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Endotoxin removal from water using microporous polyethylene chopped fibres as a new adsorbent

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SUMMARY

A new adsorbent, microporous polyethylene chopped fibre, was produced from a high density polyethylene. This can adsorb lipopolysaccharides (LPS) linearly up to 2 h, and showed the highest capacity to adsorb LPS when compared with two other polyethylene-based adsorbents and a polystyrene-based adsorbent. More than twice as much orange II and 4-nitroquinoline N oxide were adsorbed in the new adsorbent as was LPS. The adsorption isotherm of the new adsorbent for LPS was of Ln type, the correlation between adsorption and concentration of solute was proportional; whereas orange II and 4-nitroquinoline N oxide were of L type (greater adsorption than Ln type); tetrachloroethylene adsorption was of S type, less than Ln type. Adsorption of LPS to the new adsorbent increased when temperature rose, whereas adsorption of orange II and 4-nitroquinoline N oxide decreased. These data suggest that the binding of LPS to the new adsorbent is a hydrophobic interaction, whereas the binding of both orange II and 4-nitroquinoline N oxide is not. The new adsorbent has a greater potential for the removal of endotoxin from tap water than other commercially available adsorbents such as charcoal and Amberlite XAD-2.

INTRODUCTION

A large portion of pyrogens are well-recognized endotoxins (lipopolysaccharides, LPS) which are produced by Gram-negative bacteria. Attempts have been made to remove endotoxins from aqueous samples by the following methods: (*a*) physical

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and chemical adsorbents such as charcoal (Pegues *et al.* 1979), ion exchange resin (Nolan, McDevitt & Goldman, 1975), polyolefine beads (Harris & Feinstein, 1976), asbestos (Kaden, 1975), bentonites (Ditter, Urbascheck & Urbascheck, 1983), immobilized polymyxin B (Niwa, Umeda & Ohashi, 1982) and immobilized histamine (Minobe *et al.* 1982); (*b*) chemical decomposition with alkali (Niwa *et al.* 1969) and oxidizing agents (Tsuji & Harrison, 1979); (*c*) filtration by ultramembrane filters (Sweadner, Forte & Nelsen, 1977; Cradock *et al.* 1978) as well as reverse osmosis membranes (Parkinson, 1983). Adsorbents must be evaluated for their specificity and capacity.

In initial studies the microporous polyethylene hollow fibre (EHF, Mitsubishi Rayon Co., Tokyo) with unique microfibrile structure, was found to remove an LPS isolated from *Escherichia coli* 0111: B4 when a water sample was permeated through the fibre's wall membrane (Kamiki *et al.* 1982). In order to eliminate the filtration process as the cause for the removal of endotoxin, the microporous EHF was chopped up (microporous polyethylene chopped fibre, PE-CF) and evaluated as an adsorbent for endotoxin removal. In this paper we report studies concerned primarily with the *in vitro* capacities of synthetic polymers, especially the contribution of the polymer's three-dimensional conformation to the binding of endotoxins in water. We also deal with the adsorption characteristics of PE-CF for various kinds of endotoxins. The removal levels of three potentially harmful organic compounds; a diazo dye orange II, a mutagen 4-nitroquinoline N oxide (4NQNO) and a carcinogen tetrachloroethylene, are compared to the endotoxin removal level.

MATERIALS AND METHODS

Preparation of PE-CF

The microporous EHF was produced from a high density polyethylene as described by Shindo *et al.* (1983). To make PE-CF, the hollow fibre (Code: EHF 390-C, Mitsubishi Rayon Co.) was chopped up by an automatic cutter (Type D-478, Onouchi Co., Kyoto) and pieces less than 1 mm in length were sifted and collected. Some properties of PE-CF are as follows; inner diameter, 270 μm; wall membrane thickness, 55 μm; porosity, 63%; and surface area determined by nitrogen adsorption method, 31·7 m^2/g. Scanning electron micrographs (Tyne JSM-25S and JSM-35C, Nihon Denshi Co., Tokyo) of PE-CF (Top picture) and the membrane surface (Bottom picture) are shown in Fig. 1. The mean size of the lattice-like structure on the membrane surface was 0·15 μm in width and 0·6 μm in length.

