

表面亲水性粒度单分散交联 PMMA 树脂的合成及表征*

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摘 要 用种子溶胀聚合方法,合成出了粒度单分散的交联聚甲基丙烯酸甲酯微球.将微球通过水解,使其转化为表面带羧基的树脂.分别用多糖化合物 Dextran 和 DEAE-Dextran 对水解树脂表面进行包覆涂层,然后用 *n*-丁二醇双环氧丙醚分别进行交联,制备出两种表面带高交联多糖覆盖层的树脂.以两种改性树脂为填料,以人血清蛋白为试样,用 HPLC 方法对树脂的亲水性能进行了表征.研究表明,两种改性树脂均有很好的亲水性,蛋白质的回收率分别在 97% 和 99% 以上,并具有良好的机械强度和化学稳定性.

关键词 颗粒单分散微球,表面亲水性树脂, HPLC 填料

粒度单分散的高聚物微球,作为高效液相色谱(HPLC)柱填料,具有优异的分离性能,尤其对于提高色谱柱效和穿透性十分有利.目前所报道的这类填料的基质树脂,基本上都是使用种子溶胀聚合方法而制得的交联聚苯乙烯和交联聚甲基丙烯酸酯类微球^[1~4].由于这些树脂大都是呈现较强的疏水性特征,为了能制备出适合于水溶性生物分离的 HPLC 填料,对于树脂表面进行亲水化改性处理是非常必要的.本文简要介绍了新合成的以颗粒单分散交联聚甲基丙烯酸甲酯微球为基质、表面交联覆盖不同多糖类化合物的两种 HPLC 填料,并对其亲水性能进行了色谱表征.

1 颗粒单分散基质树脂的合成

颗粒单分散基质树脂是采用种子溶胀聚合方法而制得的.所使用的种子是基于分散聚合的 2 μ m 粒度单分散的线性聚苯乙烯(PS)微球.所使用的单体为甲基丙烯酸甲酯(MMA),交联剂为二乙烯苯(DVB),引发剂为过氧化苯甲酰(BPO),这些原料均以常规方法予以纯化.

将种子 PS 微球分散在含有稳定剂聚乙烯醇和表面活性剂十二烷基硫酸钠的水溶液中,然后加入预先在水相介质中被乳化的 MMA、DVB、BPO 混合乳液,在室温和搅拌条件下溶胀 6h.溶胀完毕后将体系加热到 70℃ 进行聚合.反应 24h 后将其产物充分洗涤,即可得到颗粒单分散的交联聚甲基丙烯酸甲酯(PMMA-DVB)微球.

树脂的粒度与表观形态用扫描电镜进行观

测,并按下式统计树脂的平均粒度($\bar{D}$)和粒度分散系数(ϵ):

$$\bar{D} = \sum_{i=1}^n D_i / n \quad (1)$$

$$\epsilon = \left[\sum_{i=1}^n (D_i - \bar{D})^2 / (n - 1) \right]^{1/2} / \bar{D} \quad (2)$$

式中 D_i 和 n 分别表示被测微球的粒径和数目.实验证明调节聚合的原料配比,可制得在 5~15 μ m 范围内粒度呈现单分散的 PMMA-DVB 树脂,聚合物微球的 ϵ 值均 < 2%.从图 1 所给出的扫描电镜照片中,可以直接观察到这类基质树脂的颗粒均匀性和表观形态.树脂的交联度可在 40% 以内,按其使用要求在合成中任意调节.

