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研究论文

动力学因素对液相色谱分离整体蛋白的影响

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摘要 依据液相色谱分离整体蛋白的效果与色谱柱柱长基本无关的事实, 研究了动力学因素对疏水相互作用色谱(HIC)分离整体蛋白的影响。首次提出了用于线性梯度洗脱条件下蛋白分离的“条件板高”(H)概念, 并将其用于动力学因素对分离整体蛋白的影响的表征。分别用常用的色谱柱和色谱饼对标准蛋白进行了分离, 绘制了类似于van Deemter的“条件板高”对流动相线速(u)的曲线图。发现对应于色谱柱最低“条件板高”的适合线速约为色谱饼的1/5～1/15, 且色谱饼的适合线速范围也较色谱柱宽得多。据此, 用装填有HIC填料的色谱饼(10 mm×20 mm i.d.)在12 min内便可完全分离7种标准蛋白。还用装填有HIC填料的色谱饼对重组人粒细胞集落刺激因子(rhG-CSF)进行了复性并同时纯化, 在50 min内, 仅用一步色谱法就可获得纯度≥97%的rhG-CSF, 其质量回收率为39%, 比活＞1×108 IU/mg。可以预计, 装填极细颗粒的刚性色谱填料的色谱饼可在高负荷条件下进行整体蛋白的高速和高分离度的分离、纯化并同时复性, 达到“三高”。

关键词 疏水相互作用色谱 蛋白分离 分离度 色谱饼 动力学因素 生物工程 粒细胞集落刺激因子

Effect of dynamic factors on the resolution of intact protein separation by liquid chromatography

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Abstract

Based on the fact that the resolution of intact protein separation is almost independent of column length, the effect on the resolution for intact protein separation causing from dynamic factors in hydrophobic interaction chromatography (HIC) was investigated. A concept of “conditional plate height” (H) for protein separation is firstly suggested for characterizing this effect for protein separation under linear gradient elution. Standard proteins were separated with conventional chromatographic column and chromatographic cake, and the plot of the H vs the linear velocity of mobile phase (u) was made, respectively. It was found that the obtained plot is similar to the conventional van Deemter Plot but has some differences. The optimized u corresponding to the minimum H was determined to be approximate 0.2 mm/s for the chromatographic cake and 1~3 mm/s for the conventional column. Furthermore, in comparison with the latter, optimized u value for the former has much broader range. Based on this fact, the resolutions and speeds for standard protein separation between the chromatographic cake packed with silica-base HIC material and the conventional column packed with soft HIC media were compared. The chromatographic cake (10 mm×20 mm i.d.) was found to perform a complete separation of seven standard proteins in 10 min, while with the latter (55 mm×12 mm i. d.) only five standard proteins can be completely separated in 140 min, even though the sample load for the former having bed volume of 3.14 mL, five times of that of the latter. The HIC chromatographic cake was also employed for the renaturation with simultaneous purification of the recombinant human granulocyte colony stimulating factor. The obtained purity was ≥97%, mass recovery was 39%, specific bioactivity was 1×108 IU/mg with only one step HIC in 50 min. It would be expected that when a kind of packings having very small particle size is packed into a chromatographic cake with diameter to be greater than its thickness and is employed to separate, and/or renature proteins, a result of high speed and high resolution with simultaneous renaturation under high protein loading (“three H” target) could be obtained.

Key words hydrophobic interaction chromatography protein separation resolution chromatographic cake dynamic factor biotechnology granulocyte colony stimulating factor

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