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厦门大学

硕 士 学 位 论 文

毛细管填充柱制备新工艺与电色谱研究

Single particle fritted capillary columns and their performance in electrochromatography

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摘 要

毛细管电色谱(CEC)是毛细管电泳与高效液相色谱相结合而发展起来的一种高效、快速的微分离分析技术。对于带电样品，电色谱具有电泳和色谱的双重分离作用。在 CEC 中，由于以电渗流驱动流动相，流型为塞型流，消除了以压力驱动的液相色谱中抛物线型流的峰展宽效应对柱效的影响。而且，CEC 不存在液相色谱中的压力问题，可以使用粒径较小的色谱填料以及较长的填充长度，使分离柱效大为提高。虽然 CEC 有着众多的优势，但是电渗流的稳定性却不及压力流，所以制备出强健稳定的毛细管色谱柱是非常重要的。

毛细管填充柱是CEC使用最为广泛的柱型。但是近十年来，CEC的工作都是围绕着另外一种柱型——毛细管整体柱开展的。与传统的毛细管填充柱相比，毛细管整体柱具有无需制作柱塞、柱床均一性好、通透性好、结构稳定、成本低廉等特点。但是依托于HPLC的填料工业，毛细管填充柱在固定相的选择上有着巨大的空间，而且毛细管填充柱通常可以获得优良的柱效。如果可以解决困扰毛细管填充柱的柱塞问题，相信其会有巨大的发展潜能。

传统烧结法制备的柱塞会带来一系列的问题，如柱床不均一性增加、通透性和重现性差、色谱峰展宽、气泡的产生、带电分析物的吸附等。所以急需新的柱塞工艺来解决毛细管填充柱的制备问题。单颗粒塞法是一种纯物理的柱塞方法，基于“基石效应”可以形成稳定的柱塞。柱塞长度短、通透性和重现性好、无需烧结是单颗粒塞法的优势。

本论文主要内容如下：

第一章综述了CEC和毛细管填充柱的研究现状，提出本论文的选题依据和研究目标。

第二章系统评价了单颗粒塞法制备的毛细管填充柱的稳定性。使用单颗粒塞法制备了十根毛细管填充柱，以硫脲和苯系物为样品，评价柱与柱之间的重现性。以戊苯为例，在不同电压下，十根毛细管柱保留因子、塔板高度、峰面积和不对称因子有着良好的重现性；最优柱效达到了150000/m。在整个实验过程中，没有

使用加压和温控装置，并且没有产生气泡而影响分离过程。因此，单颗粒塞法制备的毛细管填充柱有着良好的重现性和稳定性，是一种优良的毛细管填充柱的制备方法。

第三章首先对单颗粒塞法制备毛细管填充柱的过程进行了改良，使用缓冲溶液代替普通有机溶剂作为匀浆溶液。使填充过程和预平衡过程合二为一，大大缩短了从制备毛细管柱到上机运行的时间，是一种快速填充法。通过对比实验证明，使用缓冲溶液为匀浆溶剂填充的毛细管柱在柱效与稳定性上，和普通有机溶剂填充的并没有差异。同时，使用快速填充法填充的毛细管柱对中性、带电以及生物样品进行了分离，均获得了良好的分离效果。其次，使用单颗粒塞法成功制备了毛细管单级柱。如果使用传统的烧结法，制备一根毛细管单级柱至少需要烧结五次，制柱成功率极低。我们不仅成功制备出了单级柱，还对其性能进行了初步的考察。

第四章使用单颗粒塞法成功制备了聚合物基质填料的毛细管填充柱。传统的烧结法需要对毛细管柱进行高温烧结，如果使用聚合物基质的填料，高温会使填料损毁，从而无法使用。我们所使用的这种填料除了是聚合物基质以外，还是一种 SCX 的灌流填料。填料上连有的磺酸基在极低的 pH 条件下依然可以提供稳定的电渗流，以保证 CEC 的顺利进行。灌流填料与传统填料相比，不仅具有纳米尺度的扩散孔，还具有微米尺度的贯穿孔。贯穿孔的存在允许流动相直接进入填料颗粒的内部并通过，产生孔内电渗流。孔流引起的对流能够减小固定相内传质阻力，不仅能够提高分离效率，而且又能增加 EOF。我们使用这种填料制备的毛细管柱对标准多肽和 BSA 酶解物进行了电色谱分离，获得了很好的分离效果。

