Characterization of BioResolve SCX mAb

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Abstract

BioResolve SCX mAb is a novel SCX material (patent pending) developed for the chromatographic separation of charge variants of mAb-based therapeutics. The monodispersed non-porous spherical polymer particles are coated covalently with a thin hydrophilic layer and grafted with SCX functionality. An analytical method for the quantification of the thin hydrophilic coating on BioResolve SCX mAb particles has been developed. The method uses boron tribromide cleaving reagent to break ether bond of the hydrophilic coating. In addition to the hydrophilic coating, ester-containing polymer on base particle is also cleavable by boron tribromide, which can interfere the quantification. The kinetics of ether and ester cleavage by boron tribromide was studied. The cleavage conditions have been optimized to reduce of the impact of ester cleavage. The cleavage products are quantified by ^{1}H-NMR. The factors impacting the reproducibility of the measurements were investigated and addressed. The quantity of hydrophilic coating determined by this method correlates very well with chromatographic results for non-specific binding testing and column backpressure.

The mechanism of much stronger dependence of the BioResolve SCX mAb column backpressure on particle diameter than the predicted by Kozeny-Carman equation was investigated. The interstitial porosity of the dried bed was examined to deconvolute the impact from the swelling of the coating. An empirical equation has been derived based on the experimental data and Coulomb's law. Reduced separation, the mean equivalent separation distance measured in units of the mean diameter of the particles in the packing bed, used in the equation is calculated from the interstitial porosity assuming that particles are perfectly ordered. The empirical equation describes the linear relationship between the reduced separation and electrostatic energy stored in the charged particle. In other words, particles with higher electrostatic energy repel each other further away, resulting lower packing bed density. Although the interstitial porosity increases with the electrostatic energy rather than the particle diameter, larger particles tend to carry more charge and have higher energy. The linear relationship will not hold if the reduced separation exceeds 1.10. The intrinsic reduced separation in the equation is the reduced separation for the packing bed of un-charged particles, which is independent of particle diameter. The interstitial porosity of the wetted bed is calculated by applying the swelling of the coating to the dried bed. The interstitial porosity drops a lot from the dried to wetted bed with slightly more decrease for smaller particles that are charged less with lower electrostatic energy and packed more densely. As predicted by Kozeny-Carman equation, column backpressure increases rapidly with the decrease of interstitial porosity. Good agreement has been achieved between the measured column backpressure and the pressure drop calculated per the empirical equation combined with the swelling calculations when the linear relationship holds.

The BioResolve SCX mAb column has been tailored to be applicable to HPLC systems in addition to UHPLC and UPLC systems with high performance and reproducibility.