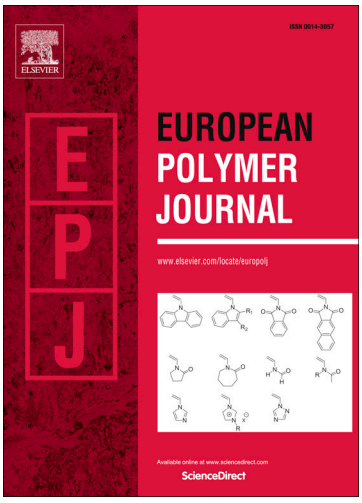

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Abstract

The present study describes the development of a fast, affordable and reliable method for hyaluronic acid detection in complex samples.

The method involves three principle steps. The first is the separation of hyaluronic acid (HA) from interfering glycosaminoglycans as well as mono- and oligosaccharides by cetyltrimethylammonium bromide fractioning. The second is subsequent digestion of HA with Streptococcus pneumoniae hyaluronate lyase to 4,5-unsaturated disaccharides (ΔHA2). The third is the reaction of ΔHA2 with 3-methyl-2-benothiazolinonehydrazone (MBTH) resulting in an intense blue-colored product. The extinction coefficient of ΔHA2-MBTH product is 34,735 mol^{-1} at 654 nm. The theoretical sensitivity of the assay is 0.07-0.09 mg/l HA. The practical sensitivity is 0.3 mg/l; the highest repeatability was achieved in the range of 3-2,000 mg/l HA (r^2=0.9994). The analysis took 25-60 minutes depending on sample complexity.