Conical track-etched Nanopore for a free-label detection of OSCS contaminants in heparin

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Over 80 years, heparin has been used as an anticoagulant in surgery and treatments of some clotting disorders. 10 years ago, the contamination by oversulfated chondroitin sulfate (OSCS) caused hundreds of death around the world. As the structures of heparin and OSCS are quite similar and the contaminant has very low concentration, traditional methods like chromatography can hardly satisfy. On the other hand, fluorescence methods extend a lot on detection accuracy until 10^{-9}% w/w of heparin, but the high cost of this method is still a limit. Here we propose a novel strategy to detect OSCS from heparin solution using conical nanopore functionalized with poly-L-Lysine. The nanopore is ionic diode-like which can modulate ion transport and thus the I-V response by varying surface charge. Briefly, a layer of poly-L-Lysine on pore surface is charged positively making the pore selective to anions. As heparin has sulfate groups, its adsorption modify pore selectivity. Playing with the equilibrium we established a quantitative relation between heparin concentration and ionic current rectification. This sensor is excellent to detect low heparin concentration (from 25 ng/ml to 3 µg/ml) using the modification of ionic current rectification. We used the properties of OSCS to inhibit the heparinase to detect it. To demonstrate that, we have followed the kinetic of heparin degradation by heparinase which has a good correlation with results obtained by classical methods. Then we have added OSCS that inhibits the heparin degradation. Our results show that the sensor can detect OSCS until a concentration of 200 pg/ml representing 0.01 % in weight in a heparin. This resolution is one order of magnitude lower than the one obtained by chromatography. For the first time, it was reached without fluorescence labelling. And by reusability study, we have shown that our sensor can work during more than 3 weeks.

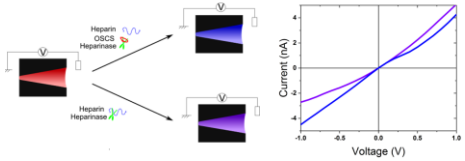

Figure 1. Principle of OSCS detection by nanopore rectification change