.	132
4.19	Actual and predicted values of adhesiveness using Equation 4.10.	133
4.20	The central composite design matrix, percentage of HEC and chitosan (CHI), and cohesiveness values. Mean \pm S.D, N=3.	136
4.21	Analysis of variance and regression parameters of central composite design for all effects.	138
4.22	Results of the <i>t</i> -test for Equation 4.11 coefficients.	138
4.23	Actual and predicted values of cohesiveness using Equation 4.11.	139
4.24	The central composite design matrix, percentage of HEC and chitosan (CHI), and viscosity value. Mean \pm S.D, N=3.	143
4.25	Analysis of variance and regression parameters of central composite design for all effects.	144
4.26	Results of the <i>t</i> -test for Equation 4.12 coefficients.	144
4.27	Analysis of variance and regression parameters of central composite design for all significant effects.	146
4.28	Results of the <i>t</i> -test for Equation 4.13 coefficients.	146
4.29	Actual and predicted values of viscosity using model Equation 4.13.	147

4.30	Actual and predicted values of gel formulation with 1.5% chitosan and 1.5% HEC, and results of physical properties of three marketed gel products. Mean \pm S.D, N=3.	150
5.1	Different formulations of gel system.	156
5.2	Biodata of healthy volunteers.	158
5.3	Skin hydration values of volunteers for Control.	163
5.4	Skin hydration values of volunteers for gel containing HEC.	165
5.5	Skin hydration values of volunteers for gel containing HEC-Gly.	167
5.6	Skin hydration values of volunteers for gel containing HEC-Chi.	169
5.7	Skin hydration values of volunteers for gel containing HEC-Gly-Chi.	171
5.8	Mean skin hydration values of different samples at 30 min.	173
5.9	Mean skin hydration values of different samples at 60 min.	174
5.10	Mean skin hydration values of different samples at 90 min.	175
5.11	Mean skin hydration values of different samples at 120 min.	176
5.12	Mean skin hydration values of different samples at 180 min.	177

LIST OF FIGURES

	Page
1.1 Molecular structure of chitosan.	2
1.2 Molecular structure of chitin.	2
2.1 Preparation of water-soluble chitosan by enzymatic hydrolysis.	32
2.2 Effect of enzyme concentration in stock solution on the solubility of chitosan. Mean \pm S.D, N=3.	40
2.3 Effect of reaction time on the solubility of chitosan. Mean \pm S.D, N=3.	43
2.4 Effect of reaction temperature on the solubility of chitosan. Mean \pm S.D, N=3.	46
2.5 Effect of pH on the solubility of chitosan. Mean \pm S.D, N=3.	49
3.1 FTIR spectra of chitosan and water-soluble chitosan.	66
3.2 Chitosan structure.	67
3.3 ^1H NMR spectrum of chitosan with insert showing enlargement of the region between 1.5 -4.0 ppm and 4.4 - 5.0 ppm.	68
3.4 ^1H NMR spectrum of water-soluble chitosan with insert showing enlargement of the region between 1.5 -4.0 ppm and 4.4 - 4.8 ppm.	68
3.5 Relative viscosity of chitosan and water-soluble chitosan at different concentration.	74
3.6 Specific viscosity of chitosan and water-soluble chitosan at different concentration.	75
3.7 Reduced viscosity of chitosan and water-soluble chitosan at different concentration.	76

3.8	Inherent viscosity of chitosan and water-soluble chitosan at different concentration.	77
3.9	Plot of reduced viscosity and inherent viscosity versus chitosan concentration for determination of intrinsic viscosity.	84
3.10	First derivative UV-spectra of variuos standard solutions of GlcNAc (a=0.005, b=0.01, c=0.02, d=0.03, e=0.04, f=0.05 mg/ml), chitosan (chi) and zero crossing point (ZCP).	87
3.11	First derivative UV-spectra of variuos standard solutions of GlcNAc (a=0.005, b=0.01, c=0.02, d=0.03, e=0.04, f=0.05 mg/ml), water-soluble chitosan (wsc) and zero crossing point (ZCP).	87
3.12	Calibration curve for the solutions of N-acetylglucosamine.	89
3.13	Correction curve for N-acetylglucosamine determination.	91
4.1	Example of texture profile analysis curve.	101
4.2	Response surface of pH generated from Equation 4.7.	115
4.3	Contour-plot of pH generated from Equation 4.7.	115
4.4	Response surface of hardness generated from Equation 4.8.	121
4.5	Contour-plot of hardness generated from Equation 4.8.	121
4.6	Response surface of compressibility generated from Equation 4.9.	128
4.7	Contour-plot of compressibility generated from Equation 4.9.	128
4.8	Response surface of adhesiveness generated from Equation 4.10.	135
4.9	Contour-plot of adhesiveness generated from Equation 4.10.	135
4.10	Response surface of cohesiveness generated from Equation 4.11.	141

4.11	Contour-plot of cohesiveness generated from Equation 4.11.	141
5.1	One of the application sites in the ventral aspect of volunteer forearm that was divided into four parts (A, B, C and D).	159
5.2	Skin hydration profiles of Control and different gel samples against time. Mean \pm S.D, N=6.	161

LIST OF PLATES

		Page
Plate 2.1	Chitosan	37
Plate 2.2	Water-soluble chitosan	37
Plate 2.3	Untreated chitosan in water	37
Plate 2.4	Water-soluble chitosan in water	37
Plate 5.1	Test area on volunteer's forearm	159

LIST OF EQUATIONS

		Page
Eq 2.1	$Concentration = \frac{M}{V} \times 100\%$	33
Eq 2.2	$Unit\ activity = M \times U$	34
Eq 2.3	$Solubility = \frac{WF}{WA} \times 100\%$	35
Eq 3.1	$\rho = \frac{0.998(M_1 + A)}{M_2 + A}$	57
Eq 3.2	$\eta_{rel} = \eta / \eta_o$	58
Eq 3.3	$\eta = \rho \times t$	58
Eq 3.4	$\eta_o = \rho_o \times t_o$	58
Eq 3.5	$\eta_{rel} = \rho \times t / \rho_o \times t_o$	58
Eq 3.6	Specific viscosity, $\eta_{sp} = \eta_{rel} - 1$	59
Eq 3.7	Reduced viscosity, $\eta_{red} = (\eta_{sp}) / c$	59
Eq 3.8	Inherent viscosity, $\eta_{inh} = \ln (\eta_{rel}) / c$	59
Eq 3.9	$[\eta] = Km.Mv^\alpha$	60
Eq 3.10	$DD(\%) = 100 - \left[\frac{A}{\frac{W - 204A}{161} + A} \times 100\% \right]$	62
Eq 3.11	$y = 1280.4x + 2.9575$	87
Eq 4.1	Hardness = H1	102
Eq 4.2	Compressibility = H1 \times L1	102
Eq 4.3	Adhesiveness = A3	103