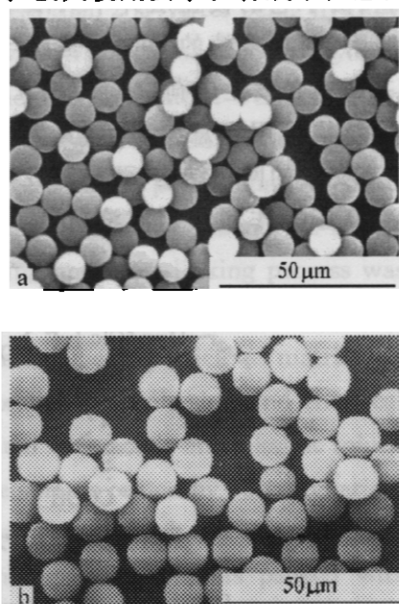
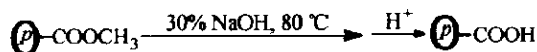


Fig. 1 SEM pictures of PMMA-DVB resins of different sizes

2 树脂表面的化学改性

欲将水溶性多糖化合物包覆交联在基质树脂的表面,首先需要对微球表面进行亲水化预处理,以增强覆盖层与微球界面之间的相容性.对于 PMMA-DVB 微球来说,最简便的预处理方法是直接进行水解,生成表面含羧基的树脂.



将两种多糖类化合物,即葡聚糖(Dextran)和二乙氨基葡聚糖(DEAE-Dextran)配成适当浓度的溶液,对水解树脂表面用浸渍方法包覆涂层;然后以 *n*-丁二醇双环氧丙醚(BDGE)为交联剂,利用其环氧基与多糖羟基之间的反应,对涂层树脂进行交联,生成两种表面带有高交联网络结构的多糖覆盖层树脂(分别称为改性树脂 I 和改性树脂 II).

为了使树脂的交联覆盖层更加均匀,涂层实际是分为两次或三次进行的,总的多糖覆盖量约为基质树脂的 7%(W/W)左右. BDGE 作为交联剂,不仅能有效地增强树脂覆盖层的稳定性,而且反应所生成的羟基化醚链链段也有助于提高树脂的亲水性能. 实验证明,这种高交联的覆盖层结构具有良好的物理和化学稳定性. 从两种改性树脂的结构设计来判断,树脂 II 的均匀性和稳定性似乎更好一些,由于它的覆盖层并不只限于两相之间的物理吸附,而且多糖的 DEAE 基团与树脂的—COOH 基团也会发生电荷之间的相互作用,该树脂的表面结构可用示意图表示(见图 2). 总的来说,由于高交联的网络结构,两种改性树脂均具备可以经受高压操作和酸碱溶液(pH 1~14)处理的特性.

3 改性树脂的 HPLC 性能表征

人血清蛋白(HSA)在不同 pH 值缓冲液体系中的分子构象是不同的,其疏水效应随缓冲体系 pH 值的增加而减少. 这种行为特征表现在色谱上则因其填料的亲水性能不同,HSA 被洗脱的能力将有明显差异. 在不同 pH 值的淋洗条件下,该蛋白样品的回收率直接与树脂的亲水性能相关联. 如果在 pH 值较低的水相淋洗液中,HSA 能被很好的洗脱,说明填料的亲水性能良好;相反如果升高淋洗液 pH 值之后,该样品的回收率仍然较低而需要加入有机溶剂(如乙二醇)才能完全被洗脱,则说明填料有一定的疏水性吸附作用. 所以可用 HSA 作为分子探针,来表征填料亲水性能.

Table 1 HSA recovery on column packed two modified resins by different mobile phases

Column packing	Recovery percent of HSA(%)		
	Mobile phase A→	Mobile phase B→	Mobile phase C
Resin I	97.52	0.06	2.42
Resin II	99.44	0.56	0

Column:8mm I. D. × 100mm; Mobile phase A:0. 1mol/L phosphate + 0. 15mol/L NaCl, pH = 3. 8; Mobile phase B: same as A, pH = 7. 0; Mobile phase C: B + 10% glycol; Flow rate: 1. 0mL/min; Detection: UV-254nm

表 1 给出了 HSA 在两种改性树脂填充柱上的回收率,其淋洗方式是一次进样用 A、B、C 三种不同的流动相依次进行洗脱,前一种流动相洗脱过后的剩余溶质组分被后一种流动相继续洗脱. 结果表明在改性树脂 I 上,用流动相 A 可使 97.52% 的 HSA 被洗脱,而难以被 A、B 两种流动相洗脱的样品只占 2.42%. 在改性树脂 II 上 99.44% 的 HSA 可被流动相 A 洗脱,而且无需流