Other adsorbents

Nonporous polyethylene chopped fibres (NPE-CF, surface area, 0·1 m^2/g) were prepared by chopping nonporous polyethylene hollow fibres which are an intermediate product of microporous EHF (Mitsubishi Rayon Co.). Polyethylene microfibres (PE-MF, SWP E620, density; 0·96 g/cm^3; average length, 1·3 mm; surface area, 8 m^2/g) were obtained from Mitsui Petrochemical Co., Tokyo. Amberlite XAD-2 and charcoal (Shirasagi WH) were obtained from Organo Co., Tokyo, and Takeda Pharmaceutical Ind., Osaka, respectively.

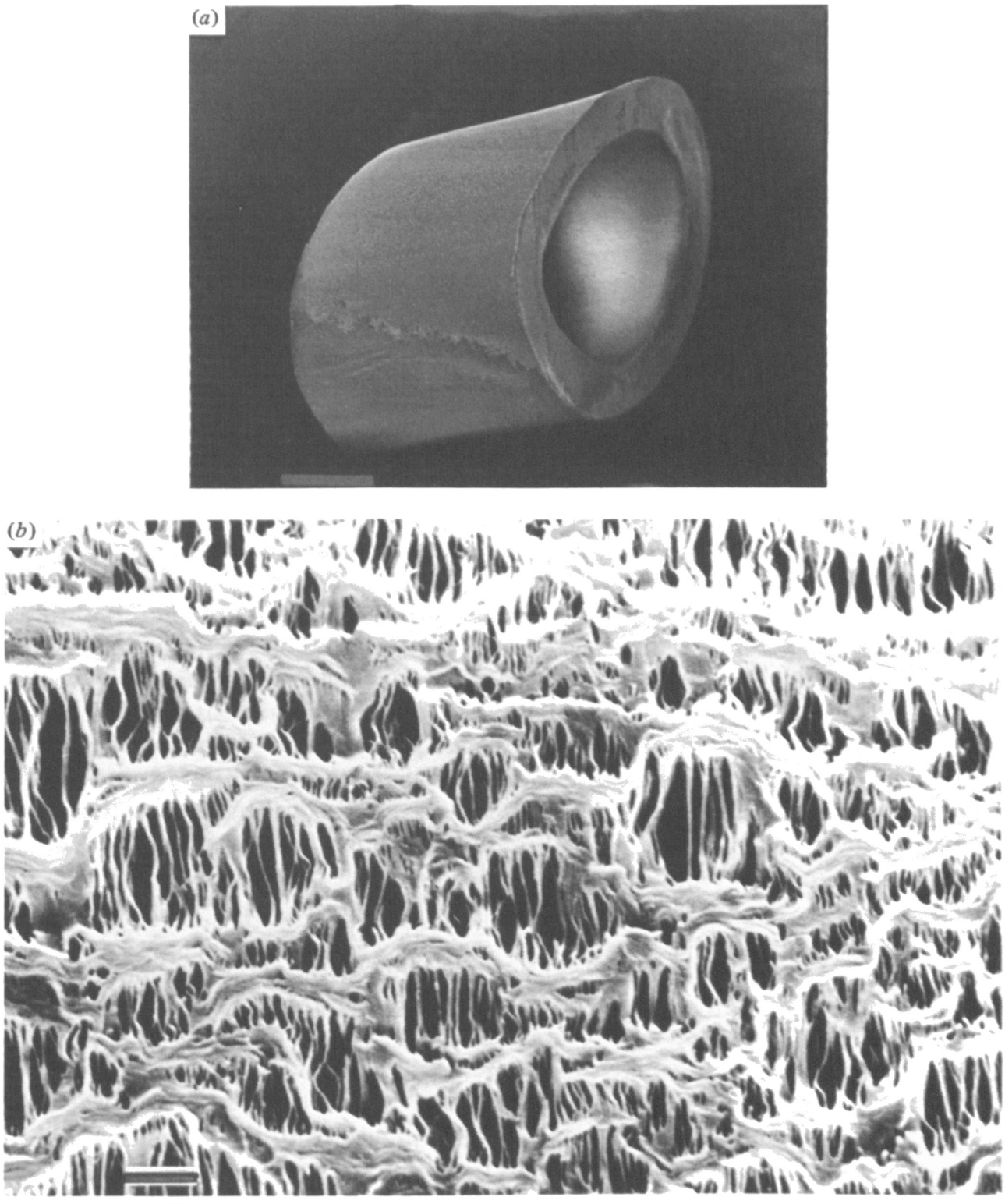

Fig. 1. Scanning electron micrographs of PE-CF, overall (*a*) and wall outer surface (*b*). Reference bars indicate 100 μm (*a*) and 1 μm (*b*).

Column chromatography

The PE-CF, PE-MF, NPE-CF and Amberlite XAD-2, 1 g, were individually packed into glass columns and were washed with 20 ml of 99% ethanol and 40 ml of distilled water before use. The flow rate was set at 2 ml/min in all chromatographic experiments. A 2 ml fraction was collected for each. Concentrations of the compounds in the eluates were then determined.

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Determination of endotoxin at picogram levels

A synthetic substrate method of *Limulus* amoebocyte lysate (Iwanaga *et al.* 1978; Fujita & Nakahara, 1982) was employed to determine the concentration of endotoxin in the test water. The endotoxin assay kit, Pyrodick®, was purchased from Seikagaku Kogyo Co., Tokyo. The reaction mixture containing the Pyrodick reagent dissolved in 0·1 ml of the attached buffer solution pH 8·0 and 0·1 ml of an endotoxin solution was incubated for 30 min at 37 °C. The reaction was stopped by adding 1·0 ml of 0·6 N acetic acid. The optical density of the resultant solution was read at 405 nm with a spectrophotometer (Type UV-250 attached to a recording unit OPI-2, Shimadzu Co., Kyoto). Endotoxin concentration in the test water was estimated from the standard curve using an LPS of *E. coli* 0111: B4 (Difco Lab., Detroit, Michigan). Endotoxin was diluted with endotoxin-free water (Otsuka Pharmaceutical Co., Naruto). All glassware for pyrogen tests was heated at 250 °C for 2 h.

