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Preparation of Cibacron Blue F3GA Bonded Poly (styrene-divinylbenzene) (PSDVB) Microbeads Used for High Performance Affinity Chromatography

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Abstract: Rigid PSDVB microbeads have been modified with poly(vinyl alcohol) (PVA) adsorbed on their surface to produce an affinity medium. Then Cibacron Blue F3GA was covalently attached to the supports. The initial concentration of PVA has effect on the adsorption of PVA. The non-specific interaction of bovine serum albumin (BSA) on the microbeads decreases with the PVA adsorbed. The pH stability test shows that the affinity medium is stable up to pH 11.0. And it has specific interaction with lysozyme, but not with pepsin.

Keywords: PSDVB microbeads, PVA, affinity medium, Cibacron Blue F3GA.

Recently high performance liquid affinity chromatography (HPLAC) has developed very quickly. HPLAC combines the speed and resolving power of HPLC with biological specificity of affinity chromatography and has been widely used as an analytical tool in biochemical research.

Cibacron Blue F3GA is the most widely used reactive triazine-based dye which has specific interaction with pyridine nucleotide-dependent dehydrogenase, kinase, blood proteins and other proteins and enzymes[1]. It is a suitable HPLAC ligand because of its reactivity and chemical stability.

In this study we used **poly(styrene-divinylbenzene)(PSDVB)** microbeads prepared in our laboratory as matrix and **Cibacron Blue F3GA** as ligand, to produce an affinity medium. Experiments have been carried out to determine the effect of **PVA** concentration on the adsorption of **PVA** on **PSDVB** microbeads, to investigate the pH stability of this medium and by using **BSA** to determine the non-specific interaction between the **PSDVB** and proteins.

Experimental

The **PSDVB** microbeads were dispersed in **PVA** solution and stirred at room temperature for 48h. The adsorbed **PVA** layer was cross-linked at room temperature using **glutaraldehyde** for 16h.

PSDVB/PVA microbeads were mixed with **Cibacron Blue F3GA** solution, then 1g of **NaOH** was added. The medium was heated for 4 h at 80℃. The microbeads were filtered and washed with distilled water and methanol several times until all unbonded dye was removed.

The **Cibacron Blue F3GA** modified **PSDVB** microbeads were suspended in different buffer solutions at different pH values and shaken for 24 h. The microbeads were centrifuged and the supernatant was examined for ligand leakage spectrophotometrically at 280nm.

BSA was dissolved in **CH_3COOH-CH_3COONa** buffer solution (pH 5.0) containing **NaCl** (ionic strength 0.01). The **BSA** adsorption capacity was determined by measuring the initial and finial concentration of **BSA** in buffer solution spectrophotometrically at 280nm.

The triazine immobilized **PSDVB** were packed into stainless-steel columns (4.6× 40 mm I.D.) using the downward slurry technique in water. All chromatographic procedures were performed with HITACHI 635A high performance liquid chromatographic equipment equipped with JASCO UVIDEC-100-Ⅳ UV spectrophotometer.

Results and discussion

There are some hydrophobic regions on **PVA** molecule, so it was thought that **PVA** was adsorbed to the **PSDVB** surface as a result of hydrophobic interaction[2]. It can be seen that the amount of **PVA** first increased with increasing initial **PVA** concentration, then reached a plateau. This plateau means that PVA adsorption on **PSDVB** microbeads is typically Langmuir type monolayer adsorption. And the maximum PVA adsorption on **PSAVB** microbeads reached 247mg/g.

Figure 1. PVA adsorption on **PSDVB** microbeads

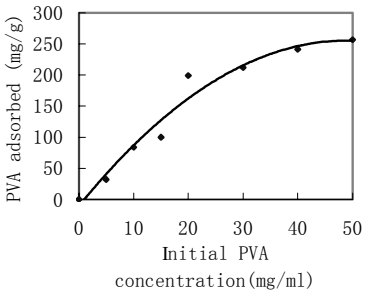
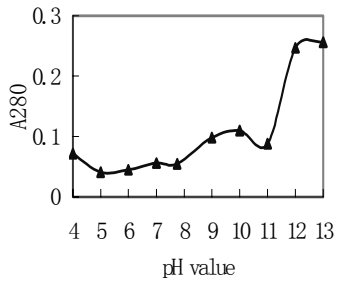


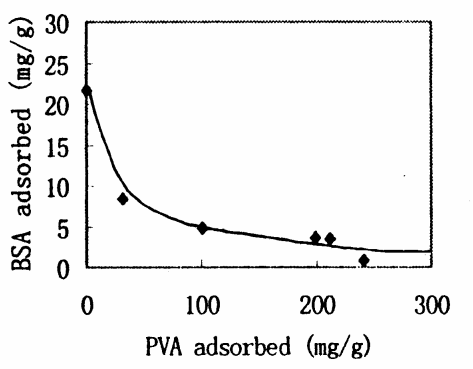
Figure 2. pH stability test



The pH stability test experiments indicated that there were no significant dye leakage from pH 4.0 to 11.0, but at pH 12.0 **Cibacron Blue F3GA** slowly leached from the surface. In spite of the dye leakage at pH 13, the medium is still stable in this situation, while silica gel cannot be used when the pH value is higher than 8.2[3].

High **BSA** adsorption capacity (up to 21.6mg/g) was achieved with **PSDVB** microbeads. This is as expected because of the hydrophobic force between the **PSDVB** and **BSA**. With absorbed **PVA** on the **PSDVB** surface, the hydrophilic groups of **PVA** mask the hydrophobic surface of the microbeads, which decrease the **BSA** non-specific adsorption. The greater the amount of **PVA** coated, the less the **BSA** adsorbed is, which is shown in **Figure 3**. It can be explained that the adsorption of **PVA** is monolayer adsorption and there are less hydrophobic regions on the **PSDVB/PVA** microbeads surface with increasing amount of adsorbed **PVA**, which decrease the hydrophobic force between the microbeads and **BSA**.

Figure 3 **BSA** adsorption on **PSDVB/PVA** microbeads

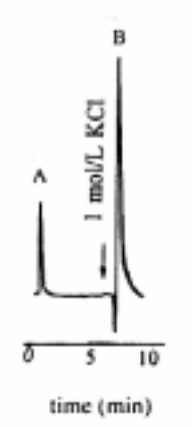


Cibacron Blue F3GA binds with a wide variety of proteins. But it has no specific interaction with **pepsin** (porcine mucosa) from pig. From **Figure 4** it can be seen that **pepsin** has no retention in the column, while **lysozyme** from chicken egg white binds to the dye and can be eluted with 1 mol/L **KCl**.

Conclusion

A new affinity medium has been synthesized by adsorbing **PVA** on the **PSDVB** microbeads surface. The initial concentration of **PVA** solution has effect on the adsorption of **PVA** to the microbeads. When the initial concentration is 40mg/g, a high adsorption amount (247mg/g) is reached. The stability test of pH indicated that this medium is stable in high pH value range. There is no obvious dye leakage from pH4.0 to 11.0. The adsorbed **PVA** decreases the non-specific interaction between **PSDVB** microbeads and **BSA**. It can be used to purify and separate proteins and enzymes, such as **lysozyme**.

Figure 4. Chromatogram of **pepsin** and **lysozyme** in dye bonded **PSDVB/PVA** microbeads



eluent: 0.1mol/L **potassium phosphate** buffer, pH 5.0; flow-rate: 0.9 ml/min; pressure: 5.0 MPa; temperature: ambient; UV detector at 280 nm (A: **pepsin**; B: **lysozyme**)

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Acknowledgment

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