Eq 4.4	Cohesiveness = A^2/A_1	104
Eq 4.5	$Y = a + bx + cy + dxy + ex^2 + fy^2$	105
Eq 4.6	$pH = + 6.10 + 0.066 *A + 0.15 *B - 0.039*A^2 - 0.089*B^2 - 0.045*A*B$	110
Eq 4.7	$pH = + 6.07 + 0.066 *A + 0.15*B - 0.084 *B^2$	112
Eq 4.8	$Hardness = +0.026 +0.034*A +0.17*B +0.027*A^2 + 0.13*B^2 +0.032*A*B$	118
Eq 4.9	$Compressibility = +0.51 +0.69*A +3.28*B +0.54*A^2 + 2.51*B^2 +0.64*A*B$	125
Eq 4.10	$Adhesiveness = +0.052 +0.16*A +0.10*B +0.10*A^2 + 0.073*B^2 +0.064*A*B$	132
Eq 4.11	$Cohesiveness = +1.25 +0.056*A -0.10*B -0.070*A^2 - 0.092*B^2 +0.031*A*B$	138
Eq 4.12	$Apparent Viscosity = +7.09+ 46.67*A+ 84.41*B+ 16.45*A^2+ 71.19*B^2+ 38.12*A*B$	144
Eq 4.13	$AV = +18.53+ 46.67*A+ 84.41*B+ 69.05*B^2+ 38.12*A*B$	146

LIST OF ABBREVIATION & SYMBOLS

°C	=	Degree centigrade
ANOVA	=	Analysis of variance
Ave	=	Average
CCD	=	Central composite design
Chi	=	Chitosan
Gly	=	Glycerin
Co.	=	Company
CV	=	Coefficient of variance
DD	=	Degree of deacetylation
GlcN	=	D-glucosamine
GlcNAc	=	<i>N</i> -acetylglucosamine
HPLC	=	High performance liquid chromatography
FTIR	=	Fourier transform infrared
HCl	=	Hydrochloric acid
HEC	=	Hemicellulase
NaOH	=	Sodium hydroxide
NMR	=	Nuclear magnetic resonance
RSM	=	Response surface methodology
SPSS	=	Statistical procedure for social science
SD	=	Standard deviation
TEWL	=	Trans-epidermal water loss
TPA	=	Texture profile analysis

UV	=	Ultra violet
UK	=	United Kingdom
USA	=	United State of America
v/v	=	volume in volume
w/v	=	weight in volume
w/w	=	weight in weight
WSC	=	Water-soluble chitosan
ZCP	=	Zero crossing point

PENYEDIAAN DAN PENCIRIAN GEL KITOSAN LARUT AIR UNTUK PELEMBAPAN KULIT

ABSTRAK

Kitosan, biopoliaminosakarida secara linear dan semulajadi, telah mendapat banyak perhatian sebagai bio-polimer berfungsi dengan aplikasi dalam bidang farmaseutikal, makanan, kosmetik dan perubatan. Namun begitu, aplikasi kitosan dihadkan oleh keterlarutannya dalam air. Matlamat kajian ini ialah untuk mengurangkan berat molekul kitosan dengan menggunakan kaedah enzim untuk meningkatkan keterlarutannya dalam air. Enzim hemiselulosa yang murah dan mudah diperolehi digunakan untuk hidrolisis kitosan. Kajian keterlarutan dijalankan untuk menentukan keadaan yang optimum untuk menyediakan kitosan larut air. Empat parameter yang dikaji ialah kepekatan enzim, suhu reaksi, masa reaksi dan pH. Tiada perubahan dalam struktur kimia untuk kitosan larut air apabila dikenalpasti dengan menggunakan FTIR dan NMR. Penentuan berat molekul dan darjah deasetilasi dilakukan dengan menggunakan viskometer dan UV-spektroskopi terbitan pertama. Terdapat pengurangan dalam berat molekul dan peningkatan darjah deasetilasi. Metodologi permukaan respons telah digunakan untuk mengoptimumkan penyediaan gel, dengan menggunakan hidoksietiliselulosa dan kitosan larut air sebagai pembolehubah input dan sifat-sifat fizikal, pH, profil tekstur (kekerasan, kelekatan, keupayaan kohesif dan kompresibiliti) dan

kelikatan jelas sebagai pembolehubah respons. Rekabentuk komposit pusat telah digunakan untuk mengurangkan bilangan eksperimen percubaan. Persamaan model untuk mengira dan meramal sifat fizikal formulasi gel, telah berjaya diterbitkan sebagai fungsi kepekatan HEC dan kitosan larut air. Nilai ramalan pH, kekerasan, kompresibiliti, kelekatan dan keupayaan kohesif adalah lebih kurang sama dengan keputusan eksperimen, kecuali kelikatan jelas yang tidak dapat diramalkan dengan tepat. Keberkesanan gel yang mempunyai kitosan larut air terhadap pelembapan kulit dikaji dengan menggunakan enam orang sukarelawan yang sihat. Nilai pelembapan kulit dikira dengan menggunakan korneometer. Didapati nilai pelembapan kulit bagi gel yang mempunyai kitosan larut air adalah setanding dengan gel yang mempunyai kitosan larut air dan gliserin. Kitosan larut air boleh digunakan sebagai oklusif, mempertahankan kehilangan air daripada permukaan kulit, oleh itu memperbaiki pelembapan kulit. Secara ringkas, penggunaan kitosan larut air sahaja adalah mencukupi untuk pelembapan kulit dan penggabungan dengan gliserin, sejenis humektan, mungkin tidak perlu.

