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Preparation of perfusive chromatographic materials via shear-induced reactive gelation

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ABSTRACT

A simple method for producing highly porous materials suitable for chromatographic applications is discussed. Starting from a dispersion of polymer core-shell nanoparticles (latex), micrometer sized clusters (100 μm) are produced via shear-induced Reactive Gelation. Thanks to their fractal structure, these aggregates exhibit highly porous structures, with pore size distribution ranging from 0.1 to several micrometers. The effect of different properties of the primary nanoparticles on the qualities of the final products is also investigated. Particle architecture, namely the ratio between the hard, highly crosslinked core, and the soft, poorly crosslinked shell, turned out to be the most important parameter to be tuned in order to obtain highly porous and mechanically resistant clusters. The final materials can be easily slurry-packed into conventional chromatographic columns. In comparison to other commercial stationary phases, these materials show not only much lower pressure drops at very high flow rates (i.e. <0.2 bar/cm at 6 mL/min), but also HETP profiles independent of fluid velocity when measured with tracers of sizes comparable to typical bio-macromolecules. Moreover, these materials, while offering the key advantage of being in a slurry form and thus easily packable and scalable, have a behavior that closely resembles that of monoliths, in which convective flow contribution dominates.

1. Introduction

Porous polymers are widely employed in a variety of different applications including separation and purification techniques [1,2], catalysis [3], thermal insulation [4] and medical tissue engineering [5]. Depending on the amount, morphology and mechanical characteristics of the desired porous material, different manufacturing methods are currently in use [6]. For example polymer-based materials with large pores are produced by suspension polymerization with the aid of a pore-generating system, mainly in the form of a porogen [6]. The resulting process involves multiple steps taking place at the same time, thus making the control of the final pore architecture very challenging. Moreover, the porogen is essential for the pore formation but needs to be fully extracted after the reaction, which may be difficult in some cases. Despite the extensive use of this approach, a reliable and comprehensive relationship between the reaction recipe and the morphological properties of the final material is far from being fully established [7–9]. Reactive Gelation, an alternative, porogen-free method for producing rigid macroporous polymer particles has been proposed previously by Marti et al. [10] and it enables the preparation of these materials in a closely controlled and step-wise manner. Starting from a stable colloidal suspension of polymer particles, additional monomer and initiator are added in order to swell them. These swollen particles, usually stabilized by ionic surfactants, are subsequently aggregated by destabilization induced in different ways: by screening the surface charges through the addition of electrolytes (Brownian aggregation), thus reducing the repulsive barrier among the particles, or by providing them enough kinetic energy to overcome the repulsive barrier (shear aggregation) [11]. Depending on the selected aggregation method, different products (*e.g.* monoliths or micrometer-size clusters) and different structures in terms of pore size distribution, have been obtained [11,12]. The last step of the Reactive Gelation consists in a post-polymerization of the previously added monomer/initiator mixture. This way the primary particles are more strongly bonded together, while retaining the porous structure and improving the mechanical strength and resistance of the final material.

Shear-induced aggregation is performed by exposing the latex to some kind of flow field. If applied without electrolyte addition, this mechanism is called pure shear-induced aggregation [13]. Owing to their fractal structure, the aggregates occupy more space while growing, which eventually leads to a percolation and the formation of a gel. It already has been shown [14] that a fraction of non-aggregated primary particles remains free when such a

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network is formed, which means that a fully percolating system can be obtained even when the aggregation is not complete. Xie et al. [15] showed that this process is affected by the size of both the primary particles and the resulting aggregates. It is also possible to carry out the aggregation process at large conversion while preserving the liquid-like behavior and limiting the system's viscosity increase [12]. This enables the use of continuous processes, which are easily scalable to large productivity. Moreover, the final dispersion of clusters and residual primary particles can be easily diluted by water addition, facilitating the subsequent steps of post-polymerization, wet filtration and packing. The final product can in fact be collected as ready-to-pack slurry of polymer particles in water.

The porous polymers produced in this work are specifically suited for chromatographic applications because they provide high transport efficiency [16]. As a matter of fact, a trade-off between pressure drop and separation performance is typically observed in chromatographic columns packed with conventional porous particles [2,16]. In contrast, particles and monoliths produced by Reactive Gelation exhibit pore sizes so large (radii larger than 200 nm) that they permit convective flow through the particle, thus reducing (or even overcoming) the intraparticle diffusion limitations even at relatively large fluid velocities [17]. This allows using large particles, thus with low pressure drop and high flowrates, while avoiding mass transfer limitations. This behavior is known as perfusion and it enables flow-independent separation performance, which represents a great improvement towards the development of high-throughput separation process [18–22]. Therefore, there is a great interest in the production of new stationary phases presenting such flow characteristics. As an example, the introduction of an additional set of very large pores in agarose beads (superporous agarose [23–26]), resulted in higher pore diffusivity and improved protein separation, in respect to conventional phases. Moreover, the same strategy, coupled this time with the very high mechanical resistance of crosslinked polystyrene [27], allowed the production of polymer-based materials able to perform separation at extremely high flow, still preserving high efficiency. On the other hand, the presence of huge pores might reduce the surface area, thus resulting in reduced binding capacity. This limitation may be overcome when brushes are grown from the pore surface [28] or nano-particles are used to cover the pore walls if they can be bound in a stable enough manner (*e.g.* by exploiting electrostatic interaction) [29]. In both cases, the effective surface area is increased, thus increasing the chromatographic performance.

