Experiments

Teaching-learning materials compiled by the PARSEL consortium
as part of an EC FP6 funded project (SAS6-CT-2006-042922-PARSEL)

Cooperating Institutions and Universities within the PARSEL-Project:

Chitin from crab shells

Source:

According to Bader, Birkholz, Chitin Handbook, R.A.A. Muzzarelli and M.G. Peter, eds., European Chitin Society. 1997. ISBN 88-86889-01-1

Equipment:

Beaker (400 ml), strainer (mesh size 3-4 mm), magnetic stirrer with heating plate, stirring rod, crystallization dish (Ø 14 cm), drying oven, balance, mortar with pestle, suction flask (500 ml), porcelain nutsch filter (Ø 9 cm)

Reagents and materials:

Reagents and materials	R-Phrases	S-Phrases	Danger symbol
Sodium hydroxide solution (w=2%)	36/38	26	 Xi
Hydrochloric acid (w=7%)			
Crab shells			

Procedure:

Do not forget safety glasses and lab coat!

1st step: coarse purification

150 g of crab shells are coarsely cleaned with water by stirring the broken shells in a 400 ml beaker with water for a few minutes. After this, the shells are filtered off. This process is repeated until sand and other soil are removed. The precleaned crab shells are dried overnight in the drying oven at 80°C.

Experiments developed by Hans Joachim Bader and Elke Birkholz (University of Frankfurt)
Additions made by: Wolfgang Gräber
Institution: IPN – Leibniz Institut für die Pädagogik der Naturwissenschaften an der Universität Kiel
Country: GER

2nd step: Protein removal

15 g of the dried shells are grinded in a mortar and transferred to a beaker. Then 250 ml of sodium hydroxide solution are added and the mixture is heated under stirring at 60-70°C for half an hour. The shells are filtered off with a strainer and the process is repeated. The filtrate should almost be clear and colorless. Then the shells are washed with demineralized water til neutral reaction. For time-saving the shells may be soaked in sodium hydroxide solution overnight after the first sodium hydroxide treatment, then filtered off and washed.

3rd step: Calcium carbonate removal

250 ml of hydrochloric acid are slowly added to the shells and the mixture is stirred at room temperature until no gas escapes anymore. As a check, 10 ml of hydrochloric acid are added. If no further generation of gas occurs, the mixture is filtered off and washed neutral with water. The product is dried overnight in the oven at 60°C.


Sodium hydroxide solution and hydrochloric acid are neutralized and poured down the sink.

Observation:

Anaylsis: Pictures of the formulas created with Chemdraw

The obtained Chitin is a dim pink-beige colored, fluffy substance. 15g of precleaned crab shells yield 3 g of chitin.

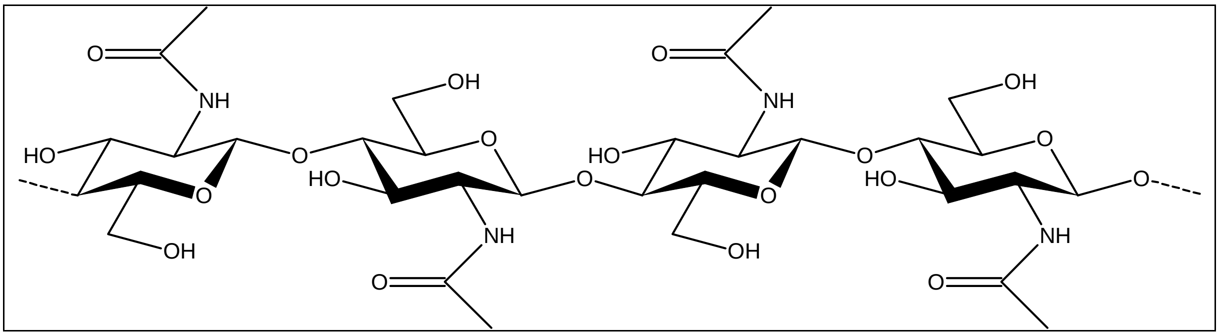

Abb.: Chitin: Poly-β-1,4-N-acetyl-D-glucosamine

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Cooperating Institutions and Universities within the PARSEL-Project:

Chitosan from Chitin by alkaline hydrolysis

Source:

According to Bader, Birkholz, Chitin Handbook, R.A.A. Muzzarelli and M.G. Peter, eds., European Chitin Society. 1997. ISBN 88-86889-01-1, with additions

