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硕 士 学 位 论 文

固载肝素导电亲和膜的制备及性能研究

Preparation and Property Research of heparin immobilized
affinity electromembrane

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摘要

本文将导电聚合物及其带有活性官能团的衍生物引入亲和膜分离领域, 分别通过掺杂和共价偶联的方式将配基肝素固载在膜上, 构建了两种具有导电能力的亲和膜, 并比较其在电刺激和非电刺激条件下的吸附、洗脱、再生性能。主要研究内容如下:

1. 掺杂肝素导电亲和膜的制备和性能表征

以聚醚砜(PES)微孔滤膜为基膜, 采用模板法, 通过化学氧化的方式将导电高分子聚吡咯(PPy)沉积到基膜表面和膜孔中。利用聚吡咯的可掺杂特性将配基肝素固载到膜上, 制备具有理想形貌和物化性能的掺杂肝素的导电亲和膜。膜的电导率为 1.73×10^{-4} S/cm, 肝素配基的含量为 0.894 mg/g 膜。

2. 掺杂肝素导电亲和膜的吸附、洗脱、再生性能研究

以凝血酶为目标蛋白, 采用恒电位法对膜施加电刺激, 考察在电刺激和非电刺激条件下吸附速率的变化以及电位值、温度、初始浓度对平衡吸附量的影响。结果显示电刺激能够显著的提升导电亲和膜的吸附速率和吸附容量; 膜对凝血酶的平衡吸附量随着温度和电刺激强度(电位值)的增加而增加; 膜对凝血酶展现了良好的特异吸附性; 电刺激和非电刺激下的吸附均符合 Freundlich 等温吸附模型。对掺杂肝素导电亲和膜吸附凝血酶后的洗脱和再生性能进行研究。实验结果显示采用促溶剂 2.0 M NaSCN 溶液能够对膜上吸附的凝血酶进行高效的洗脱, 洗脱率达到 93.1%; 膜在经历了 5 次吸附—洗脱循环后吸附能力仅仅减小了 4%, 表明掺杂肝素的导电亲和膜具有良好的可重复利用性。

3. 共价偶联肝素导电亲和膜的制备和表征

以尼龙微孔滤膜为基膜, 采用模板法, 通过化学氧化将聚(吡咯-吡咯-3-羧酸)共聚物沉积到基膜表面和膜孔中。利用膜上引入的羧基首先与间隔臂乙二胺反应, 再利用乙二胺上的氨基与配基肝素分子反应, 制备出具有良好形貌的共价偶联肝素的导电亲和膜。膜的电导率为 3.5×10^{-7} S/cm, 肝素配基的含量为 1.897 mg/g 膜。

4. 共价偶联肝素导电亲和膜的吸附、洗脱、再生性能研究

在与掺杂肝素的导电亲和膜相同的条件下考察所制备膜的吸附性能, 实验结

果显示电刺激能够显著的提升导电亲和膜的吸附速率和吸附容量; 吸附速率和吸附容量随着温度的增加而增加, 随着电刺激强度先增加后减小; 膜对凝血酶展现了良好的特异吸附性; 电刺激和非电刺激下的吸附同样符合 Freundlich 等温吸附模型。对共价偶联肝素导电亲和膜的洗脱再生能力进行研究, 采取 2 M NaSCN 为洗脱剂, 结果显示该洗脱剂能够对膜上所吸附的凝血酶进行高效的洗脱, 洗脱率为 93.3%; 经过 5 次吸附-洗脱循环后吸附能力仅仅减小了 3%, 表明共价偶联肝素的导电亲和膜具有良好的可重复利用性。

5. 对电刺激促进吸附的机理进行分析; 比较了两种膜的吸附性能, 结果表明采用共价偶联方式制备的导电亲和膜的吸附性能更优。

关键词: 导电亲和膜; 电刺激; 掺杂; 共价; 凝血酶

Abstract

In this paper, conductive polymer and its ramification with reactive functional group were introduced into the field of affinity membrane chromatography. Two kinds of novel heparin-immobilized affinity electromembranes were constructed by doping and covalently coupling, respectively. The affinity adsorption, elution, regeneration properties of these two kinds of affinity electromembranes, with and without electrical stimulation, were researched. The main research contents were as follows:

1. Preparation and characterization of heparin-doped affinity electromembrane

The polyether sulfone (PES) microporous membrane was chosen as a support matrix, using the template method, then, conductive polymer polypyrrole (PPy) was deposited on the surfaces and inner pores of the PES membrane by chemical oxidation. Because conductive polymer has the characteristic of doping, the ligand heparin could be incorporated into its skeleton during polymerization. The constructed affinity membrane has the ideal morphology and physicochemical properties. The conductivity value of the membrane was 1.73×10^{-4} S/cm. The heparin content of the membrane was 0.894 mg/g membrane.

