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Removal of Endotoxin from Human Serum Albumin Solutions by Hydrophobic and Cationic Charged Membrane

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Abstract: A novel matrix of macropore cellulose membrane was prepared by chemical graft, and immobilized the cationic charged groups as affinity ligands. The prepared membrane can be used for the removal of endotoxin from human serum albumin (HSA) solutions. With a cartridge of 20 sheets affinity membrane of 47 mm diameter, the endotoxin level in HSA solution can be reduced to 0.027 eu/mL. Recovery of HSA was over 95%.

Keyword: Hydrophobic and cationic charged membrane, endotoxin removal.

Removal of endotoxin from medicine injection is very important, because endotoxin with potential biological activity causes pyrogenic and shock reactions in mammals on intravenous injection even as low as nanogram amounts. Endotoxin, a constituent of potential contaminant of physiological fluids and aqueous solutions and very stable at extreme temperature and pH values. For removing endotoxin from solutions of biomolecules, such as HSA, adsorption techniques are used[1]. Many methods for endotoxin removal were reported, but they all had their disadvantages [2-3]. We attempted to prepare a novel affinity membrane and made it suitable for the endotoxin removal from biological products or parenteral liquids. In this work, we prepared a type of hydrophobic and cationic charged membrane that can be used for endotoxin removal from HSA solutions and ascites. With a cartridge of 20 sheets affinity membrane, the endotoxin level can be reduced to a minimum of 0.027 eu/mL, and the recovery of HSA was over 95%.

Experimental

Material and Reagents Cellulose Membrane of filter-paper, Xinhua No.1 was purchased from Hangzhou Xinhua Paper Manufactory (Hangzhou, China). Glycidylmethacrylate (GMA) was analytical grade reagent from Luoyang Hengguang Chemical Reagent Factory (Luoyang, China). 1-Vinylimidazole, N,N,N',N'-tetramethylethylenediamine and 1,6-dichloro hexane were purchased from Fluka (Switzerland). Tachypleus Amebocyte Lysate (TAL) for endotoxin detection was from Marine organisms products

Factory of Zhanjiang China and endotoxin standard was from National Institute for the Control of Pharmaceutical and Biological Product (Beijing, China). HSA was from Centeon (German).

Apparatus Peristaltic pump BT01-100 was from Lange Constant Flow Pump Corporation (Baoding, China), Bacterial endotoxin test apparatus BET-16 was from Electronic Instrument Factory of Tianjin University (Tianjin, China).

Membrane Preparation The cellulose membrane was put into 100 mL deionized (D.I.) water and heated to 70°C, occasionally stirred.8 mL GMA, 0.4 g amonium per-sulfate and 0.6 g sodium thiosulfate were added to the reactor and the reaction lasted for 1 h. Then the reaction was terminated and the membrane was washed with D.I. water and dried. After graft, 8 mL 1-vinylimidazole was added and the reaction lasted for 1-3 h. The membrane was washed with D.I. water. This membrane was named as A. Then the membrane A was put into 100 mL D.I. water (70°C) again. 10 mL N,N,N',N'-tetramethylethylene-diamine and 10 mL 1,6-dichlorohexane were added to the reactor and the reaction was permitted to proceed overnight, occasionally stirred. Then the product was washed and dried. This membrane was named as B.

Adsorption experiment The sample was prepared by putting 60 eu endotoxin and 1 ml 20% (200mg/mL) HSA into 100 mL 0.9% NaCl injection (pH 5.8, I=0.15M), then the concentration of endotoxin was 0.6 eu/mL and the concentration of HSA was 2 mg/mL in the sample. The sample was pumped to the cartridge at a flow-rate of 0.5 mL/min at room temperature. The filtrate was collected at 30, 90, 150, 200 minutes and 2 mL was collected for each time. Endotoxin amounts of the collected fractions were determined by BET-16 Apparatus.

Endotoxin determination Kinetic-Turbidimetric Method was used in Endotoxin quantity determination by bacterial endotoxin test apparatus BET-16. The minimum detection limit was as low as 0.001eu/mL. The endotoxin contents of every collected fraction were determined according to the standard curve (lg T=2.12964+(-0.56006LgC), r=0.9992) and the removal efficiency can be calculated.