Equipment:

Round-bottomed three-necked flask (250 ml, two NS 14, one NS 29), connector (cone to hose coupling), reflux condenser, ground-glass thermometer (NS 14), heating coil, magnetic stirrer, stirring rod, balloon, water pump, suction flask, porcelain nutsch filter (Ø 9 cm), filter paper, crystallization dish (Ø 14 cm), drying oven, balance

Reagents and materials:

Reagents and materials:	R-Phrases	S-Phrases	Danger symbol
Sodium hydroxide solution (w=50%)	35	26-36/37/39-45	 C
Chitin			

Procedure:

Do not forget safety glasses, safety gloves and lab coat!

In a round-bottomed flask 150 ml sodium hydroxide solution are added to 2 g of chitin. The apparatus closed air-tightly with a balloon (the literature recommends working with a protection gas (Nitrogen). For school purposes, this can be done without). With backflow and under stirring, the mixture is heated at 125°C for one hour. The mixture is allowed to cool down and then 100 ml of water are added. The next day the mixture is filtered off and the residue is washed with water to neutral reaction and dried in the oven at 60°C.

Remark: The ground joint has to be well-greased (possibly using teflon hulls)!

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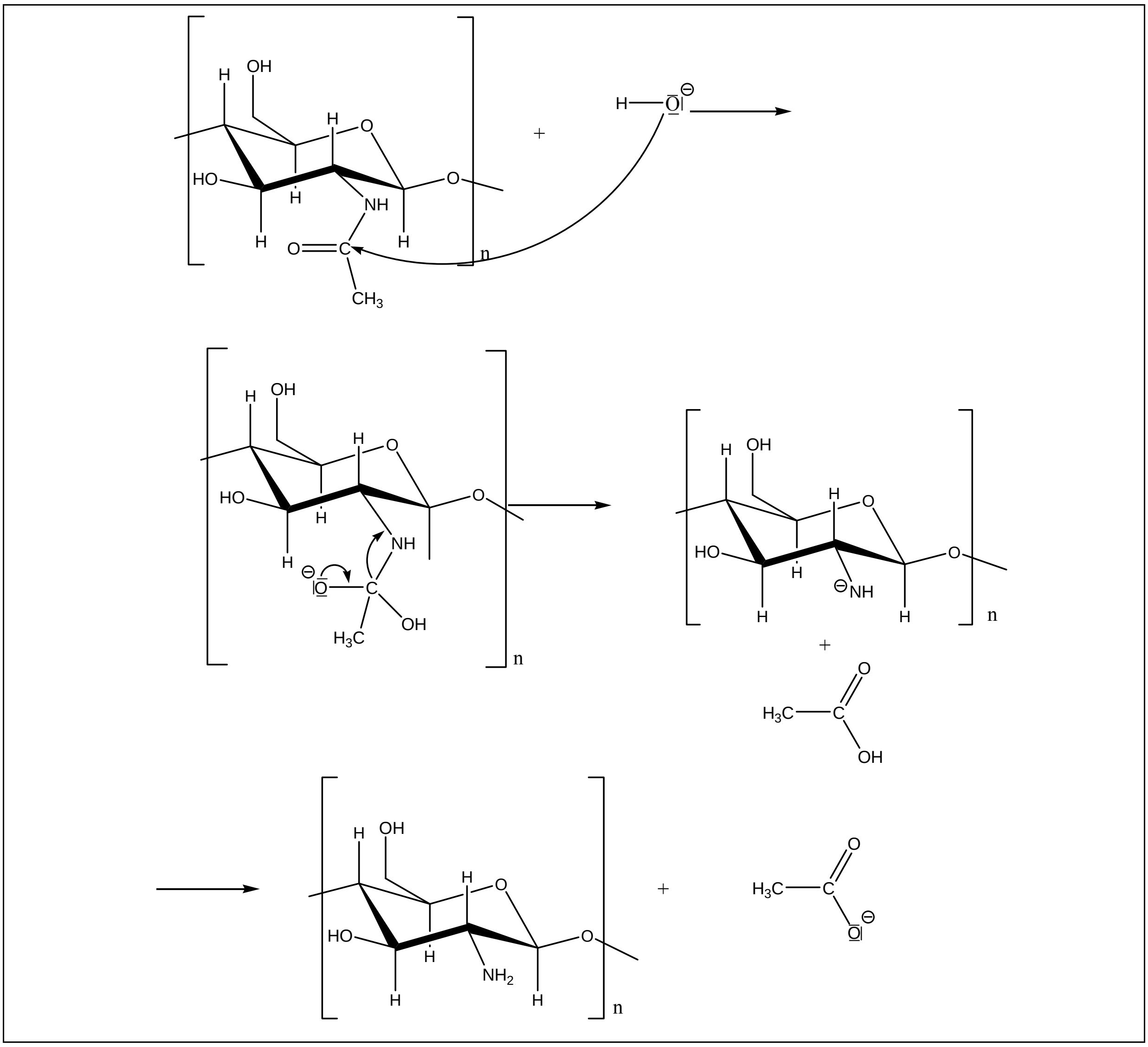
Mother liquor and washing water are neutralized and poured down the sink.

