報告番号	甲　第　13798　号

主　論　文　の　要　旨

論文題目　**Study on Fabrication of Cibacron Blue-Modified Nanofiber Fabric and Its Application for Protein Separation**
(シバクロンブルー修飾ナノファイバー不織布の作製およびタンパク質分離への応用に関する研究)

氏　　名　刘　松(LIU　Song)

論　文　内　容　の　要　旨

In this research, polyvinyl alcohol (PVA) nanofiber fabrics were prepared through electrospinning, and the affinity of the PVA nanofiber fabrics was enhanced using Cibacron Blue F3GA (CB). Bovine serum albumin (BSA) and bovine hemoglobin (BHb) were used as model proteins for separation study. The effects of the physical and chemical structure of nanofiber fabrics and adsorption environmental conditions on the adsorption properties of proteins were clarified. Also, the adsorption mechanism of proteins on the surface of PVA nanofiber fabrics was examined.

In chapter 1, the development history of nanofiber fabrics is reviewed, common protein separation methods are introduced, and the research background of the topic is

presented. Nanotechnology is a technology for developing, synthesizing, characterizing and applying materials and devices by modifying their size and shape at the nanoscale. Nanotechnology is a multidisciplinary science involving physics, chemistry, materials science and other engineering sciences. Compared to the bulk material, nanomaterials exhibit new magnetic, optical, electrical, mechanical, and chemical properties due to their shape and size. Applications of nanomaterials are spreading in almost all branches of science and technology. Nanofibers are narrowly defined as fibers with a diameter equal to or less than 100 nm. However, in a broad definition, fibers with a diameter less than 1 μm (1000 nm) are usually regarded as nanofibers. Nanofibers have the characteristics of high specific surface area, large porosity, superior mechanical strength, and wide source of raw materials and so on. Therefore, they have received extensive attention from scientists all over the world and play an important role in the field of nanotechnology. Electrospinning is a versatile, convenient, and simple method for fabricating nanofibers.

Protein products not only have a wide range of applications in daily life such as food engineering and daily chemicals, but also play a vital role in the frontier fields of biomedicine, medical diagnosis and proteomics. Protein products are mainly obtained through two sources, one is the extraction from natural products; the other is the large-scale production using biotechnology and fermentation engineering. Protein extraction, separation and purification processes are necessary for the both sources. To

ensure the efficacy and safety of protein products, the fields of food engineering, bioreagents and biopharmaceuticals have high requirements for the purity of protein products, which must meet the requirements of food specifications, reagent standards and pharmacopoeias. In the production process of protein products, the separation and purification steps directly determine the purity and biological activity of the target protein. The tedious process of biological product recovery dramatically impacts the final product cost because separation and purification accounts for 70% to 80% of the total biological process cost.

The separation and purification of proteins are primarily based on differences in the characteristics of proteins, including molecular size and shape, acid-base properties, solubility, adsorption properties, and biological affinity for other molecules. The ultrafiltration process, which is the most widely applied in protein separation, is difficult to control the selectivity for proteins with similar sizes. Although chromatography is characterized by high precision in protein separation, it has the disadvantages of high cost, slow flow rate, large pressure drop, and not easy for large-scale industrial production.

In chapter 2, fabrication of CB-modified PVA nanofiber fabrics was introduced. Electrospinning polymer solution was prepared by dissolving PVA in water, and maleic acid was added as a cross-linking agent. Then PVA nanofibers were prepared using the electrospinning method. The PVA nanofiber fabric surface was observed by SEM, and

the resultant nanofiber fabric showed a distinctive highly porous structure with micro-scale interstitial spaces. The diameter distribution of the fibers was counted, and the average diameter was calculated. The PVA nanofiber fabric surface was subsequently modified with the reactive dye of CB through a series of surface modification steps. CB molecule contains electrophilic triazine chloride groups capable of reacting with the nucleophilic group -OH of PVA molecule. The chemical structures of the original and CB-modified PVA nanofiber fabrics were detected with FTIR. The characteristic spectra of the CB molecules, such as benzene ring, C-N, and sulfonic acid groups, were observed on the CB-modified PVA nanofiber fabrics, which confirms that CB molecules were successfully fixed onto the PVA nanofiber fabrics.

