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研究快报

基于固定化pH梯度整体材料的芯片自由流等电聚焦电泳模式的构建

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摘要 芯片自由流电泳对于来源稀少的重要生物样品的连续预分级和微制备具有重要的意义。本文在自由流芯片的微分离腔内,通过原位光引发聚合反应制备了聚丙烯酰胺整体材料,并进行了pH梯度的固定化,从而构建了基于固定化pH梯度整体(M-IPG)材料的芯片自由流等电聚焦模式(μFF-IEF)。利用该新型分离模式,实现了异硫氰酸荧光素(FITC)标记的最小等电点相差0.33的甘氨酸、脯氨酸和赖氨酸混合物的分离,且分离结果优于传统的μFF-IEF。实验结果表明,通过发展基于M-IPG材料的μFF-IEF模式,不仅可以避免在缓冲溶液中添加两性电解质对后续采用其他模式分离和质谱鉴定的干扰,而且可以获得较高的分离和富集能力,有望在微量样品的连续分离和制备方面发挥重要作用。

关键词 芯片自由流电泳 等电聚焦 固定化pH梯度 整体材料 氨基酸

Microchip free flow isoelectric focusing with immobilized pH gradient on monolithic materials

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Abstract

Microchip free flow electrophoresis (μFFE) is a significant microscale technique for the continuous pre-fractionation and the preparation of valuable biological samples. In our recent work, monolithic polyacylamide (PAM) materials were polymerized in microchamber by ultraviolet (UV) initiated polymerization. With the further immobilization of a stable pH gradient on the monolith, a novel microchip free flow isoelectric focusing (μFF-IEF) with monolithic immobilized pH gradient (M-IPG) materials was developed, by which fluorescein-5-isothiocyanate (FITC) labeled glycin, proline and lysine, with a minimum pI difference of 0.33 units, were well separated with a resolution higher than that performed by traditional μFF-IEF. Our experimental results demonstrate that by μFF-IEF with M-IPG, not only the interference of mobile carrier ampholytes in buffer, usually indispensable in traditional μFF-IEF, on the further separation by other techniques and the identification by mass spectrometry (MS) could be avoided, but also the improved resolution and detection sensitivity could be obtained compared with traditional μFF-IEF. Therefore, such a novel technique might be promising in microscale consecutive separation and preparation of samples.

Key words microchip free flow electrophoresis isoelectric focusing immobilized pH gradient monolithic materials amino acids

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