PREPARATION AND CHARACTERIZATION OF WATER-SOLUBLE CHITOSAN GEL FOR SKIN HYDRATION

ABSTRACT

Chitosan, a natural and linear biopolyaminosaccharide, has received much attention as a functional biopolymer with applications in pharmaceuticals, food, cosmetics and medicines. Nevertheless, chitosan application is limited by its solubility in aqueous solution. The aim of this study is to reduce the molecular weight of chitosan using enzymatic method to increase its aqueous solubility. Cheap and commercially available hemicellulase enzyme was used to hydrolyze the chitosan. Solubility test was carried out to determine the optimum condition to prepare water-soluble chitosan. Four parameters investigated were concentration of enzyme, reaction temperature, reaction time and pH. There was no alteration in the chemical structure of water-soluble chitosan when identified using FTIR and NMR. Determination of molecular weight and degree of deacetylation was performed using viscometry and the first derivative UV-spectroscopy. There was a reduction in the molecular weight and an increase in the degree of deacetylation. Response surface methodology was employed in the optimization of gel preparation, with hydroxyethylcellulose and water-soluble chitosan as input variables and the physical properties, pH, texture profile (hardness, adhesiveness, cohesiveness and compressibility) and apparent viscosity as response

variables. Central composite design was used to minimize the number of experimental trials. Model equations to calculate and predict the physical properties of gel formulations, were successfully derived as functions of concentration of HEC and water-soluble chitosan. The predicted values of pH, hardness, compressibility, adhesiveness and cohesiveness were closely similar to the experimental results, except apparent viscosity, which could not be reliably predicted. The efficacy of gel containing water-soluble chitosan on skin hydration was studied using six healthy human volunteers. The skin hydration was quantified using a corneometer. It was found that the skin hydration effect of gel containing water-soluble chitosan was comparable with gel containing water-soluble chitosan and glycerin. Water-soluble chitosan could act as an occlusive, preventing water loss from the skin surface, thus improving skin hydration. In short, the use of water-soluble chitosan alone is sufficient for skin hydration and combination with glycerin, which is a humectant, might not be necessary.

CHAPTER 1

INTRODUCTION

1.1 Chitosan

Chitosan is a heteropolymer consists of $\beta(1-4)$ 2-acetamido-2-deoxy- β -D-glucopyranose (N-acetylglucosamine) and 2-amino-2-deoxy- β -D-glucopyranose (D-glucosamine) units, randomly or block distributed throughout the biopolymer. The chain distribution is dependant on the processing method used to derive the biopolymer (Dodane and Vilivalam, 1998; Kumar, 2000; Khor and Lim, 2003). It is the N-deacetylated derivative of chitin, but the N-deacetylation is almost never complete (Kumar, 2000; Santos *et al.*, 2005). Chitin and chitosan are names that do not strictly refer to a fixed stoichiometry. Chemically, chitin is known as poly-N-acetylglucosamine, and in accordance to this proposed name, the difference between chitin and chitosan is that the degree of deacetylation in chitin is very little, while deacetylation in chitosan occurred to an extent but still not enough to be called polyglucosamine (Muzzarelli, 1973).

The structural details of chitin and chitosan are shown in Figures 1.1 and 1.2 respectively. Chitosan has one primary amine and two free hydroxyl groups for each monomer with a unit formula of $C_6H_{11}O_4N$. This natural biopolymer is a glucosaminoglycan and is composed of two common sugars, glucosamine and N-acetylglucosamine, both of which are constituents of mammalian tissues (Khan, 2001; Snyman *et al.*, 2002).

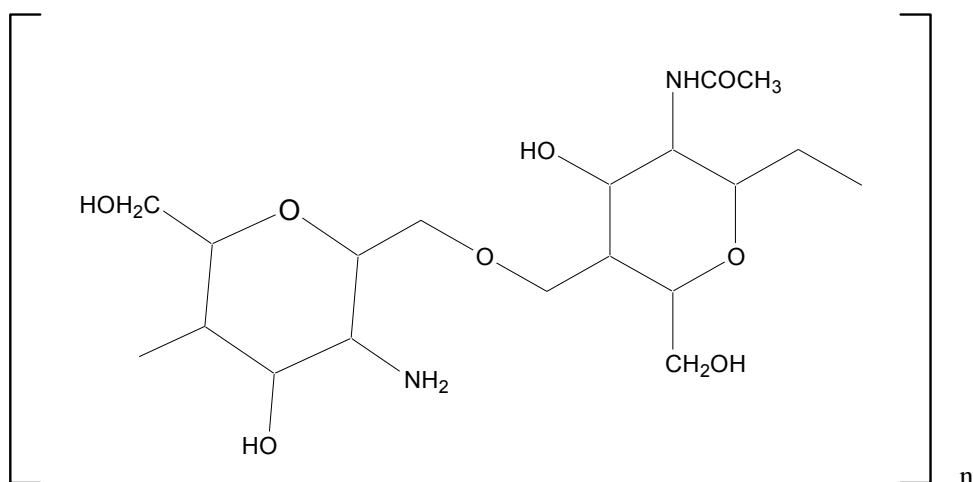


Figure 1.1: Molecular structure of chitosan

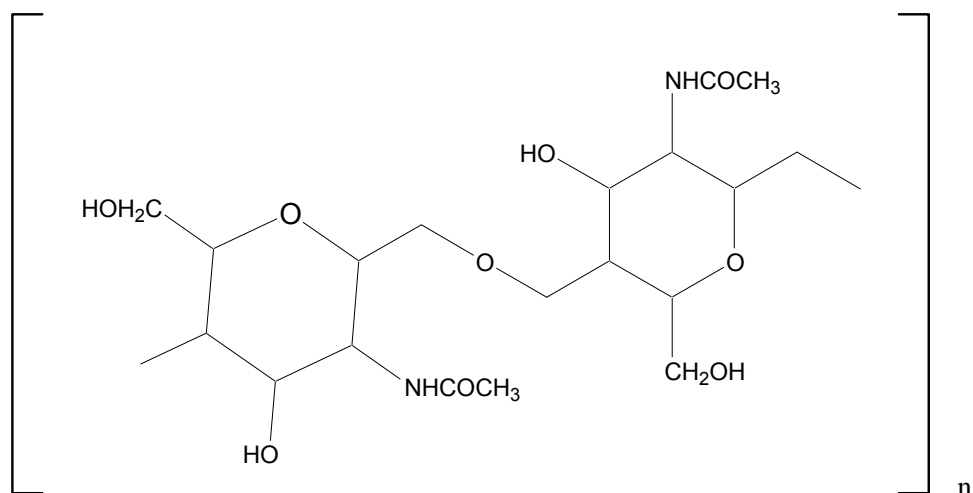


Figure 1.2: Molecular structure of chitin

Chitosan is the second abundant polysaccharide next to cellulose (Duarte *et al.*, 2002; Sinha *et al.*, 2004), but it is the most abundant natural amino polysaccharide and is estimated to be produced annually almost as much as cellulose (Kumar, 2000). Chitosan can be chemically considered as analogues of cellulose, in which the hydroxyl at carbon-2 has been replaced by acetamido