In chapter 3, the static adsorption performance of CB-modified PVA nanofiber fabrics on BSA was investigated. The adsorption amount increased with the increase of the initial concentration of BSA. The equilibrium isotherm data was analyzed by the Langmuir and Freundlich isotherms, respectively. And the Langmuir isotherm fitted the results better than the Freundlich isotherm, which shows that the BSA adsorption on the nanofiber surface is a monolayer adsorption. The saturation adsorption amounts of the PVA nanofiber fabrics were 179 mg/g and 714 mg/g before and after CB modification, resulting in four times increase. The effects of pH value and ionic strength on the adsorption capacity were studied. pH value mainly affects the morphology and surface charge of protein molecules, thus affecting the electrostatic interaction between protein

molecules and nanofiber surface. Increasing ionic strength could intense the binding of CB molecules to the PVA fabric surface by hydrophobic interaction, which results in the decrease of CB coupling density accessible to BSA molecules. Moreover, as the salt concentration increased, it can lead to coordination of the deprotonated sulfonic acid groups of CB with sodium ions of the salt (NaCl), which leads to low protein adsorption.

In the desorption test with 1.0 M NaCl at pH 10, the desorption ratio of BSA-adsorbed PVA nanofiber fabrics reached 97.3%. The affinity PVA nanofiber fabrics showed no significant decrease in adsorption performance during repeated adsorption-desorption cycles and exhibited excellent reusability. These results reveal that CB-modified PVA nanofiber fabrics are superior candidates for affinity adsorption of proteins.

In chapter 4, the effects of adsorption conditions on the BSA dynamic adsorption process, such as modification of CB, BSA concentration of the permeate solution, and permeation rate, were examined in a continuous system. Adsorption results were fitted using the adsorption kinetics of the pseudo-first-order kinetic and the pseudo-second-order kinetic. All kinetic parameters and correlation coefficient values were compared between the pseudo-first-order kinetic and the pseudo-second-order kinetic. The higher correlation coefficient values of the pseudo-second-order kinetic model demonstrated that the adsorption rate of BSA on the nanofiber surface is not

proportional to the concentration driving force but proportional to the square of the driving force, and the adsorption rate is controlled by the chemical adsorption mechanism. In order to provide a design basis for adsorption operation, the static and dynamic adsorption efficiencies were compared by the fitting curves according to the experimental data. The dynamic adsorption was more efficient and time-saving than the static adsorption in the concentration range of the experiment. These results can provide a reference for process scale-up design.

In chapter 5, selective adsorption studies were performed using the CB-modified PVA nanofibers with BHb and BSA as model proteins. The molecular weights of BHb and BSA are 64500 and 67000, respectively. And the sizes of BHb and BSA are too close to be effectively separated by conventional filtration operations. The static and dynamic adsorption behaviors of the PVA nanofiber fabrics on BHb before and after CB modification were investigated.

Amino acids are the basic units that make up proteins and are linked by dehydration and condensation to form peptide chains. Proteins are biological macromolecules composed of one or more polypeptide chains. Proteins are amphiphilic molecules because of the difference in carboxyl and amino groups. The positive and negative charges of proteins are exactly equal at a certain pH, where the net charge is zero. And this pH is named the isoelectric point of the protein. Each protein has its own isoelectric point. The effect of pH on the adsorption properties of BHb and BSA was investigated.

The maximum adsorption amounts for BHb and BSA on the CB-modified PVA nanofiber fabrics appeared at pH 6.8 and 5.0, respectively. However, the maximum difference of the adsorption amounts for the two proteins was observed at pH 6.8, which is corresponding to the isoelectric point of BHb. The selective separation experiments of the BHb-BSA binary solution were carried out at pH 6.8, and a high selectivity factor of 5.45 for BHb was achieved. Finally, the reusability of the nanofiber fabric was examined by three adsorption-elution cycle tests. This research demonstrated the potential of the CB-modified PVA nanofiber fabric in protein adsorption and selective separation.

In chapter 6, the research of the thesis is summarized comprehensively. This research demonstrates that Cibacron Blue-modified PVA nanofiber fabrics with simple preparation process, low cost and high protein adsorption capacity have great potential for protein separation and purification.