第五章总结了本论文的研究工作，探讨了工作中的不足，并对将来的研究工作进行了展望。

关键词：电色谱；柱技术；毛细管填充柱；柱塞

Abstract

Capillary electrochromatography (CEC) is a hybrid of capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC). Ionic analytes can be separated in CEC, combining two separation mechanisms: chromatography and electrophoresis. In CEC, the plug-like flow profile in EOF reduces dispersion of the band of solute as it passes through the columns compared to parabolic pressure-driven flow in HPLC. The electrically driven flow rate is independent of particle diameter and column length so that, in principle, smaller particles and longer columns can be used, further increasing column efficiency. Although EOF has lots of advantages, it is not as stable as pressure-driven flow and fabricating robust electrochromatographic columns are very important.

Packed column is a kind of widely used electrochromatographic columns. The past ten years or so have seen a great number of CEC works performed on monolithic columns, due to simplicity in column fabrication, good permeability and stable structure. The rich library of HPLC packing material provides a wide range of choices for chromatographic separations performed in electrodriven mode. And packed columns can obtain higher efficiency. If the problem of column fritting could be solved, we believe that packed columns will get broad development space.

The sinter-fritting method in column fabrication highlights several problems, such as introduced nonuniformity to the chromatographic bed, poor porosity and reproducibility, increased band broadening, bubbles formation and adsorption of polar analytes onto the frits. So developing a simple and robust fritting technology is extremely urgent. This is due to the unique property of single particle fritting technology, where no heating is required during the whole preparation process. In fact, the fritting is based on keystone effect. The characteristics of single particle fritting are the heat-free fritting process, good porosity and reproducibility and short frit length.

This dissertation including following contents:

In Chapter one, the research status of CEC and packed columns were reviewed, especially focusing on the fabrication of packed columns, and then the research proposal was made.

In Chapter two, we investigated a single particle fritting technology, to immobilize particulate chromatographic material inside capillary tube in a sinter-free manner to produce robust capillary columns. To evaluate the performance of single particle fritted columns, a batch of ten columns was prepared. Single particle fritted columns present significantly improved column-to-column reproducibility (n = 10) in peak efficiency, retention factor, peak area and asymmetry. The excellent median peak efficiency reached 150000 plates m^{-1}. In the experiment, each single particle fritted column was subjected to repeated runs for a long term without thermostatting and pressurisation. During this course, no bubbles generation or current failure was observed. Single particle-fritted capillary columns have high stable and efficient CEC separations. The single particle fritting technology was a kind of excellent fritting technologies.

In Chapter three, first, we directly used CEC mobile phase as the packing solvent and prepared capillary columns based on single particle fritting technology. In this new strategy, column packing and conditioning is conducted in one run. The time of column preparation reduced sharply. We evaluated the performance of the single particle fritted columns packed with a CEC mobile phase by comparison with the columns packed with organic solvent. The experiment indicated that packing columns with CEC mobile phase did not influence the columns' efficiency, while the preparation throughput is significantly improved. The rapid prepared columns were demonstrated to be efficient for CEC separations of neutral, charged and biomolecules with excellent peak efficiencies. Then, we fabricated simplex capillary columns successfully based on single particle fritting technology. If simplex columns were fabricated by sinter-fritting method, the columns need heat five times at least. The success rate of fabricating columns was quiet low. We fabricated and evaluated them preliminary.

In Chapter four, we fabricated polymer particles columns successfully based on single particle fritting technology. If these columns were fabricated by sinter-fritting method, polymer particles were damaged by hot heating. These packing materials were not only polymer particles but also perfusive ones with strong cation exchange (SCX). These SCX groups exhibited a high and stable EOF at a low pH. Perfusive particles with increased pore diameters were employed such that the double layer was not overlap and produced intraparticle EOF. In such a case, solutes experience two distinct regions: interparticle and intraparticle regions. With these stationary phases, a large portion of the total flow appears to be through the pores of particles, thereby increasing the separation efficiency through a further decrease of the flow inhomogeneity and through enhancement of the mass transfer kinetics. We used capillary columns with these stationary phases to separate peptide standard mixture and BSA enzymatic hydrolysate in CEC, and obtained excellent separating effects.

Chapter five summarized achievements of this dissertation as well as remaining flaws, and also outlines the future proposed research work on packed columns.

Keywords: electrochromatography, column technology, packed column, frit