Observation:

Analysis: Pictures of the formulas created with Chemdraw

The obtained Chitosan is a dim pink-beige colored, fluffy substance very similar to Chitin. 2 g of Chitin yield 1,5 g of chitosan.



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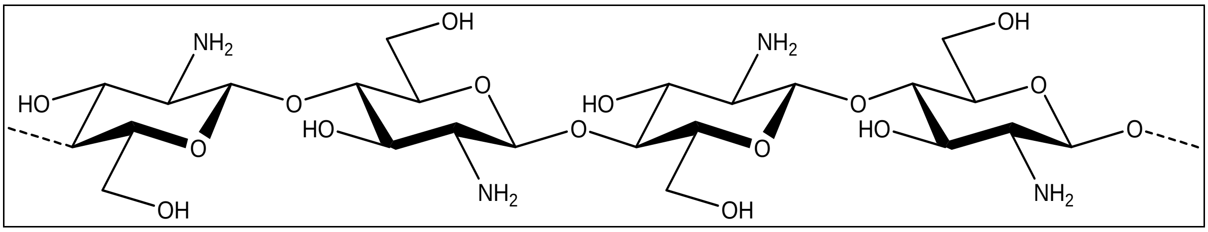

Chitosan: Poly-β-1,4-D-glucosamine

Source of errors:

Links:

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Cooperating Institutions and Universities within the PARSEL-Project:

Differentiation of chitin and chitosan

Source:

According to Bader, Birkholz, Chitin Handbook, R.A.A. Muzzarelli and M.G. Peter, eds., European Chitin Society. 1997. ISBN 88-86889-01-1
Finger, H.: Chitin und Chitosan – Neue Rohstoffe auf dem Weg zur industriellen Nutzung, WS 1999/2000, Marburg, http://www.chemie.uni-marburg.de/~reiss/downloads/expvortr/646.pdf

Equipment:

2 watch glasses, 2 Pasteur pipettes.

Reagents and materials:

Reagents and materials	R-Phrases	S-Phrases	Danger symbol
Chitin			
Chitosan			
I_2/Kl-solution (0,2 g I_2 in 100 ml Kl-solution (w=5%))			
Sulphuric acid (w=1%)			

Procedure:

Do not forget safety glasses and lab coat! Work at the extractor hood!

Some flakes of chitin or chitosan are put onto a watch glass. 2-3 drops of I_2/KI solution are added and the mixtures are acidified with 2-3 drops of sulphuric acid.

Chitin and chitosan treated are added to the waste jar for solids.

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Experiments

Observation:

After addition of I_2/KI solution, the chitosan changes color to dark brown and the solution becomes colorless. On addition of sulphuric acid, the dark brown color turns dark purple.

On the opposite side, chitin remains unchanged on addition of iodine solution, which retains its brownish-yellow color. Also, the acidification with sulphuric acid has no consequence.

Analysis:

A definite analysis of the experiment does not exist, but a suggestion for an analysis, which shall be presented here: Like the reaction of iodine with starch, chitosan is meant to establish a host-guest complex. To make this clear, the complex is illustrated below. The starch exists in a helix-formed conformation, which creates void spaces, in which the poly-iodine chains are inserted to. This is meant to happen similarly with chitosan. Chitin, on the other hand, is to be sterically inhibited in that regard (residues of acetylamine). However, a helix-formed order of the monomers, which is given with starch by α-1,4-links, is a requirement for this. In chitosan, however, the monomers are β-1,4-linked (according to Finger).