In this work, a high-throughput method for producing highly porous and mechanically resistant polymer particles via reactive gelation is presented. The primary particles are aggregated through pure shear-induced aggregation by pumping the latex through a microfludizer, followed by post-polymerization. A comprehensive analysis of the effect of different features of the primary particles (architecture and size) on the morphology of the final material and its performance as chromatographic matrix is conducted, aiming at identifying the optimal operating conditions required to fulfill desired specifications. Pulse chromatography experiments using tracers of different sizes have been run on the produced materials and a comparison with commercially available polymer-based resins, both perfusive and non-perfusive, in nonretaining conditions, is presented.

2. Experimental

2.1. Materials

The following chemicals have been employed without further treatments: styrene (STY, Sigma Aldrich, 4-ter-butylcatechol as stabilizer, purity ≥99 wt%), divinylbenzene (DVB, Sigma Aldrich, technical grade, >80 wt%), sodium dodecyl sulfate (SDS, Sigma Aldrich, BioUltra ≥99 wt%), potassium persulfate (KPS, Fluka, purity ≥99 wt%), 2-2‘-azo(2-methylpropinitirile) (AIBN, Fluka, purity ≥98 wt%), sodium chloride (for analysis, Merck), sodium phosphate dibasic, sodium phosphate monobasic (Sigma Aldrich). Dextran standards with different molecular weights (Sigma Aldrich) have been used for packing testing. Ultra-pure grade water has been prepared by Millipore Synergy (Millipore, Billerica, MA, U.S.A.). Deionized water to be used for the polymerization reactions has been de-oxygenated by degassing under vacuum and subsequent saturation with nitrogen. Commercial resins POROS® 50 HS and Eshmuno® CPX have been purchased from ThermoFisher Scientific and Merck respectively and have been packed following the packing procedures provided by the manufacturers. The monolith CIM® r-Protein A DISK has been purchased from BIA Separations and used following the instructions of the producer.

2.2. Synthesis of the primary particles

The primary particles are produced via a semi-batch emulsion polymerization protocol. The procedure is divided into two steps: core and shell synthesis. In the first phase a hard, highly crosslinked (20 wt%) seed of nanoparticles is produced. In the second step, a much softer, slightly crosslinked (1 wt%) shell is grown around the previously formed particles. These two steps are done in series, thus feeding the monomer mixture required for the shell production directly after completion of the core synthesis. Several latexes have been produced with different particle architectures. Namely, the ratio between core and overall particle radii as well as the total particle sizes were varied. Details of all the recipes used in each synthesis are reported in Appendix A, while the applied general procedure is presented hereinafter.

2.2.1. Core synthesis

A mixture of water and surfactant (SDS) is initially charged into the glass reactor and the temperature set to 70 °C (Initial Charge, IC). Once this set-point is reached, a solution of water and initiator (KPS) is injected (Initiator Shot 1, IS1). In order to guarantee starved polymerization conditions, an emulsion of styrene, DVB, water, and surfactant is fed over the reaction time using an HPLC pump (Charged Feed 1, CF1). Moreover, a solution of water and KPS is continuously fed as well, as the total reaction time is longer than the half-life time of the initiator at the reaction temperature (Initiator Feed, IF). The conversion is frequently measured to ensure monomer-starved conditions.

2.2.2. Shell synthesis

A new monomer solution (Charged Feed 2, CF2), this time composed only of styrene and DVB, is then fed to the system right after terminating the core synthesis in the same reactor. The previous initiator feed is disconnected, while a shot of water and KPS is added to keep the reaction running (Initiator Shot 2, IS2). Again, the conversion is monitored to ensure starved operation. After the new monomer addition is complete, the synthesis is left running in batch until full conversion is obtained.

2.2.3. Latex characterization

During production, the latex is monitored in terms of particles size distribution (by dynamic light scattering (DLS) using a Zetasizer Nano ZS from Malvern) and monomer conversion (from the dry mass fraction of the sample) [30]. In the latter case, the latex is spread over quartz sand and analyzed at 120 °C in air using a HG53 Moisture Analyzer from Mettler-Toledo.