or amino groups (Krajewska, 2004). As a point of difference from other abundant polysaccharides, chitin and chitosan contain nitrogen in addition to carbon, hydrogen and oxygen. Chitin and chitosan are of commercial interest due to their high percentage of nitrogen (6.89%) compared to synthetically substituted cellulose (1.25%) (Muzzarelli and Muzzarelli, 1998; Kumar, 2000). As most of the present-day polymers are synthetic materials, their biocompatibility and biodegradability are much more limited than those of natural polymers such as cellulose, chitin, chitosan and their derivatives. However, these naturally abundant materials also exhibit a limitation in their reactivity and processability. Chitosan is recommended as suitable functional material, because this natural polymer has excellent properties such as biocompatibility, biodegradability, non-toxicity and adsorption properties. Recently, much attention has been given to chitosan as a potential polysaccharide source (Kumar, 2000). Chitosan can be degraded by soil microorganisms and water microorganisms. This makes chitosan environmental friendly. This was acknowledged by the US Environmental Protection Agency when it exempted chitosan from tolerance level testing (Hennen, 1996).

1.2 Production of chitosan

Chitin and chitosan are obtained from the shells of crustaceans such as crabs, prawns, lobsters and shrimps, the exoskeletons of insects, and the cell walls of fungi such as *aspergillus* and *mucor* where it provides strength and stability (Dodane and Vilivalam, 1998; Kumar, 2000; Khor and Lim, 2003; Krajewska, 2004; Sinha *et al.*, 2004; Qin *et al.*, 2006). Crab and shrimp shell wastes are currently utilized as the major industrial source of biomass for the large-scale production of chitin and chitosan. Processing wastes from marine food factories help to recycle the wastes and make the derivatives or by-products for use in other fields. These crustacean shell wastes are composed of protein, inorganic salts, chitin and lipids as main structural components. Therefore, extraction of chitin and chitosan was mainly employed by stepwise chemical methods (Kim and Rajapakse, 2005). In the first stage, chitin production was associated with food industries such as shrimp canning. In the second stage, the production of chitosan was associated with fermentation processes, similar to those for the production of citric acid from *Aspergillus niger*, *Mucor Rouxii*, and *Streptomyces*, which involved alkali treatment yielding chitosan. Briefly, shells were ground to smaller sizes and minerals, mainly calcium carbonate, were removed by extraction (demineralization, decalcification) with dilute hydrochloric acid followed by stirring at ambient temperature. The protein was extracted (deproteinisation) from the residual material by treatment with dilute aqueous sodium hydroxide and thereby prevents contamination of chitin products from proteins. The resulting chitin was deacetylated in 40 - 45% sodium hydroxide at 120°C for 1- 3 hours with exclusion of oxygen, and followed by purification

procedures to form chitosan with a cationic nature. The alkali removed the protein and the deacetylated chitin simultaneously. Depending on the alkali concentration, some soluble glycans would be removed.

In the deacetylation process, some of the acetyl groups were removed from the molecular chain of chitin. This shortened the chain lengths of the chitin molecule, eventually leaving behind a polymer with a complete amino group called chitosan. This treatment produces 70% of deacetylated chitosan (Kumar, 2000; Khan, 2001; Krajewska, 2004; Kim and Rajapakse, 2005). Methods based on alkaline treatments were employed to achieve N-deacetylation, as N-acetyl groups cannot be removed by acidic reagents as effectively as with alkaline treatment. However, partial deacetylation could occur under this harsh treatment (Muzzarelli, 1973). The extent of deacetylation mainly depends upon alkali concentration, time and temperature employed throughout the process. For example, increasing temperature or strength of sodium hydroxide solution can remove acetyl groups, resulting in a range of chitosan molecules with different physicochemical properties and applications (Khan, 2001).

According to Kumar (2000), to produce 1 kg of 70% deacetylated chitosan from shrimp shells, 6.3 kg of HCl and 1.8 kg of NaOH are required in addition to nitrogen, water (1.4 tons). Commercially, chitosan is available in the form of dry flakes, solution and fine powder (Duarte *et al.*, 2002; Sinha *et al.*, 2004). The hydrolysis of chitin with concentrated acids under drastic conditions produces relatively pure D-glucosamine (Kumar, 2000). In India, the Central Institute of

Fisheries Technology, Kerala, initiated research on chitin and chitosan. From their investigation, they found that dry prawn waste contained 23% and dry squilla contained 15% chitin. Chitin and chitosan are now produced commercially in India, Japan, Poland, Norway and Australia (Kumar, 2000).

It is likely that future sources of chitin and chitosan will come from biotechnology innovation, especially when medical applications are the focus (Khor and Lim, 2003). Thus, production and utilization of chitosan constitutes an economically attractive means of crustacean shell wastes disposals, which is sought worldwide.

1.3 History of chitosan

The history of chitosan dates back to the last century since its discovery in the late 1850s and when Rouget discussed the deacetylated form of chitosan in 1859 (Hennen, 1996; Dodane and Vilivalam, 1998; Kim and Rajapakse, 2005). Research on the uses of chitin and chitosan flourished in 1930s and early 1940s, but the rise of synthetic fibers, like the rise of synthetic medicines, overshadowed the interest in natural products. Interest in natural products, including chitin and chitosan, gained resurgence in the 1970s and continued to expand ever since (Hennen, 1996; Kim and Rajapakse, 2005). Since the mid-1960s, a considerable amount of research on chitin and chitosan has emanated from Asia. Japan has been the undisputed leader, but other Asian nations, namely Korea, Singapore, Taiwan and Thailand have also made notable contributions. More recently, China has joined the club to become an increasingly major

research source for chitin and chitosan in Asia. The focus at the time was to better understand these materials and reports covered the whole spectrum of research, from better production and purification methods, to the derivatization chemistry and a myriad of applications (Khor and Lim, 2003).