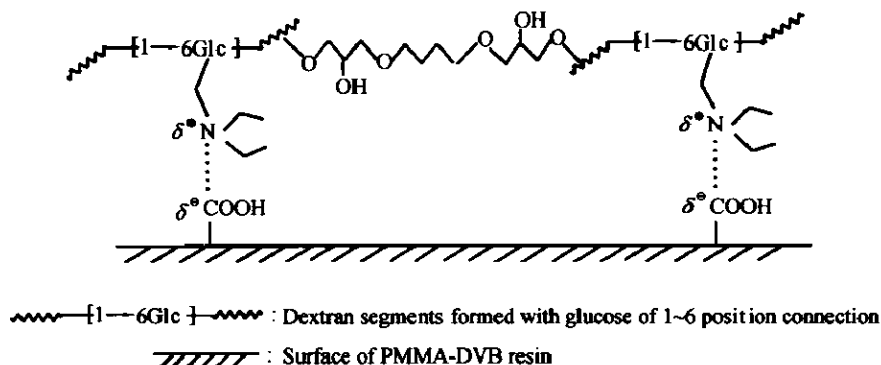


Fig. 2 Surface structure of resin- II

动相 C, 只用流动相 A、B 就能达到 100% 的洗脱。
这充分说明两种改性树脂具有优异的亲水性能。

此外, 从色谱动力行为来看, 两种树脂均已具备高
柱效、高渗透性填料的特性。

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PREPARATION AND CHARACTERIZATION OF MONOSIZED CROSS-LINKED PMMA RESINS WITH HYDROPHILIC SURFACE

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Abstract This paper presents the novel monosized resins with hydrophilic surface coated polysaccharides, including synthesis of the matrix beads, modification of the particle surface and characterization of HPLC properties of the modified products. The monosized matrix beads based on poly(methyl methacrylate-co-divinylbenzene) (PMMA-DVB) were synthesized by seed swelling polymerization. The linear polystyrene particles as seeds were swollen by an emulsifying mixture of monomer, cross-linker and initiator in the aqueous solution containing stabilizer at the room temperature, then the swelling products were polymerized in the same solution system at 70°C, finally the polymerization products were fully washed with water. The particle parameters and apparent morphology of the matrix beads were characterized by scanning electron microscopy (SEM). The results show that the polymerization method is suitable to the preparation of monosized PMMA-DVB beads with particle diameters of 5~15 μm , the particle dispersion coefficient of the polymerization products with different cross-linking densities is less than 2%. After being hydrolyzed with a 30% (W/V) NaOH solution at 80°C, the PMMA-DVB beads were transformed into the hydrolyzed resins containing carboxylic groups on the particle surface. Dextran and DEAE-Dextran were coated respectively by the impregnation method, and then were cross-linked respectively with 1,4-butanediol diglycidyl ether as cross-linker. Two new modified resins with covering layer of cross-linked polysaccharides on the particle surface were obtained. The polysaccharides content of the modified resins was about 7% (W/W) of the matrix beads. In order to get a uniform covering layer, the coating and cross-linking process was generally divided into two or three times to accomplish with same operation method. SEM observation showed that the physical morphology of the two modified resins still kept monodisperse particle as matrix beads. Owing to highly cross-linking network structure, the modified resins exhibit good physical and chemical stability. The particles can withstand high pressure operation, can be treated with acidic and alkaline solutions (pH = 1~14). Monodisperse particle size, good particle rigidity and the surface coated by polysaccharides make the modified resins as HPLC packings possessing high column efficiency, high permeability and excellent hydrophilic properties. The experiments show that on chromatographic columns packed with the two modified resins, human serum albumin possesses good elution behavior in elution system of phosphate buffer (pH = 3.8), the protein recovery is over 97% and 99% respectively.

Key words Monosized beads, Resin with hydrophilic surface, HPLC packings