Chemical modification of commercial chitosan and its anticoagulant activity

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INTRODUCTION: Chitosan is a polysaccharide obtained from chitin deacetylation, which is isolated from crab and shrimp shells. Chitosan structure consists in a linear polysaccharide β (1→4)-linked GlcNH2 and GlcNAc units. The presence of these functional groups allows the preparation of a variety of products through chemical modification, such as sulfation. Sulfated polysaccharides have many pharmacological activities, which include anticoagulant properties, but the mechanism that these compounds interact with factors of coagulation cascade still unknown. MATERIALS AND METHODS: Chitosan sulfation was carried out by the Terbojevich method (1989) and monitored by 1,9-Dimethyl-Methylene Blue (DMMB). The product was analyzed by high performance size exclusion chromatography (HPSEC), NMR and anticoagulation activity was determined by intrinsic pathway (aPTT). RESULTS AND DISCUSSION: Chitosan sulfation was confirmed by DMMB dye binding assay. HPSEC showed a homogeneous peak of 14 KDa with 4.59 of polydispersity and DS of 0.49. 2D NMR analysis showed that sulfate groups is localized in C3 and C6, due the NMR signals at 4.85 and 4.45 ppm (1H NMR) and 76.3 and 67 ppm (13C NMR), respectively. Anticoagulant activity of chitosan sulfate was dose-dependent; doubling clotting time at initial concentration (0.5µg/µL), Suwan and collaborators (2009) showed that 1 µg of N-sulfate chitosan was not capable to double clotting time. CONCLUSION: The sulfation method is efficient to produce chitosan 3,6-di-O-sulfate and it becomes to prolong the clotting time. These results guide to pursue the understanding of interaction between sulfated polysaccharide and clotting factors.

Palavra chave: hexosamine, clotting time, NMR
Patrocínio: FAPESP, CNPq and CAPES