



Chitosan Modifications and Studies of Structure-Activity Relationships

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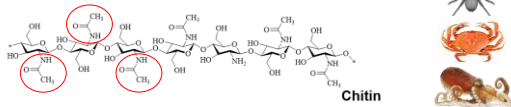
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Chitin

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Chitin

- Linear homopolysaccharide
 - Poly β -1,4-N-acetylglucosamine
- Structural polysaccharide in animals and fungi
 - Not in plants (not in mammals)
- Insoluble and similar to cellulose in plants
- The shells of crustaceans (and fungi) is the main commercial source
- Raw material for making chitosan, N-acetylglucosamine and glucosamine



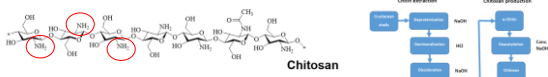
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Chitosan

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Chitosan

- Natural biopolymer found in some fungi
- Mostly obtained by deacetylation of chitin
- Obtained in few steps from steps from crustacean shells
 1. Deproteinization by treatment with weak NaOH
 2. Demineralization by treatment with HCl
 3. Deacetylation by treatment with strong (50%) NaOH
- Polycationic and soluble in acetic aqueous solutions



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Chitosan Applications

- Industry
 - Water treatment
- Agriculture
 - Plant Protection
- Food processing
 - Preservative
 - Flocculant
- Health Nutraceutical
 - Anti-obesity
 - Cholesterol lowering
- Pharmaceutical-Biomedical
 - Wound healing
 - Bone growth
 - Nanoparticles
 - Gene-delivery
 - Antimicrobial



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Chitosan in scientific research

- Chitosan may currently be the most studied polymer in scientific research

Biopolymers		Synthetic polymers	
Biopolymer name	Number of 2023 publications* naming the polymer in the title	Polymer name	Number of 2023 publications* naming the polymer in the title
Chitosan	5,547	Polyethylene (PE)	2,485
Cellulose	5,288	Polyurethane (PU)	2,132
Starch	2,937	Polypropylene (PP)	1,315
Collagen	2,359	P. m. methacrylate (PMMA)	713
Hyaluronic Acid	1,956	Polylactic Acid (PLA)	689
Chitin	492	Polycaprolactone (PCL)	445
Dextran	485	Polydimethylsiloxane (PDMS)	328
Elastin	143	Polytetrafluoroethylene(PTFE)	178

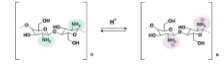
*Based on a search in ISI Web of Science, use the search term shown in bold. The result reported in this table is the number of documents registered in Web of Science where the search term appears in the document's title in 2023.

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Chitosan limitations

- Limited structural variation
- Charge is dependent on pH
- Low solubility at neutral pH
 - Usually dissolved in dilute organic acids or HCl
 - Divalent anions (like phosphate) may cause precipitation
- Low solubility in organic solvents
 - Limits processing and chemical modification
- Purity is important
 - Proteins (especially allergens)
 - Endotoxin (affects cellular assays)



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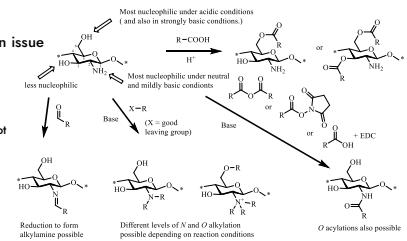


Chitosan Derivatives



„Direct“ Modification

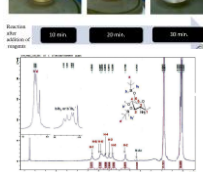
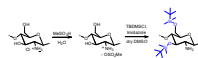
- Simple reactions
 - Often one step
- Lack of selectivity an issue
- Lack of detailed characterization a common issue in publications
 - Sometimes DS is not reported



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TBDMS chitosan

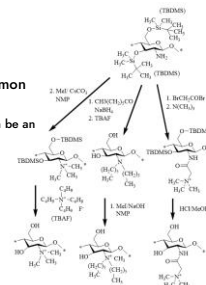


OV Rönansson, C. Maläner, J. Holappa, STh. Sigurdsson, M. Mässon Carbohydr. Res. 2008, 343, 2076-2082
 OV Rönansson, J. Holappa, C. Maläner, H. Stenroos, M. Rönkä, S. E. Nevalainen and M. Mässon. Eur. Polym. J. 2010, 46 (6), 1253-1257
 W. Song, X.S. Gao, D.X. Rönansson, M. Mässon, J.F. Mano. Carbohydr. Polym. 2010, 81 (1) 140-144



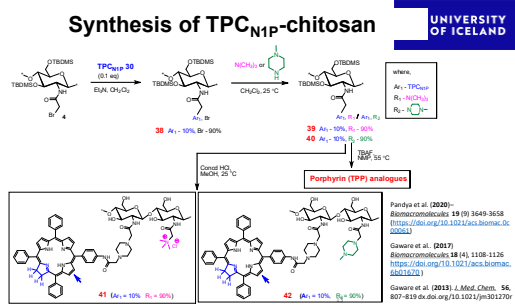
Synthesis using TBDMS chitosan as precursor

