

Experiment 2: Chitosan from chitin by alkaline hydrolysis

Duration: First day: 70 minutes, second day: 30 minutes without drying time.

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

Reagents and materials: sodium hydroxide solution, $w(\text{NaOH}) = 50\%$, chitin, nitrogen bomb.

Procedure: In a round-bottom flask 150 ml sodium hydroxide solution are added to 2 g of chitin. The apparatus is flushed with nitrogen and then it is closed air-tightly with a nitrogen-filled balloon. Now the mixture is heated under stirring to at 125°C for one hour. The mixture is allowed to cool 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 .

Result: The chitosan obtained is an almost colorless and fluffy substance resembling chitin. The conversion of 2 g of chitin yields 1.5 g of chitosan. *Waste disposal:* Mother liquor and washing water are neutralized and poured down the sink.

Analysis of chitin and chitosan

Like starch chitosan forms with iodine an inclusion complex, which has a purple colour in acidic medium. On the other hand, the chitin is unable to accommodate iodine molecules (experiment 3). In contrast to alkaline hydrolysis the glycosidic bonds of chitin between the sugar units are cleaved by acidic hydrolysis. The amide bond in chitin is preserved. Degradation of chitin leads to N-acetylglucosamine and corresponding oligomers. Hydrolysis of chitosan yields glucosamine and its oligomers (experiments 4 and 5):

The aldehyde groups of the glucosamine and N-acetylglucosamine are oxidized by Fehling's solution to carboxylic acids, whereas the divalent copper is reduced to univalent copper, which precipitates as brick-red colored copper(I) oxide in alkaline medium. The free aldehyde groups of chitosan are not sufficient for the Fehling's test (experiment 4), and, due to insolubility, the test with chitin is not feasible. The determination of free amino groups in chitosan is performed according to Slyke (Sommerfeld and Bader, 1995).