Many researchers have focused chitosan as a source of potential bioactive material during the past few decades. However, chitosan has several drawbacks to be utilised in biological applications, including its poor solubility under physiological conditions (Kim and Rajapakse, 2005). Therefore, several efforts have been reported to prepare functional derivatives of chitosan by chemical modifications and partially hydrolysed chitosan. Chemically modified chitosan structures results in improved solubility in water and general organic solvents have been reported by some researchers (Francis and Matthew, 2000; Kubota *et al.*, 2000; Kumar, 2000; Xie *et al.*, 2002; Chen and Park, 2003; Kim and Rajapakse, 2005).

Partially hydrolyzed chitosan using chemical and enzymatic methods have also been reported recently. Partially hydrolysed chitosan by enzymatic methods seems to have enhanced biochemical significance compared to the chitosan from which they derive, and are more easily handled (Ilyina *et al.*, 1999; Zhang *et al.*, 1999; Muzzarelli *et al.*, 2002; Kittur *et al.*, 2003; Qin *et al.*, 2003). Efforts to improve chitosan solubility continued and this in turn increases its applications. Khor and Lim (2003) reported that the applications of chitosan especially in the biomedical area have become more streamlined and concentrated.

1.4 Properties of chitosan

Most of the naturally occurring polysaccharides, for instance, cellulose, dextran, pectin, alginic acid, agar, agarose and carragenans, are neutral or acidic in nature, whereas chitin and chitosan are examples of highly basic polysaccharides (Kumar, 2000). This polymer is known to be nontoxic, odourless, biocompatible in animal tissues and enzymatically biodegradable (Zong *et al.*, 2000). Their unique properties include polyoxysalt formation, ability to form films, chelation with metal ions and optical structural characteristics (Kumar, 2000).

The main parameters influencing the characteristics of chitosan are its molecular weight and degree of deacetylation, which affect the solubility, rheological and physical properties. Various grades of chitosan are available commercially, which differ primarily in the degree of deacetylation and molecular weight. Different conditions such as type and concentration of reagents, time and temperature employed throughout the processing can affect the physical characteristics and performance of the final chitosan product (Khan, 2001). However, both DD and molecular weight can be further modified. For example, DD can be lowered by reacetylation (Shigemasa *et al.*, 1996; Muzarrelli and Muzzarelli, 1998; Brugnerotto *et al.*, 2001; Duarte *et al.*, 2002; Wan *et al.*, 2003) and molecular weight can be lowered by acidic or enzymatic depolymerisation (Zhang *et al.*, 1999; Jia and Shen, 2002; Qin *et al.*, 2003).

1.4.1 Molecular weight

Chitosan usually refers to a family of polymers that are characterized by the number of sugar units per polymer molecule (n), which defines its molecular weight (Dodane and Vilivalam, 1998). The physico-chemical properties, which include viscosity, solubility, adsorption on solids, elasticity, and tear strength, are dependant on the molecular weight of the polymer concerned (Khan, 2001). Chitosan has received much attention as a functional biopolymer for diverse applications. These functions have been revealed to be dependent not only upon their chemical structure but also the molecular size (Qin *et al.*, 2003). Crystal size and morphological character of its prepared film can be affected by the molecular weight of chitosan. It was shown that crystallinity of membrane increased with a decrease in chitosan molecular weight (Khan, 2001). It has been reported that the molecular weight of chitosan products is dependant on the deacetylation process and would decrease as the time of deacetylation increased (Francis and Matthew, 2000). Depending on the source and preparation procedure, the average molecular weight of chitosan may range from 50 to 1000kDa (Francis and Matthew, 2000), 3.8 to 2000 kDa (Sinha *et al.*, 2004) or 50 to 2000kDa (Chenite *et al.*, 2001).

1.4.2 Degree of deacetylation

Chitosan is a semi-crystalline polymer and the degree of crystallinity is a function of the degree of deacetylation. Crystallinity is maximum for both chitin (i.e. 0% deacetylated) and fully deacetylated (i.e. 100%) chitosan (Francis and Matthew, 2000). Despite those specific chemical designations, the names 'chitin' and 'chitosan' actually correspond to a family of polymers varying in the acetyl content measured by the degree of deacetylation (DD) (Duarte *et al.*, 2002). Chitosan is the universally accepted non-toxic N-deacetylated derivative of chitin, where chitin is N-deacetylated to such an extent that it becomes soluble in dilute aqueous acids (Kumar, 2000). The process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin, leaving behind a complete amino group (-NH₂). Chitosan versatility depends mainly on this high degree of chemically reactive amino groups. To increase the amine group content of chitosan and higher deacetylation, chitosan (for example, DD > 90%) is subjected to repeated alkaline treatment (Wan *et al.*, 2003). Increasing either the temperature or strength of the alkaline solution can also enhance the removal of acetyl groups from chitin (Khan, 2001).

An important parameter to examine closely is the degree of deacetylation in chitosan, it is the ratio of 2-acetamido-2-deoxy-D-glucopyranose to 2-amino-2-deoxy-D-glucopyranose structural units (Kumar, 2000). The degree of deacetylation of chitosan, which determines the content of free amino groups, can be employed to differentiate between chitin and chitosan. When the number of 2-amino-2-deoxy-D-glucopyranose units is more than 50%, the biopolymer is

termed chitosan. Conversely, when the number of 2-acetamido-2-deoxy-D-glucopyranose units is higher, the term chitin is used (Nystrom *et al.*, 1999; Brugnerotto *et al.*, 2001; Khor and Lim, 2003). According to Kumar (2000), chitosan is the fully or partially N-deacetylated derivative of chitin with typical degree of deacetylation of more than 0.35. While Khan (2001) reported that chitin with DD of 75% and above is generally known as chitosan but Montembault *et al.* (2005) reported that chitosan has the DD of 60% and above. Tommeraas *et al.* (2002) reported that the commercially available chitosan usually has the range between 0 to 0.3 fraction of N-acetyl unit. This ratio has a striking effect on the performance of chitosan in many of its applications. It has been reported that DD has prominent roles in the biochemical significance of chitosan. The DD of chitosan has been shown to correlate with its solubility in acidic solution and the crystallinity of its membrane (Khan, 2001; Duarte *et al.*, 2002). Conversion of chitin into chitosan increases DD, and thereby alters the charge distribution of chitosan molecules (Francis and Matthew, 2000). It is known that the charge density along the chain increases with an increase in the DD, and that chain flexibility of chitosan molecules can be manipulated by changing the DD (Nystrom *et al.*, 1999). Commercially available chitosan has degree of deacetylation ranging from 50 to 90% (Francis and Matthew, 2000), 66 to 95% (Sinha *et al.*, 2004) or 40 to 98% (Chenite *et al.*, 2001).