2. Adsorption, elution and regeneration properties of heparin-doped affinity electromembrane

Thrombin was chosen as the target protein. All of the electrical stimulation experiments were conducted at the constant potential. Adsorption rate and the effects of potential value, temperature and initial concentration of thrombin on the equilibrium adsorption were investigated. The results showed that the electrical stimulation can significantly enhance the adsorption rate and adsorption capacity; adsorption rate and adsorption capacity increase with temperature and electrical stimulation intensity (potential value); the heparin-doped affinity electromembrane has low nonspecific adsorption of foreign protein and high specific adsorption of thrombin.; and the adsorption both with and without electrical stimulation were lined with the Freundlich adsorption isotherm model well. Investigating the elution and

regeneration properties of heparin-doped affinity electromembrane, the results showed the thrombin adsorbed on the membrane can be efficiently eluted by 2.0 M NaSCN and the eluted ratio can be reached 93.1%; after five adsorption-elution cycles were repeated, adsorption capacity of heparin-doped affinity electromembrane decreased by only 4%. The heparin-doped affinity electromembrane possesses excellent regeneration properties.

3. Preparation and characterization of heparin covalent coupled affinity electromembrane

The nylon microporous membrane was selected as support matrix. Using the template method, poly(pyrrole-pyrrole-3-COOH) (P(Py-Py-3-COOH)) copolymer was deposited on the surfaces and inner pores of nylon membrane by chemical oxidation. First, the space arm, ethylenediamine, was attached to the carboxyl of membrane, and then the heparin was immobilized on the membrane by coupling with the amino group of ethylenediamine. The constructed affinity membrane has the ideal morphology and physicochemical properties. The conductivity value of the membrane was 3.5×10^{-7} S/cm. The heparin content of the membrane was 1.897 mg/g membrane.

4. Adsorption, elution and regeneration properties of heparin covalent coupled affinity electromembrane

The adsorption properties of heparin-coupled affinity electromembrane were investigated under the same condition with heparin-doped affinity electromembrane. The results showed that the electrical stimulation can significantly enhance the adsorption rate and adsorption capacity; adsorption capacity increased with the temperature; adsorption capacity increased with the electrical stimulation intensity (potential value) at the early stage, then decreased; and the heparin covalent coupled affinity electromembrane has high specific adsorption of thrombin. The adsorption, both with and without electrical stimulation, was lined with the Freundlich adsorption isotherm model well. Investigating the elution and regeneration properties of heparin doped affinity electromembrane, the results showed the thrombin adsorbed on the membrane can be efficiently eluted by 2.0 M NaSCN solution, and the eluted ratio can be reached 93.3%. After five adsorption-elution cycles were repeated, adsorption

capacity of heparin-coupled affinity electromembrane decreased by only 3%.

5. The mechanism of electrically facilitated adsorption was analyzed and the affinity properties of the heparin-doped and heparin-coupled affinity electromembrane were compared. The results showed the adsorption properties of heparin-coupled affinity electromembrane were better than the heparin-doped electromembrane.

Keywords: Affinity electromembrane; Electrical stimulation; Doping; Covalent coupling, Thrombin

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第一章 绪论

1.1 亲和膜技术研究进展

生物大分子如多肽、蛋白质、核苷酸等的分离、提取、精制是生物工程的一个重要的组成部分，所需费用往往占到总成本的 50%或更多，因此，对生物大分子分离纯化进行研究是一项非常有意义的课题。对于天然的或者通过人工发酵获得的生物大分子，在初始阶段往往都包含着复杂的、种类繁多的组分，通常都要经过一系列的分离纯化手段才能得到能够用于实际应用的较高纯度的产品。在纯化过程中往往是要在很大体积的低浓度溶液中提取出少量的生物活性物质^[1]，因此，在分离过程中要求尽量避免过于繁琐的工艺以保持目标物质的活性，达到对目标物质的有效分离。亲和膜技术由于兼具膜分离速度快、可连续操作和亲和分离技术高选择性、特异性的特点^[2]，是用于生化分离和蛋白质纯化的一个非常重要的技术手段。

1.1.1 亲和色谱分离过程

亲和色谱是利用生物分子之间的特异性相互作用力，将其中一种生物分子固定作为配基用于从复杂体系中特异性的分离另一种生物分子的技术。传统的亲和柱色谱一般是以微球型或无定性的介质，如葡聚糖、琼脂糖、大孔硅胶、陶瓷等为填料。研究表明该种色谱能够成功的用于复杂组分中目标物质的分离，但由于溶液中待分离物质的分离速度受其在亲和介质颗粒之间的扩散速度、亲和介质内的扩散速度以及轴向的扩散速度影响，如图 1-1^[3]，待分离物质的扩散路径需要穿过扩散的沟路以及介质的微孔，扩散路径和时间都非常长^[4]，这大大的降低了待分离物质和亲和色谱配基之间的质量传递效率，造成分离过程压降大、处理量小和分离速率低。通常正是由于亲和色谱过程的耗时、单位时间处理量低的缺点导致在亲和纯化分离过程中很多生化物质的降解和失活^[5]。

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