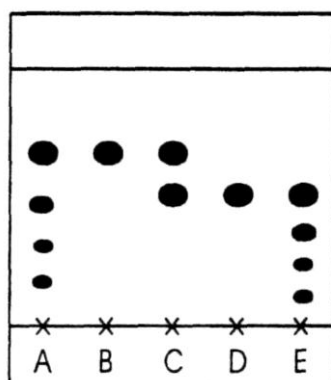


## Experiment 5: Determination of the hydrolyzates of experiment 4 by chromatography

*Duration:* 40 minutes.

Thin layer chromatography (TLC) on a silica gel plate with mobile solvent made of 10 % of water, 15 % of methanol, 25 % of water-free acetic acid and 50 % of 1,2-dichloroethane produces the result shown in Figure 1. (Spray reagent: 0.5 g of thymol are added to a mixture of 5 ml sulphuric acid ( $w(\text{H}_2\text{SO}_4) = 96\%$ ) and 95 ml of ethanol; reference solution: 10 mg of N-acetylglucosamine added to 20 ml of water, 10 mg of glucosamine hydrochloride are added to 20 ml of water, 10 mg of N-acetylglucosamine and 10 mg of glucosamine are added to 20 ml of water).

*Observation* (cf. Figure 1): The main spot in the chromatogram of the filtrate obtained from the acidic hydrolysis of chitin corresponds to the spot of the N-acetylglucosamine reference on regarding its position and brownish colour. Both chromatograms of the filtrates show below the main spots further spots, which are graded downwards and which are paler in intensity. Those indicate different oligomers of N-acetylglucosamine and glucosamines consisting of 2 to 4 sugar units.



**Figure 1.** Chromatogram of products obtained in hydrolysis of chitin and chitosan. A: Filtrate of acidic hydrolysis of chitin, B: N-acetylglucosamine reference, C: N-acetylglucosamine/glucosamine reference, D: glucosamine reference, E: filtrate of acidic hydrolysis of chitosan.