

ABSTRACT

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Title of Doctoral Thesis **Studying the distribution of substituents in derivatives based on hyaluronan**

Derivatives based on hyaluronan have found applications in various areas of medicine. Prepared derivatives are commonly characterized by their molar mass and their degree of substitution, however, such parameters are unable to distinguish among derivatives with different distributions of substituents along the chain, which may exhibit different macroscopic properties. For this reason, methods to determine the distribution of substituents along the chain are needed. The presented thesis deals with the determination of distribution of amino groups in deacetylated hyaluronan. The theoretical part starts with a description of hyaluronan structure and of its derivatives. Next, the application, the preparation, and the characterization of deacetylated hyaluronan are discussed, focusing particularly on its depolymerisation into oligosaccharides using deaminative cleavage or enzymes that degrade hyaluronan. Finally, examples of liquid chromatographic methods used to analyse hyaluronan oligosaccharide are presented. The experimental part describes the preparation of samples of deacetylated hyaluronan, the determination of their molar masses using size-exclusion chromatography, and the determination of their degree of deacetylation using proton nuclear magnetic resonance spectroscopy. Besides, the main attention is paid to the development of two methods to determine distribution of the amino groups. The first method is based on deaminative cleavage with nitrous acid and subsequent labelling of obtained oligosaccharides with 3-methyl-1-phenyl-2-pyrazoline-5-one. The second method is based on enzymatic cleavage with hyaluronidase from *Streptococcus pneumoniae* and the same subsequent labelling. To analyse the labelled oligosaccharides, reversed phase liquid chromatography is employed in both methods. Subsequently, the experimental oligosaccharide distributions are compared with theoretical random distribution models. The comparison indicate that deacetylation of hyaluronan does not occur completely randomly. Specifically, deacetylation of specific N-acetyl-D-glucosamine reduce the probability of deacetylation in the neighbouring disaccharide unit.