1.4.3 Solubility of chitosan

Chitosan is a semi-crystalline polymer, a weak base, which is insoluble in water, alkali or aqueous solution above pH 7, and common organic solvents due to its stable and rigid crystalline structure. Chitosan is normally polydispersed and has the ability to dissolve in certain inorganic and organic acids such as hydrochloric acid, phosphoric acid, lactic acid, propionic acid, succinic acid, acetic acid, tartaric acid, citric acid and formic acid at certain pH values after prolonged stirring (Muzzarelli, 1973; Sugimoto *et al.*, 1998; Francis and Matthew, 2000; Zong *et al.*, 2000; Khan, 2001; Krajewska, 2004; Perez-Orozco *et al.*, 2004; Chung *et al.*, 2005; Qin *et al.*, 2006). Nitric acid could dissolve chitosan, but after dissolution, white gelatinous precipitate would occur (Muzzarelli, 1973). Sulphuric acid does not dissolve chitosan because it would react with chitosan to form chitosan sulphate, which is a white crystalline solid (Muzzarelli, 1973). The solubility of chitosan also depends on the pK_a of these acids and their concentrations. Investigation of chitosan dissolution characteristics revealed that its dissolution rate varied according to the type of acid used (Sugimoto *et al.*, 1998; Khan, 2001; Perez-Orozco *et al.*, 2004). Chitosan behaves as a sphere in aqueous acetic acid solution or as an expanded random coil in urea (Muzzarelli and Muzzarelli, 1998; Perez-Orozco *et al.*, 2004).

Chitosan degrades before melting, which is typical for polysaccharides with extensive hydrogen bonding. This makes it necessary to dissolve chitosan in an appropriate solvent system to impart functionality (Kumar, 2000). A mixture of

dimethylformamide and dinitrogen tetroxide at a ratio of 3:1 has been reported to be a good solvent for chitosan (Muzzarelli, 1973; Khan, 2001). For each solvent system, polymer concentration, pH, counter-ion concentration and temperature would affect the solution viscosity (Khan, 2001). For example, at pH value below 4, most of the amino groups of chitosan are supposed to be protonated, and since this effect promotes electrostatic repelling between charged groups of the same sign, it leads to enhanced swelling of the polymer network (Nystrom *et al.*, 1999). While Muzzarelli and Muzzarelli (1998) reported that at pH 5.2, an unstable structure is generated. Upon neutralization with an excess NaOH, the ionic strength of the solution increases and therefore the size of the aggregates decreases due to compaction of the macromolecular coils. The free amino groups form intermolecular hydrogen bonds with the oxygen of the adjacent chains. At pH value greater than 6.5, which is approximately the pK_a of the amino group in chitosan, the size of the aggregates increases and phase separation occurs. The polymer coagulates and can be recovered as an amorphous solid (Muzzarelli and Muzzarelli, 1998; Nystrom *et al.*, 1999).

The uniqueness of chitosan depends on the distribution of the acetyl groups remained along the chain but mostly depends on the free amino ($-NH_2$) groups which is important in forming conformational features through intra and / or intermolecular hydrogen bonding. This makes it soluble in acidic solutions below pH of approximately 6.5 and thereby overcoming associative forces between chains. Amino groups make chitosan a cationic polyelectrolyte ($pK_a \approx 6.5$), one of the few found in nature. In contrast, other polysaccharides are either neutral or negatively charged. The basicity gives chitosan singular

properties: chitosan is protonated upon dissolution in aqueous acidic medium at $\text{pH} < 6.5$, but when dissolved possesses high positive charge on $-\text{NH}_3^+$ groups and the resultant soluble polysaccharide is positively charged. As a result, it adheres to negatively charged surfaces. Chitosan aggregates with polyanionic compounds, and chelates heavy metal ions. Both the solubility in acidic solution and aggregation with polyanions impart chitosan with excellent gel-forming properties (Brugnerotto *et al.*, 2001; Chenite *et al.*, 2001; Khan, 2001; Yang *et al.*, 2002; Krajewska, 2004; Santos *et al.*, 2005; Qin *et al.*, 2006).

Even though chitosan is known to have important functional activities, the poor solubility of chitosan is the major limiting factor in its utilization. This interferes with the biomedical application of chitosan, especially at the physiological pH value (7.4) where chitosan is insoluble and ineffective as an absorption enhancer (Snyman *et al.*, 2002). Hence, improving the solubility of chitosan is crucial if this plentiful resource is to be utilized across a wide pH range. Despite this limitation, various applications of chitosan and modified chitosan have been reported (Kubota *et al.*, 2000; Kumar, 2000; Chen and Park, 2003; Kim and Rajapakse, 2005). Chitosan possesses distinct chemical and biological properties. In its linear polyglucosamine chains of high molecular weight, chitosan has reactive primary amino and hydroxyl groups, amenable to chemical modification and provide a mechanism for side group attachment using a variety of mild reaction conditions (Francis and Matthew, 2000; Krajewska, 2004). Modification of chitosan provides a powerful means to promote new biological activities and to modify its mechanical properties. The general effect of addition of a side chain is to disrupt the crystal structure of the

material and hence increase the amorphous fraction. This modification generates a material with lower stiffness and often altered solubility (Francis and Matthew, 2000).

Various studies were conducted to make water-soluble derivatives of chitosan by chemical modification techniques, such as PEG-grafting (Ouchi *et al.*, 1998; Sugimoto *et al.*, 1998; Gorochovceva and Makuska, 2004), sulfonation (Francis and Matthew, 2000), partial N-acetylation (Kubota *et al.*, 2000), N-acetylation (Kumar, 2000; Francis and Matthew, 2000), chitosan carrying phosphonic and alkyl groups (Ramos *et al.*, 2003), hydroxypropyl chitosan (Xie *et al.*, 2002), branching with oligosaccharides (Tommeraas *et al.*, 2002), chitosan-saccharide derivatives (Yang *et al.*, 2002; Chung *et al.*, 2005), O-succinyl-chitosan (Zhang *et al.*, 2003) quaternisation (Snyman *et al.*, 2002) and carboxymethylation chitosan (Chen and Park, 2003).