Determination of adsorption isotherm

The adsorbents to be used were bagged in polyethylene mesh fabric (80 mesh) in amounts of 50, 100, 200 and 400 mg by a heat-sealing technique. Each bag was dipped in 99% ethanol for 1 min, transferred into 70% ethanol (500 ml) and left for 1 min, transferred into distilled water (500 ml) and shaken for 1 min, and finally transferred to distilled water (500 ml). Each bag was put into a test tube containing 10 ml of the test solution. The test tube was shaken intermittantly for exactly 1 h and then the bagged adsorbent was removed. The polyethylene mesh fabric itself was tested as a control. The concentration of the compound was determined in the remaining solution. An adsorption isotherm was obtained using at least 12 points at different determinations by using Freundlich's equation (Freundlich, 1926);

$$\log (X/M) = \frac{1}{n} \log \mathrm{Ci} + \log \mathrm{K},$$

where X/M = adsorbed material, mg/100 ml/g of adsorbent; Ci = equilibrated concentration of solute, mg/100 ml; K = a constant; n = the adsorption coefficient.

Quantitative determination of adsorbing solutes

The concentrations of solutes were determined from the standard curves measured by a spectrophotometer at 214 nm for LPS, 225 nm for Orange II and 365 nm for 4NQNO. Tetrachloroethylene was determined by gas chromatography (Type G-1800 with FID detector at 120 °C, Yanagimoto Co., Kyoto).

Endotoxin removal from tap water by various adsorbents

Each individual adsorbent was packed into plastic columns of varying sizes as described in Table 3 and tap water of Nagoya City with temperature range of 15–20 °C was continuously passed through the column at a flow rate of 300 ml/min. When the cumulative flux of water reached 2 m^3 total for a column, the treated water sample was aseptically collected and the concentration of endotoxin was determined.