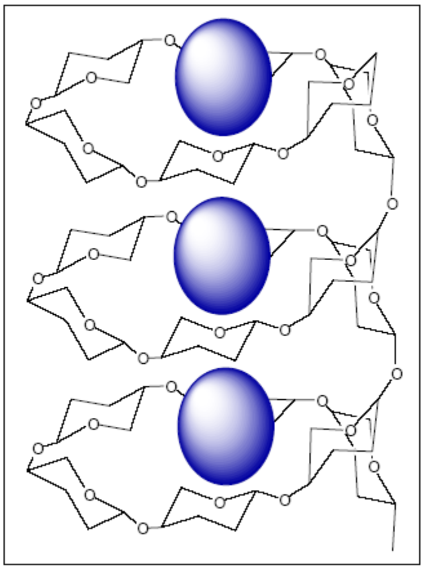

Fig.: Iodidine-Starch host-guest complex

Source of errors:

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Cooperating Institutions and Universities within the PARSEL-Project:

Preparation of chitosan films

Source:

According to Bader, Birkholz, Chitin Handbook, R.A.A. Muzzarelli and M.G. Peter, eds., European Chitin Society. 1997. ISBN 88-86889-01-1

Equipment:

2 beaker (250 ml), magnetic stirrer with heating plate, stirring rod, Pasteur pipette, small-meshed strainer, 2 plastic plates (30 cm x 30 cm) or rinsing bowl

Reagents and materials:

Reagents and materials	**R-Phrases**	**S-Phrases**	**Danger symbol**
Chitosan			
Acetic acid (w=12%)	36/38	26	 Xi
Tetraethylene glycol			

Procedure:

Do not forget safety glasses, safety gloves and lab coat! Work at the extractor hood!

Two beakers are filled each with 2 g of chitosan and 100 ml of acetic acid. Under slight heating and stirring the chitosan is dissolved. After cooling one of the solutions is poured through a strainer onto a plastic plate or on the backside of a rinsing bowl. The content of the second beaker is mixed with 0.2 g of tetraethylene glycol; the mixture is stirred for some minutes. Afterwards this solution is poured through a strainer onto a plastic plate or on the backside of a rinsing bowl too. The slightly viscous solutions are not smoothed down. The solvent is allowed to vaporize under the extractor hood overnight.

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Experiments

 Immediately after use, the strainer should be cleaned with running water.

Observation:

After the vaporization of the solvent in both experiments a flexible, tear resistant and transparent film remains, which is easily peeled off the plate. The film with additional tetraethylene glycol is softer than the pure chitosan film

Analysis: Pictures of the formulas created with Chemdraw

For the creation of transparent films, the film-creating characteristics of the macro molecule chitosan are used, which are caused by intra- and inter-molecular hydrogen bonds. Tetraethylene glycol can serve as a softening agent in this case, as it inserts itself into the chitosan molecules under the creation of hydrogen bonds.