New interest has recently emerged on partially hydrolyzed chitosan where molecular weight of chitosan decreases which in turn makes it readily soluble in water due to their shorter chain lengths and free amino groups in D-glucosamine units (Ilyina *et al.*, 1999; Qin *et al.*, 2003). The low viscosity and greater solubility of such chitosan at neutral pH have attracted the interest of many researchers to utilize chitosan in its lower molecular weight form (Kim and Rajapakse, 2005).

1.5 Applications

Chitosan is inexpensive and possesses important physiological properties such as biocompatible, biodegradable, non-allergenic and non-toxic for mammals (Sugimoto *et al.*, 1998; Duarte *et al.*, 2002; Falk *et al.*, 2004; Kim and Rajapakse, 2005; Qin *et al.*, 2006). Chitosan is a versatile biopolymer and therefore its derivatives have shown various functional properties, which make them possible to be used in many fields including, food, cosmetics, biomedicine, agriculture, environmental protection, wastewater management and fibre industries (Duarte *et al.*, 2002; Kim and Rajapakse, 2005).

Chitosan has been found to have an LD50 of over 16 grams/day/kg body weight in mice (Hennen, 1996). To put these data in context, chitosan was compared to common sugars, it appears that chitosan was less toxic than these substances. Mice are not men. For safety purposes, the data gathered in mice were divided by 12 to get the human equivalent. The relative LD50 in humans then would be 1.33 grams/day/kg. Given that an average person weighs 150 pounds or 70 kg, this means that the toxic amount for a person would be greater than 90 grams per day. Conservatively, one could feel very safe with the level below 10%, or 9 grams per day. Clinical studies have used 3-6 grams per day of chitosan with no adverse effects (Hennen, 1996).

1.5.1 Pharmaceutical and medical

A wide variety of medical applications for chitosan and chitosan derivatives have been reported over the last three decades (Kumar, 2000). Chitosan has been considered for pharmaceutical formulation and drug delivery applications in which attention has been focused on its absorption-enhancing, controlled release and bioadhesive properties (Dodane and Vilivalam, 1998).

Indeed, chitosan is known for being biocompatible allowing its use in various medical applications such as topical and ocular applications, implantation or injection. Moreover, chitosan is metabolized by certain human enzymes, especially lysozyme, and is considered as biodegradable. Due to its positive charges at physiological pH, chitosan is also bioadhesive, which increases retention at the site of application (Berger *et al.*, 2004). Chitosan has been used extensively to prepare microspheres for oral and intra-nasal delivery. Chitosan polymer has also been proposed as a soluble carrier for parenteral drug delivery (Gomez and Duncan, 1997). Chitosan is a versatile carrier for biologically active species and drugs due to the presence of free amino groups as well as its low toxicity. Gomez and Duncan (1997) reported that chitosan polymers when used as soluble polymeric carriers for intravenous administration have the potential to induce cellular toxicity.

There are many studies showing that chitosan accelerates wound healing in many clinical cases. It was reported that chitosan granules could enhance

regeneration of normal skin in open wounds. It has been suggested that chitosan may be used to inhibit fibroplasias in wound healing and to promote tissue growth and differentiation in tissue culture (Kumar, 2000). Chitosan is used as raw material for man-made fibres, filament, powder, granule, sponge, and composite with cotton or polyester in most studies. Medical product made of chitosan is useful as absorbable sutures and wound-dressing materials. It appears that chitosan, having structural characteristics similar to glycosamino glycans and could be considered for developing such substratum for skin replacement (Kumar, 2000; Kweon *et al.*, 2003). Chitosan could also inhibit the growth of tumor cells by exerting immuno-enhancing effects. Results of some related studies suggested that, the observed antitumor activities were not due to direct killing of tumor cells, but might be due to increased production of lymphocytes, leading to manifestation of antitumor effect through proliferation of cytolytic T-lymphocytes (Qin *et al.*, 2002; Kim and Rajapakse, 2005).

Chitosan has also been shown to have antacid and antiulcer activities, which prevent or weaken drug irritation in the stomach. The anti-ulcer activity is due to its capacity to bind free gastric acid and to a significant ability to act as demulcent. Also, chitosan matrix formulations appear to float and gradually swell in an acidic medium (Muzzarelli, 1973; Kumar, 2000; Falk *et al.*, 2004).

1.5.2 Biotechnology

Chitosan has one amino group and two hydroxyl groups in the repeating hexosamide residue. Chemical modification of these groups and the regeneration reaction gives to various novel bio-functional macromolecular products having the original organization or new types of organization. Chitosan is reported to suppress viral infections in various biological systems. Cationic charges of amino groups in chitosan may have additional functions to activate the immune and defence systems in plants and animals (Kim and Rajapakse, 2005). Chitosan has been used as cell-stimulating materials in plants, animals and human. Extracellular lysozyme activity was shown to be enhanced in *in vitro* cultures of several mammalian cells after treatment with chitosan and its derivatives. As a result, connective tissue formation was stimulated, and the self-defence function against microbial infection was enhanced at the cellular level (Kumar, 2000).

Several chitosan dressing materials have been developed commercially for the healing treatment. Chitosan has been used as an antibacterial and antifungal agent. The cationic amino groups of chitosan probably bind to anionic group of microorganisms and fungi, such as *Escherichia coli*, *Fusarium oxysporum*, *Alternaria arbusti*, *Helminthosporium papulosum*, and *Aspergillus nidulans* resulting in growth inhibition (Kumar, 2000; Xie *et al.*, 2002; Hai *et al.*, 2003; Qi *et al.*, 2004; Chung *et al.*, 2005; Kim and Rajapakse, 2005; Qin *et al.*, 2006).

Chitosan has also been used as fat trapper since it can attach itself to fat in the stomach before it is digested. Like some plant fibres, chitosan is not digestible; therefore it has no calorie value. No matter how much chitosan is ingested, its calorie count remains at zero. This is a very important property for any weight-loss product. It could trap the fat and prevent its absorption by the digestive tract. Fat in turn binds to the chitosan fibre, forming a mass, which the body cannot absorb, and then gets eliminated by the body. Chitosan fibre differs from other fibres in that it possesses a positive ionic charge, which gives it the ability to bind chemically with the negatively charged lipids, fats and bile acids (Hennen, 1996; Kumar, 2000; Kim and Rajapakse, 2005).