Chemicals

For the major examination an LPS from *E. coli* 0113 was chosen from our stock because this LPS has been well characterized (Niwa *et al.* 1969; Ribi *et al.* 1966) and has been used as the US Reference Endotoxin, EC-2. The other two LPSs were purchased from Wako Pure Chemical Ind., Osaka. In order to dissolve LPS, the LPS was suspended in 10 mM Tris-HCl, pH 7·1 and treated with a sonicator (Water-bath Type, Nippon Rikagaku Kikai Co., Tokyo) at maximum power for 10 min at 70 °C. Orange II, 4NQNO, tetrachloroethylene were purchased from Katayama Chemical Ind., Osaka. These compounds were dissolved in endotoxin-free water. All chemicals were of analytical-reagent grade.

RESULTS

PE-CF adsorption capacity of various compounds by column chromatographic technique

Three polyethylene-based adsorbents and a polystyrene-based adsorbent Amberlite XAD-2 were examined for their capacities to adsorb LPS (*E. coli* 0113), orange II and 4NQNO by the column chromatographic technique. The adsorption capacity of each adsorbent for each individual compound was calculated from the elution profiles in Fig. 2, and the result shown in Table 1. PE-CF had the highest capacity to adsorb LPS when compared with the other three adsorbents, PE-MF, NPE-CF and Amberlite XAD-2. Orange II was removed to the greatest extent with a column of PE-CF. On the other hand, PE-MF revealed little removal of LPS, orange II and 4NQNO. NPE-CF adsorbed the least amount of LPS when compared with the other three adsorbents. Amberlite XAD-2 was capable of binding less LPS, orange II, or 4NQNO than PE-CF. Interestingly, PE-CF showed an increase in the capacity to bind LPS when the temperature rose, for instance to 70 °C.

Adsorption capacity of PE-CF for LPS as a function of incubation time

The PE-CF was examined for its capacity to bind LPS as a function of incubation time. An adsorption curve was almost linear up to the first 2 h. At 1 h of incubation, approximately 10 mg of LPS was adsorbed per gram of PE-CF. Adsorption reached a plateau after 24 h (Fig. 3). Adsorption capacities of PE-CF for LPS (0113) in 10 mM Tris-HCl buffer at pH 5·1 and pH 9·1 under 1 h of incubation time were then compared to the control pH 7·1. Adsorption capacities were 95% at pH 5·1 and 91% at pH 9·1 as compared to the control, respectively (data not shown). For the later experiments for the adsorption isotherms, pH 7·1 and 1 h of incubation time were adopted.

Experimental adsorption isotherms

PE-CF was further studied for its adsorption isotherms for three kinds of LPS, orange II, 4NQNO and tetrachloroethylene (Fig. 4). Conditions of adsorption experiments in Fig. 4 were as described in Table 2. For LPS, the adsorption of PE-CF in the presence of 0·5 M NaCl (3) was obviously higher than that of the control without NaCl (1). Ethanol concentration at 5% (4) decreased adsorption. An