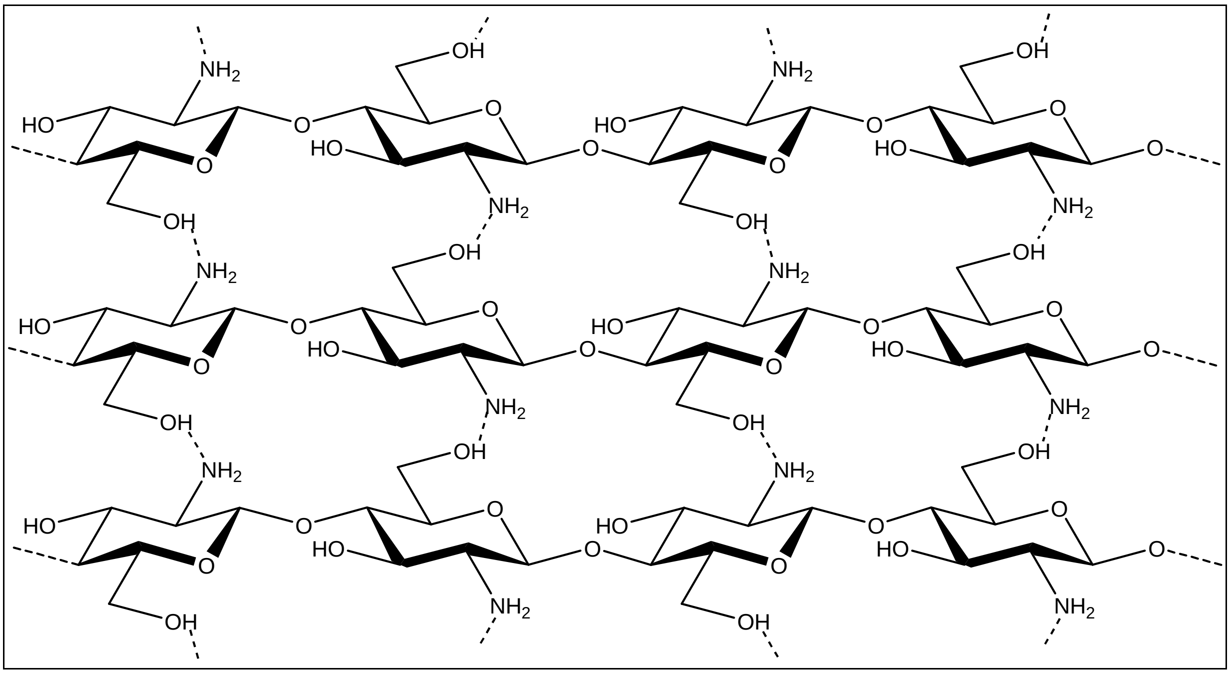

Fig.1: Chitosan film without softening agent

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Experiments

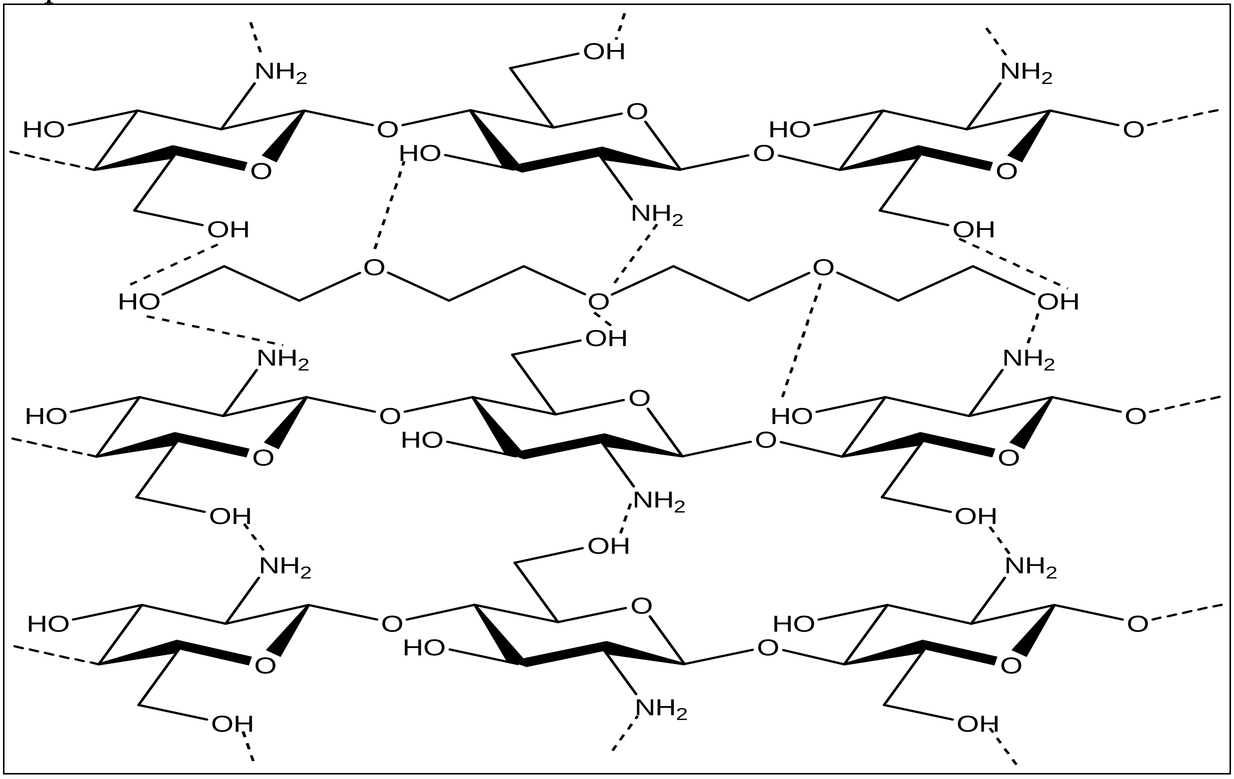

Fig 2: Chitosan film with tetraethylene glycol as softening agent

Source of errors:

If the solutions are poured directly onto plastic plates without using strainer, it is possible that chitosan particles not dissolved completely cause thickenings and uneven patches. Besides, pouring through strainer prevents the formation of bubbles.