HSA concentration determination The concentrations of HSA was determined from the absorbance measurement at 280 nm by spectrophotometer. According to Beer's Law (A=abC), the recovery (C_i/C_0) was obtained by calculating A_i/A_0.

Application Membrane B has ever been used in the endotoxin removal of ascites from serious liver patient.

Results and discussion

Because endotoxin contains negative phosphate ester groups, the cationic charged substituents on the membrane can interact with them. Endotoxin also contains hydrophobic long-chain fatty acids[4], the hydrophobic alkyl membrane can adsorb it. So the cationic groups and hydrophobic groups on this membrane interact to synergistically capture and remove endotoxin. Comparing with membrane B, membrane A has less alkyl and cationic charges, so it exhibits lower removal efficiency than membrane B (**Table 1**,**Table 2**).

Table 1 The results of membrane A used for removal of endotoxin from HSA solution

time(min)	endotoxin		HSA recovery(%)
	concentration(eu/mL)	removal efficiency(%)	
30	0.0656	89.1	94.0
90	0.0656	89.1	96.0
150	0.0656	89.1	96.6
200	0.0619	89.7	97.2

Table 2 The results of membrane B used for removal of endotoxin from HSA solution

time(min)	endotoxin		HSA recovery(%)
	concentration(eu/mL)	removal efficient(%)	
30	0.0329	94.5	93.3
90	0.0280	95.4	97.8
150	0.0270	95.5	98.5
200	0.0270	95.5	98.9

HSA having negative charge at PH=5.8 also can interact with cationic charged membrane, so it can inhibit the interaction of endotoxin with the cationic charge sites on the membrane. Under such condition, the hydrophobic groups on the membrane assist in the removal of endotoxin. From **Table 1,2,3** we can conclude that this type membrane adsorbed a small amount of HSA and most part of endotoxin. As the membrane was used in the low concentrations of endotoxin and HSA, the endotoxin removal efficiency and HSA recovery were not very high, but the concentration of endotoxin in the filtrate was very low. We can suppose that if it was used in high concentration, the endotoxin removal efficient and HSA recovery can be improved.

In endotoxin removal from ascites, the ascites has not very high endotoxin content, but we can get some information from the experiment. From **Table 3**, we can conclude that membrane B can effectively remove endotoxin from ascites without affecting other substance recovery. So we can consider to apply it to remove endotoxins from patients' ascites.

Table 3 The results of membrane B used for removal of endotoxin from ascites

substance	concentration in sample	concentration in filtrate	removal efficiency(%)	recovery(%)
specific gravity	1.020	1.020		
endotoxin	0.135 eu/mL	0.023 eu/mL	82.8	
protein	2.65 mg/dL	2.54 mg/dL		95.8
IgG	1430 g/L	1480 g/L		103.5

Comparing with the histidine and polymyxin B as ligands in our previous work[5], the hydrophobic and cationic charged membrane has the same ability to removal endotoxin to very low concentration. But it has an obvious advantage, that is, this ligand is very cheaper.

From the results, it can be concluded that hydrophobic and cationic charged

membrane can be considered as a useful tool for the removal of endotoxin from HSA solutions, but if it's used in clinic, more work should be done.

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References

1. C. Hirayama, M. Sakata, Y. Yugawa, H. Ihara, *Journal of Chromatography A*, **1994,** *676*, 267 .
2. A. C. Issekutz, *Journal of Immunological Methods*, **1983**, *61*, 275.
3. K. C. Hou , R. Zaniewski, *Journal of Parenteral Science & Techmology*, **1990**, *44* , 204.
4. M.Wakita,T. Adachi,J. Oda, M.Hashimoto ,.*Bull. Chem .soc.Jpn.*, **1996,** *6*9 ,1017 .
5. W. Guo, Z. H. Shang, Y. N. Yu, L. M. Zhou. *Chinese Chemical Letters*, **1995,** *6*(10), 919.

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