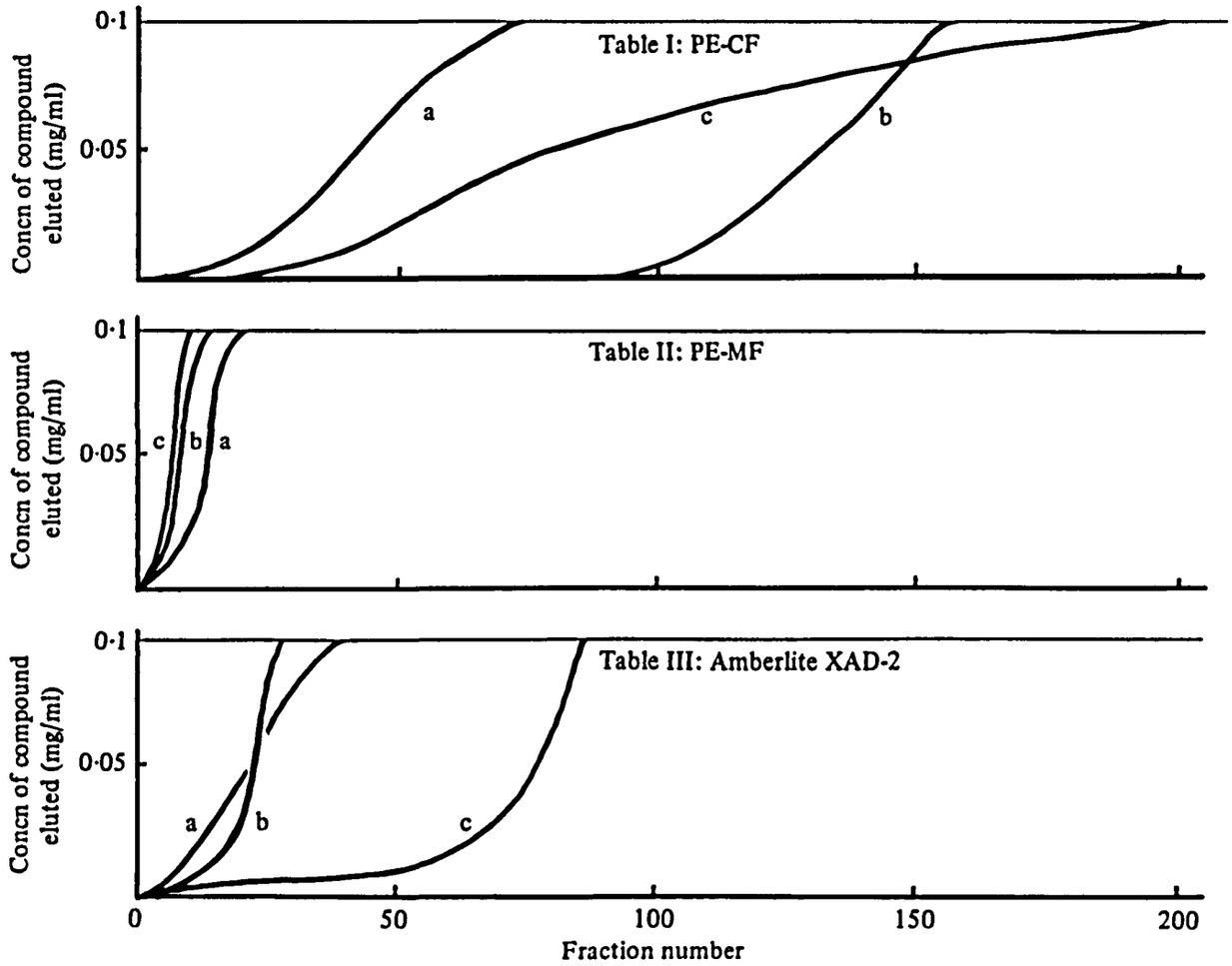

Fig. 2. Column chromatographic profiles of LPS, orange II and 4NQNO for three adsorbents. Table I: PE-CF, 1 g, column size: 0·9 × 8 cm. Table II: PE-MF, 1 g, column size: 0·9 × 10 cm (at beginning) to 0·9 × 6 cm. Table III: Amberlite XAD-2, 1 g, column size: 0·9 × 2·5 cm. All were done at 25 °C. (a) LPS (0113), (b) orange II, (c) 4NQNO. Axis: The concentration of the compound in the fraction was spectrophotometrically determined and calculated in reference to the unit of comparison (mg/ml).

Table 1. *Adsorption capacity of various adsorbents for LPS, orange II and 4NQNO using column chromatography*

	Amount adsorbed (mg/g) by			
Adsorbed solute	PE-CF	PE-MF	NPE-CF	Amberlite XAD-2
LPS (0113)	4·3 5·1*	1·3	0·4	2·2
Orange II	13·1	0·6	<0·1	2·1
4NQNO	9·5	0·8	<0·1	7·2

At 25 °C; *70 °C.

elevated temperature (2) showed significantly stimulated binding as compared to the control temperature (1). Similar results were obtained for the three LPS. All adsorption isotherms except the condition 2 seemed linear and were quite similar to the Ln type of adsorption isotherm as demonstrated by Alligham, Giles & Neustadter in 1954. That is, the correlation between X/M and Ci is proportional. NPE-CF had less adsorption capacity of LPS (0113) in comparison to PE-CF. For