If too much of the softener is added, the two components separate and after some time the softener forms oily drops on the film.

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Cooperating Institutions and Universities within the PARSEL-Project:

Clarification of fruit juices

Source:

According to Bader, Birkholz, Chitin Handbook, R.A.A. Muzzarelli and M.G. Peter, eds., European Chitin Society. 1997. ISBN 88-86889-01-1

Equipment:

Beaker (100 ml), magnetic stirrer, stirring rod, centrifuge, centrifuge tubes, Pasteur pipette, light source (e.g. laser).

Reagents and materials:

Reagents and materials	R-Phrases	S-Phrases	Danger symbol
Chitosan			
Naturally cloudy juice (e.g. apple juice)			

Procedure:

Do not forget safety glasses and lab coat!

50 ml of naturally cloudy apple juice are mixed with 0,1 g of chitosan and the mixture is stirred for 5 minutes. Then the solution is centrifuged for 10 minutes. Parallel, 50 ml of naturally cloudy apple juice without additional chitosan are centrifugated as blank test.

The solutions are poured down the sink.

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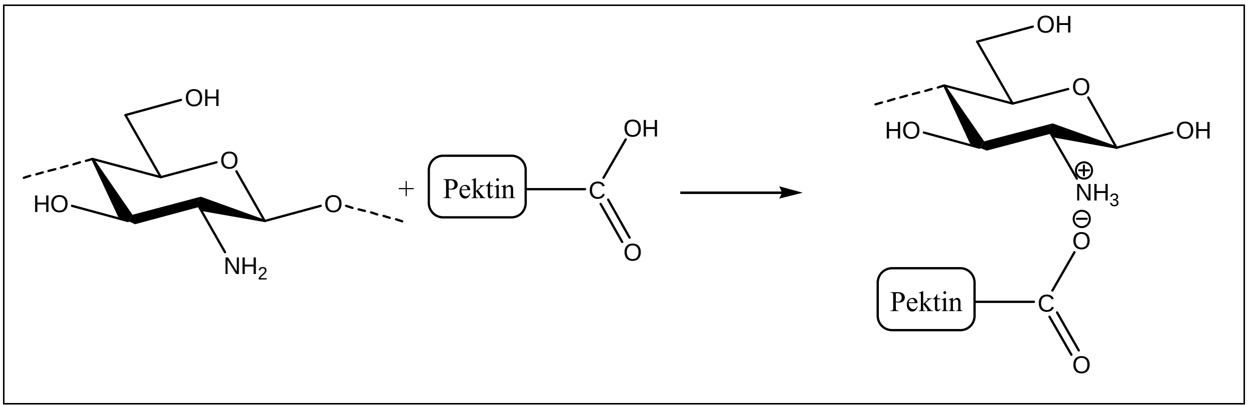
Experiments

Observation:

The centrifugate of the apple juice mixed with chitosan is completely clear and no Tyndall effect is visible. In opposite, the centrifugate of the untreated apple juice shows same cloudiness as before. The Tyndall effect is visible.

Analysis: Pictures of the formulas created with Chemdraw

Fruit juice without addition of chitosan does not show any clarification when filtrated and centrifugated as it is a suspension, in which natural poly-anionic tannins and other matter exist colloidally suspended. Fruit juice does not show a Tyndall effect after adding chitosan and centrifugating it. This is due to the poly-cationic characteristics of chitosan. As a poly-cation, chitosan builds ionic macromolecular complexes with these compounds which can be centrifugated off.



Source of errors:

If too much chitosan is added, the centrifugate remains cloudy. .

Links:

Tyndall effect: An effect of light scattering by colloidal particles or particles in suspension. It is named after the 19th century Irish scientist John Tyndall. It is similar to Rayleigh scattering, in that the intensity of the scattered light depends on the fourth power of the frequency, so blue light is scattered more strongly than red light. An example in everyday life is the blue colour sometimes seen in the smoke emitted by motor bikes. The phenomenon is best explained as the particle size is much greater than the wavelength of light.. *Source:* http://en.wikipedia.org/wiki/Tyndall_effect

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