Hyperpolarized solvatochromic nanosensors embedded in agarose gel towards heparin sensing in blood

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Heparin is a polyanionic anticoagulant extracted from natural sources and used in numerous surgical procedures. Its typical concentration in the blood steam ranges from 0.1 to 5 IU/mL and varies with time due to its clearance from the body. Too low of a heparin concentration increases clotting risk, whereas excessive levels can induced uncontrolled bleeding. Thus, it is crucial to be capable of quickly measuring heparin levels. The effects of heparin may be reversed via polyionic binding with protamine, an arginine-rich protein that can be used as an antidote in the case of heparin overdose.

The current gold standard of heparin determination is the anti-Xa assay. Since it is a fluorescence-based method, it requires significant sample processing times as it cannot be performed in whole blood. The demand for a more suitable analysis tool encouraged the development of more convenient heparin quantification methods.

Meyerhoff and coworkers achieved pioneering work on protamine and heparin detection since 1994 with ion selective electrodes[1] and optical sensors.[2] Our group recently developed emulsion-based particles sensitive towards protamine containing a solvatochromic dye as signal transducer.[3] The nano-optodes showed a significant absorbance shift in the presence of protamine, no pH cross response and were successfully used to quantify heparin levels in hospital patients' plasma. However, this approach still required sample treatment since it was unsuitable for whole blood detection owing to its high background absorbance.

Agarose gels have been previously used to embed optodes for ion detection.[4] Our protamine sensors were embedded in agarose gel, poured in commercially available polystyrene cuvettes and pictures were taken after protamine addition on top of the gel. The nanosensors showed the desired absorbance shift in presence of protamine when embedded in the gel. Here, however, the mixing between protamine and the nanoparticles was limited by protamine diffusion into the gel. Previous works using embedded nanosensors to detect cations utilized a distance-based readout. Such approach could not be applied to protamine or heparin sensing because physiological concentrations were much smaller. Thus, an intensity-based quantification method was developed here with spectral unmixing,[5] allowing us to monitor the signal change induced by protamine diffusion over time. This method gave promising results for both protamine and heparin quantification in whole blood samples.

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[3] Y. Soda et al., *Chem. Sci.* **2021**, 12 (47), 15596–15602.
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