- DCM or NMP as solvent
- Quantitative reactions common
- Good control of DS
 - Incomplete deprotection can be an issue
- Mw reduction

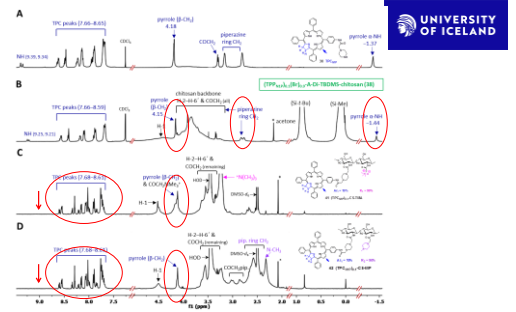


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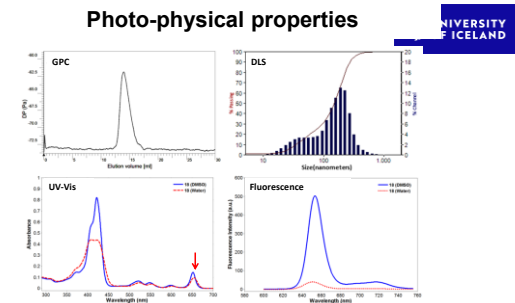
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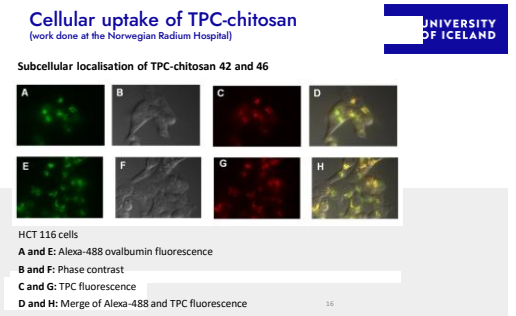
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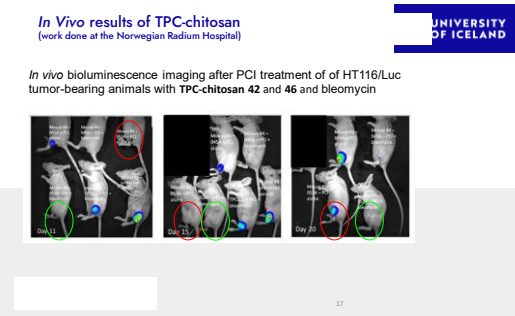
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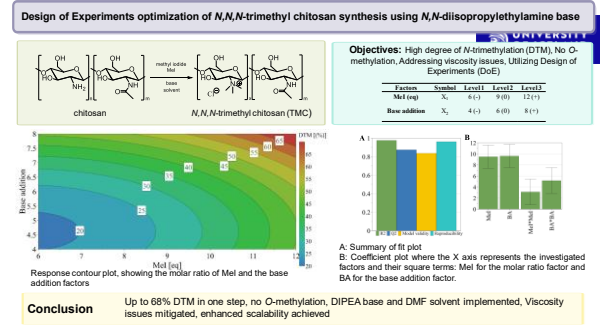
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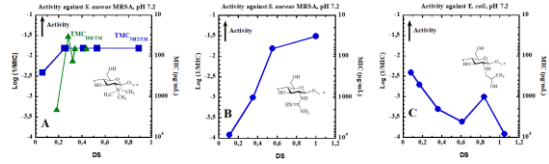


Structure-activity relationship
Degree of substitution



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TMC – Guanidyl Chitosan – Hydroxypropyl Chitosan



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Molecular Weight



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The Quantitative Molecular Weight-Antimicrobial Activity Relationship for Chitosan Biopolymers and Derivatives

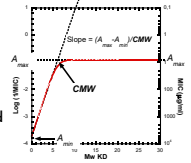


- A bilinear equation was formulated to describe the molecular weight-antimicrobial activity association for chitosan and its derivatives:

$$\text{Log}\left(\frac{1}{\text{MIC}}\right) = A_{\text{max}} + \frac{(A_{\text{max}} - A_{\text{min}})}{\text{CMW}} \times \text{Mw} - \text{Log}\left(\frac{\frac{A_{\text{max}}}{10^{\text{CMW}} \times \text{Mw}} + 10^{\text{A}_{\text{max}}}}{\frac{A_{\text{min}}}{10^{\text{CMW}} \times \text{Mw}} + 10^{\text{A}_{\text{min}}}}\right)$$

- The shape of the activity vs. Mw graph is then determined by three constants:

- $A_{\text{max}} = \text{Log}\left(\frac{1}{\text{MIC}_{\text{min}}}\right)$
- $A_{\text{min}} = \text{Log}\left(\frac{1}{\text{MIC}_{\text{max}}}\right)$
- CMW (critical molecular weight for maximum activity)