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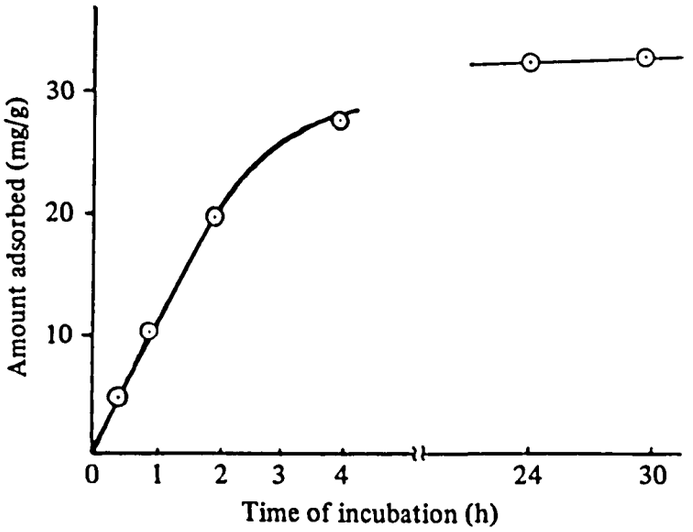

Fig. 3. Adsorption kinetic of PE-CF for LPS. LPS (0113) at initial concentration of 100 mg/100 ml, pH 7·1, was used. The adsorbent (300 mg/bag) was put into 10 ml of the solution and incubated for an indicated time at 25 °C and the LPS concentration remained was determined. Each point is a mean of duplicate determinations.