Chitosan has also been used as material for enzyme immobilization. Its desirable characteristic for immobilizing enzymes include high affinity to proteins, availability of reactive functional groups for direct reaction with enzymes and for chemical modification, hydrophilicity, mechanical stability and rigidity, and ease of preparation in different geometrical configuration that provide the system with permeability and surface area suitable for a chosen biotransformation (Krajewska, 2004). In agriculture, chitosan has been described as a plant antivirus, an additive in liquid multicomponent fertilizers and it has also been investigated as plant regulator and agro-products preservative in agriculture (Dodane and Vilivalam, 1998; Hai *et al.*, 2003).

1.5.3 Wastewater treatment

As environmental protection is becoming an important global problem, the relevant industries pay special attention to the development of technology, which does not cause environmental problems. It has been reported that wound dressing made of chitosan fibres has application in wastewater treatment (Kumar, 2000; Sinha *et al.*, 2004). Chitosan has been used for about three decades in water purification process. When chitosan is spread over oil spills, it holds the oil mass together making it easier to clean up the spill. Water purification plants throughout the world use chitosan to remove oils, grease, heavy metals, and fine particulate matter that cause turbidity in wastewater streams (Hennen, 1996). Due to the easy availability of free amino groups in chitosan, it carries a positive charge and thus reacts with many negatively charged surfaces. Chitosan spontaneously forms water-insoluble complexes with anionic polyelectrolytes. Therefore, chitosan has been shown to be an effective coagulating agent for treatment of food, processing waste effluents from vegetable, poultry, and egg breaking plants and for conditioning of activated sludge produced from biological treatment of waste (Wu *et al.*, 1976; Sinha *et al.*, 2004). Chitosan also undergoes chelation with metal ions. The removal of heavy metal ions by chitosan through chelation has received much attention (Kumar, 2000; Sinha *et al.*, 2004). Chitosan is one of the optimum decolourization methods for all wastewater streams. Due to its unique molecular structure, chitosan has an extremely high affinity for many classes of dyes, including dispersed, direct, reactive, acid, sulfur and naphthol dyes. The rate of diffusion of dyes in chitosan is similar to that in cellulose (Kumar, 2000).

1.5.4 Cosmetics

Chitosan has different molecular weights and degree of deacetylation, which is tailored for use in different cosmetic fields such as skin care, deodorants and hair care. Chitosan has been noted for its application as a film-forming agent and hydrating agent in cosmetics in view of its durable moisturizing effect on the skin. By reducing the trans-epidermal water loss, it increases water-binding capacity and skin moisture. It also improves the sensorial parameters and the dermatological compatibility of formulations (Dodane and Vilivalam, 1998; Muzzarelli and Muzzarelli, 1998; Klingels *et al.*, 1999). The film-forming ability of chitosan assists in imparting a pleasant feeling of smoothness to the skin and in protecting it from adverse environmental conditions and consequences of the use of detergents. Chitosan was found to be superior to hyaluronic acid as far as lasting hydrating effects are concerned (Muzzarelli and Muzzarelli, 1998). Klingels *et al.* (1999) reported that chitosan is a multifunctional active ingredient for the skin with additional advantages in sun protection and lip care. Sunscreen products must care for the skin to prevent drying out and should exhibit prolonged water-resistance. Complete water resistance is not achievable. However, water resistance can be increased by addition of hydrophobic waxes / oils, film formers or cationic polymers. For this reason, Klingels *et al.* (1999) conducted a test to determine whether chitosan as a cationic polymer could also increase the water resistance of UV filters in a sunscreen formulation. It was found that chitosan significantly increased water resistance. The chitosan film improved the adhesion of the UV filters and thus protected them against

washing off. The protection provided by the chitosan containing formulation was thus correspondingly enhanced (Klingels *et al.*, 1999).

Lipstick is one of the most widely used decorative cosmetics. Lip-care sticks are also used by many consumers. The main components of lipstick are waxes and oils. However, lip-care ingredients have also been increasingly used such as UV filters, and lipophilic agents (allantoin, bisabolol and vitamin E). Hydrophilic substances, such as moisturizers, are rarely used because the stick compound itself is generally occlusive and retains moisture. Lip-care sticks are mainly used to treat brittle, chapped lips. Chitosan activates various skin cell types and acts on the wound-healing process. Klingels *et al.* (1999) obtained results from a test with fibroblasts which revealed positive effects of chitosan with regards to improved cell adhesion. These properties support the use of chitosan as an active lip-care ingredient and not just to protect the lips against drying out. The addition of chitosan makes lips softer and also supports long-term colour adhesion (Klingels *et al.*, 1999).

Klingels *et al.* (1999) reported that chitosan is also suitable as a “deodorant-active” component in deodorants and as a styling polymer in hair cosmetics. With its antimicrobial properties (Klingels *et al.*, 1999; Kumar, 2000), it may be an added advantage to use chitosan in deodorants since it inhibits the activity of enzyme-producing bacteria. It is suitable for maintaining the spray ability of deodorant containing chitosan and has the antimicrobial effects at the same time. Various tests were carried out to assess the effectiveness of chitosan against odour-producing bacteria. A study was conducted to provide information on the

compatibility and sensorial properties of a formulation and not just on its deodorizing effect. The deodorizing effect and skin compatibility of the chitosan formulation were judged to be better when compared with triclosan. This result can be explained by the additional effects of chitosan, such as improved skin compatibility. In another comparative study, the fragrance adhesion and intensity of perfume oil in a brand-name deodorant formulation with and without added chitosan were assessed by a perfume expert and an expert laboratory panel. The chitosan containing formulation was rated much more highly by both groups. Chitosan retains the perfume fragrance for a longer period and with greater intensity and at the same time masks the odour of perspiration over a longer period of time. Chitosan can be used as a sole deodorizing component and may also be combined with other commercially available deodorizing agents (Klingels *et al.*, 1999).

Chitosan is the only natural cationic gum that becomes viscous on being neutralized with acid. These materials are used in creams, lotions and permanent waving lotions and nail lacquers (Kumar, 2000; Krajewska, 2004). Chitosan has been suggested as emulsifiers in cosmetics and pharmaceuticals. Modifying chitosan by introducing the phosphoric and alkyl groups onto its structure resulted in the presence of hydrophobic and hydrophilic group that controls solubility properties. In many cases, emulsion stabilization is achieved by the addition of specially designed polymers, which have hydrophilic and hydrophobic segments. For this reason, chitosan has been suggested as an emulsifier because it is absorbed at the interfacial surface, thus stabilizing the emulsion (Ramos *et al.*, 2003).