论文

双孔色谱介质的制备和质粒DNA快速色谱纯化

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摘要 以甲基丙烯酸缩水甘油酯为单体,二甲基丙烯酸乙二酯为交联剂,环己醇与十二醇为有机致孔剂,200g/L的碳酸钙悬浮液为内水相(超孔致孔剂),用二次乳化法制备了(W/O)/W乳液,通过紫外光引发悬浮聚合生成两类孔型高分子微球(DEA-B).DEA-B孔径为双峰分布,范围分别为50~200nm和500~5000 nm. 其体积平均粒径、湿密度与不含超孔的微孔介质(DEA-M)接近.在流速为0.5mL/min(150cm/h)及相同的洗脱条件下,比较了所制备的微孔色谱介质填充的色谱柱与双孔色谱介质填充的色谱柱对5.4 kb质粒DNA的分离效果.结果表明,仅双孔介质色谱柱可以纯化质粒DNA,并且可以在1500cm/h的高流速下得到色谱纯的质粒DNA,证明该双孔色谱介质可以用于质粒DNA的高速分离.

关键词 两类孔型介质 双乳化 悬浮聚合 质粒 色谱

分类号

# PREPARATION OF A BIPOROUS POLYMERIC ADSORBENT FOR RAPID CHROMATOGRAPHIC PURIFICATION OF PLASMID DNA

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Abstract Preparation of a biperous polymeric stationary phase for high-speed flowthrough chromatography was reported Using calcium carbonate suspension(200 g/L)and organic solvent(mixture of cyclohexanol and dodecanol)as the porogenic agents, a novel hiperous matrix has been prepared by ultraviolet photo-polymerizing monomers of glycidyl methacrylate and ethylene glycol dimethacrylate by(W / O) / W emulsification. The matrix was modified with diethylamine to create an anion exchanger(DEA—B). It was found that there was two families of pores (micropores and superpores)in DEA-B,but there was only microperes in the microporous beads(DEA—M)prepared by O / W emulsification. The two families of pores in DEA-B were in the range of 50—200 nm and 500—5000 nm,respectively,while the pores of DEA—M were mainly in the range of 50—200 nm. The hydrated density and particle size of DEA—B were 1. 08 g / mL and 46. 3 µm,respectively,similar to those of DEA-M(1. 10 g / mL and 45. 2 µm). Operated at a mobile phase velocity of 150 cm / h,the DEA-B column could be used to purify a 5. 4 kb plasmid DNA. but the DEA-M column failed to do that at the same condition. Then,HPLC pure plasmid DNA was were obtained with the DEA-B column operated at 1500 cm / h. The results indicated that DEA-B column was promising for high-speed chromatography purification of plasmid DNA.

**Key words** Biporous matrix (W/O) /W emulsification Suspension polymerization Plasmid DNA Chromatography

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