SYNTHESIS OF "CLICKABLE" MACRO-POROUS MATERIALS FOR ULTRAFAST PURIFICATION OF MONOCLONAL ANTIBODIES

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Porous polymers are topical materials and have gained a lot of interest in both academic and industrial research because they combine particular features of porous materials with those of synthetic polymers. Nowadays, porous polymers are used for a variety of different application fields such as catalysis, gas storage, or as separation materials. Various manufacturing methods can be applied to produce macroscopic porous polymeric particles, but most of them require the utilisation of a suspension polymerisation process in the presence of a porogen. This method often negatively affects the control of the final morphologies and requires a tedious work up as the porogen has to be fully extracted. In previous works, we have already presented “reactive gelation” as an alternative, porogen-free method to produce macro-porous materials by controlled aggregation of colloidal polymer nanoparticles under high shear\(^1,2\). Nevertheless, the production of macroporous polymers, which allow an easy post-functionalization had yet to be proven to address relevant applications like the chromatography of therapeutic proteins for instance.

Herein, we present the emulsion polymerisation of highly crosslinked styrene-co-vinyl benzyl azide core-shell nanoparticles (~40 nm), their aggregation at high shear rates (10^6 1/s), and the hardening of the obtained fractal structures by post-polymerisation. This process results in chemically and physically stable macroporous microclusters with average size around 80 microns and pore sizes up to 10 µm. Thanks to the azide groups in the shell, the macroporous polymer scaffold can easily be post-functionalized by exploiting the concept of copper catalysed alkyne-azide cycloaddition (CuAAC). Among a vast number of functionalities that have been clicked onto the porous base material, we have also immobilised \textit{staphylococcus aureus} protein A, well known as an affinity ligand for the capturing of monoclonal antibodies. By immobilising protein A on a macroporous scaffold, we are addressing the most significant bottleneck of large-scale production of therapeutic proteins, namely the purification of such. Indeed, the separation of the target protein from its cell impurities (host cell proteins, DNA, enzymes, etc.) often consists up to 80 % of the overall production costs because conventional chromatographic materials are not mechanically stable and only possess small pores (up to 150 nm). These smaller pores result in a diffusion-limited flow behaviour, making the downstream process costly and time-consuming. Our base material combines mechanical stability and perfusive flow behaviour, thereby providing protein separation at very high flow rates up to 1800 cm/hr. This excels conventional industrial materials by far, which can usually be used between 300-600 cm/hr. Noteworthy, the dynamic binding capacity is independent of the process rate, giving 10 g/l at 1800 cm/hr. The developed protein A prototype has also been proved stable under alkaline conditions, showing recovery above 80 % after 80 cycles with 0.1 M NaOH solution. However, since the industrial downstream process has a few other chromatographic steps following the capturing (such as ion-exchange chromatography and hydrophobic interaction chromatography), also polyelectrolytes and aliphatic molecules have successfully been attached to the macroporous base material. Addressing all types of chromatography needed during the purification of therapeutic proteins with just one base material highlights the true potential of this material, and might pave the way for perfusive protein chromatography.

In summary, we have demonstrated emulsion polymerisation towards clickable core-shell nanoparticles, their aggregation under shear yielding macroporous particles, and their application as chromatography resins for the ultra-fast downstream of therapeutic proteins. Since the click chemistry protocol allows easy functionalization, the proposed process is expected to be suitable for other application fields as well.