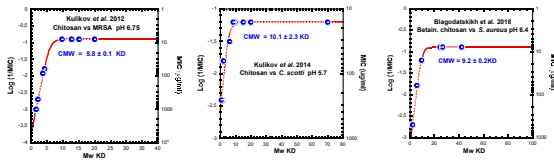


Már Másson (2024). The quantitative molecular weight-antimicrobial activity relationship for chitosan polymers, oligomers, and derivatives. *Carbohydrate Polymers* 337 121159

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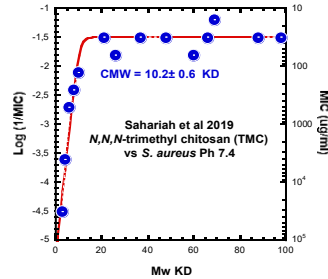


Data from 2012 to 2018
S. aureus, fungi – chitosan, chitosan derivative



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The 2019 study
TMC



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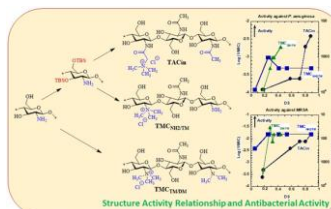


Structure-Activity Relationship (SAR) for antimicrobial action of cationic chitosan derivatives

- Degree of substitution (DS)
- Charge position (CP)
- Lipophilicity (LP)
- Multiple substituents (MS)
- Molecular Weight (MS)

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DS and activity of trimethylated derivatives against MRSA and P. aeruginosa

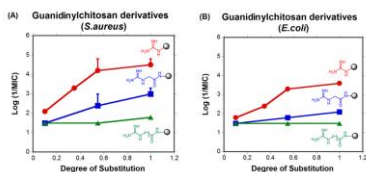


S. Rathnam, S. Ólafsdóttir, S. Jónsdóttir, M.Á. Hjalmsdóttir, M. Másson. Int. J. of Biol. Macromol. 160 548 2020

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DS and Antimicrobial activity



Priyanka Sahariah, Bjarni M Óskarsson, Marthá Á Hjalmsdóttir, Már Másson Carbohydrate Polymers 127, 407-417, 2015

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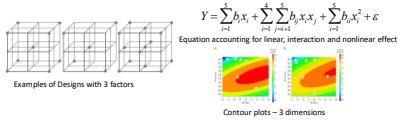
CP and antimicrobial N-acyl derivatives



Compound	Structure	MIC (µg/ml)		MBC (µg/ml)		M _w
		<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	
TAC-10T		10	10	100	100	417
TAC-10P		10	10	100	100	417
TAC-10S		10	10	100	100	417
TAC-10T		10	10	100	100	417
TAC-10P		10	10	100	100	417
TAC-10S		10	10	100	100	417
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TAC-10S		10	10	100	100	417
TAC-10T		10	10	100	100	417
TAC-10P		10	10	100	100	417

Design of experiment (DOE)

- Mathematical tool – statistical model
- To reduce number of required experiments.
 - High number of input parameters
 - Can evaluate many output parameter simultaneously
 - Optimization and response contour plots



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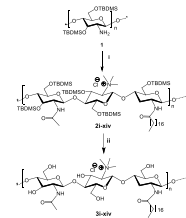


The Design Matrix

Table 1. Equivalent quantities of reagents used for synthesis of chitosan derivatives

Compound	Reagents (equivalents)		
	Acetyl chloride	Stearoyl chloride	Methyl iodide
3i	0.95	0	5
3ii, 3iii	0*	0	5
3iv	0.65	0.3	5
3v	0*	0.3	5
3vi	0.65	0	5
3vii	0.85	0.1	5
3viii	0.75	0.2	5
3ix	0*	0.2	5
3x	0.25	0.3	5
3xi, 3xii, 3xiii, 3xiv	0.425	0.15	5

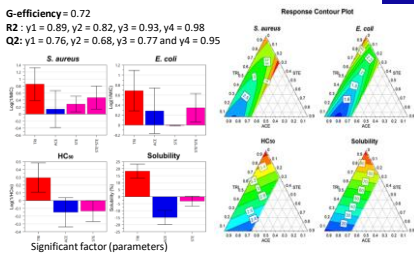
*Acetyl chloride was not added in this case since starting chitosan contains 5% acetylation.



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Contour plots

G-efficiency = 0.72
 R²: y₁ = 0.89, y₂ = 0.82, y₃ = 0.93, y₄ = 0.98
 Q²: y₁ = 0.76, y₂ = 0.68, y₃ = 0.77 and y₄ = 0.95



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Optimization

- Criteria:
 - high antimicrobial activity (>4 for *S. aureus*), (>3 for *E. coli*),
 - high solubility (>60)
 - low hemolytic toxicity (<3).

Proposed and observed values for optimized setpoint of all the responses.

Optimized chitosan derivative	Ratio of factors	<i>S. aureus</i> log(1/MIC)	<i>E. coli</i> log(1/MIC)	Hemolytic activity log(1/HCa)	Solubility (%)
Proposed derivative	TRI=0.63, ACE=0.08, STE=0.29	4.80	2.77	3.00	75
Observed derivative	TRI=0.67, ACE=0.05, STE=0.28	4.19	2.69	2.81	75



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