ENDOTOXIN REMOVAL BY MEMBRANE AFFINITY CHROMATOGRAPHY

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SUMMARY
Endotoxin was removed by membrane affinity chromatography. Macropore cellulose membrane was prepared from filter paper by alkaline treatment and chemical crosslinking, and was used as stationary phase for affinity chromatography. The matrix membrane was derived by hexamethylenediamine and activated by glutaraldehyde before histidine was immobilized as affinity ligand. A membrane cartridge containing 40 sheets of affinity membrane was also prepared, which can be used to remove pyrogen from aqueous solutions.

INTRODUCTION
During the last ten years, biological engineering has been developed very fast, and has produced more and more important functional proteins and factors. But these pharmaceutics seemed difficult and expensive to be used in practice because of the difficulties in the purification procedure, especially the depyrogenation procedure.

Bacterial endotoxins are the lipopolysaccharides (LPS) derived from the outer membranes of gram negative bacteria. The highly pyrogenic nature of bacterial endotoxin made the terms pyrogen, endotoxin and LPS synonymous. Endotoxin is known to cause febrile reactions in animals with symptoms of high fever, vasodilation, diarrhea and in extreme cases, fatal shock. There are many methods known for removing or reducing the level of endotoxin in fluids. The chemical decomposition of pyrogen with acid, alkali, or oxidizing agent is commonly practiced in sterilizing the system. Filtration using ultrafiltration membrane or depth type filters are popular means of depyrogenating biological solutions[1]. However, none of the above proved to be perfect, an effective and economical depyrogenation method is still in urgent need.

Affinity chromatography is a unique method in the separation technology since it is the only technique that enables the purification of almost any biomolecule on the basis of its own biological functions rather than individual physical or chemical properties. It is one of the few methods that can remove pyrogen from varied sample to a relatively low level[2]. But the inherent defaults of classical affinity chromatography, such as low operating flux and long separation time, seriously limit its application in depyrogenating. To solve this problem, in this work, we adopted a new