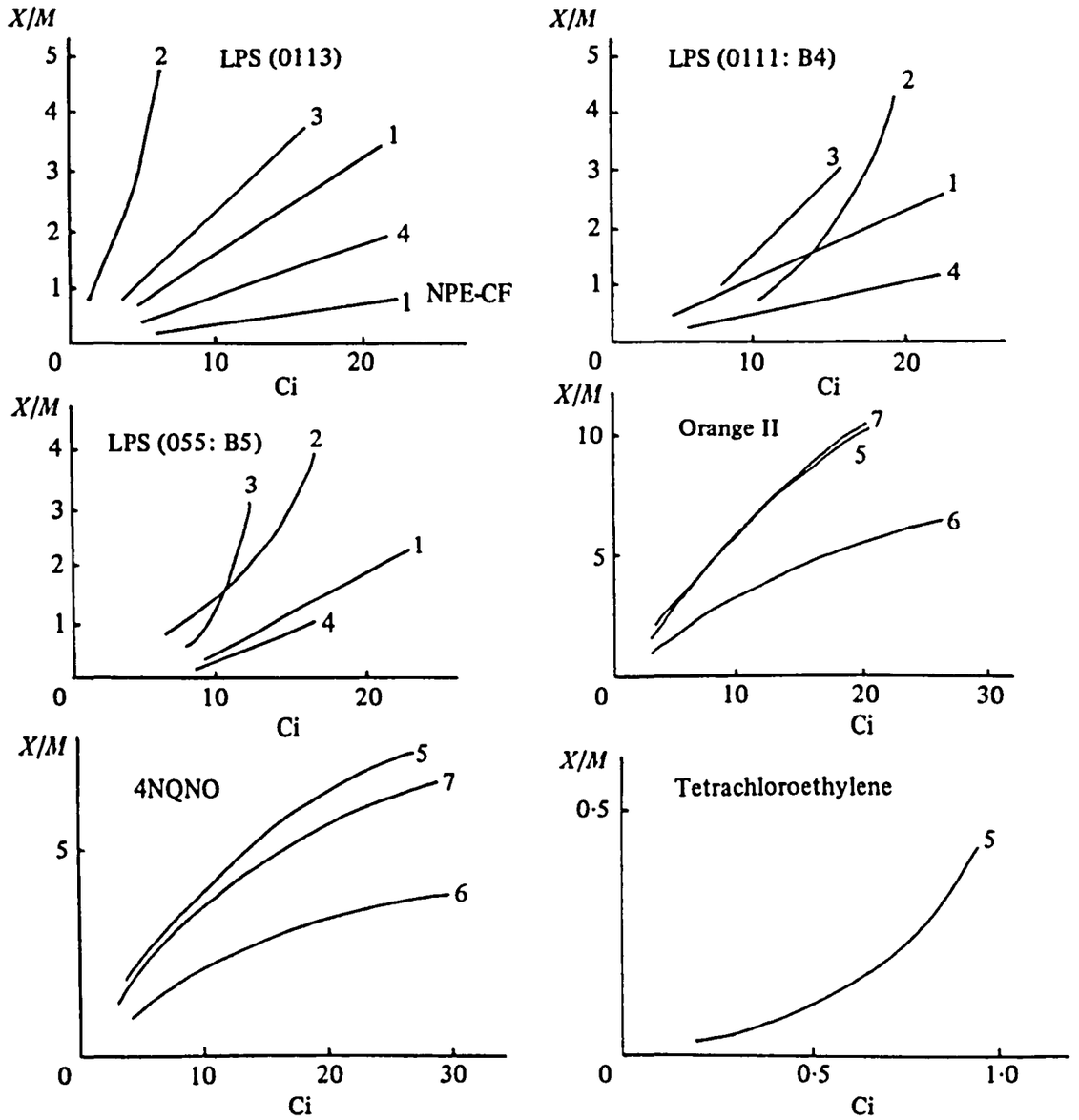

Fig. 4. Adsorption isotherms for PE-CF and NPE-CF of LPS, orange II and 4NQNO. Figures indicate conditions, see conditions in Table III. X/M, mg/g; Ci, mg/100 ml.

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Table 2. *Experimental affinity values of PE-CF and NPE-CF for six compounds. Data were calculated by Freundlichs' equation*

	Solute											
	LPS (0113)		LPS (0111: B4)		LPS (055: B5)		Orange II		4NQNO		Tetrachloro-ethylene	
Adsorbent condition	1/*n*	log K	1/*n*	log K	1/*n*	log K	1/*n*	log K	1/*n*	log K	1/*n*	log K
PE-CF												
1. pH 7·1* 25 °C	0·87	−0·55	1·09	−1·08	0·87	−0·55	—	—	—	—	—	—
2. pH 7·1 70 °C	1·37	−0·06	3·90	−3·98	2·54	−2·16	—	—	—	—	—	—
3. pH 7·1 25 °C 0·5 M NaCl	1·10	−0·42	2·48	−1·84	1·60	−1·17	—	—	—	—	—	—
4. pH 7·1 25 °C 5% EtOH	0·64	−0·41	3·57	−4·25	1·82	−1·63	—	—	—	—	—	—
5. In H_2O 25 °C	—	—	—	—	—	—	0·82	−0·04	0·81	−0·25	1·34	−0·55
6. In H_2O 70 °C	—	—	—	—	—	—	0·71	−0·09	1·06	−0·97	—	—
7. In H_2O 25 °C 0·5 M NaCl	—	—	—	—	—	—	0·92	−0·13	0·77	−0·31	—	—
NPE-CF												
1. pH 7·1 25 °C	0·98	−1·17	—	—	—	—	—	—	—	—	—	—

* 10 mM Tris-HCl buffer.

Table 3. *Concentration of endotoxin in tap water following treatment with various adsorbents*

Treated with	Weight (g)	Column size (cm)	Concentration of endotoxin (ng/ml)
None	—	—	4·71
PE-CF	120	4·5 × 20	0·09
Charcoal	650	8·0 × 20	4·50
Amberlite XAD-2	120	4·5 × 20	2·17

other compounds, orange II and 4NQNO, the adsorption of PE-CF in the presence of 0·5 M NaCl (7) seemed to have very little effect as compared to the control (5). PE-CF revealed less adsorption capacity for both orange II and 4NQNO when the temperature rose (6). Adsorption isotherms of PE-CF for these organic compounds was of the L (Langmuir) type. The PE-CF had very little adsorption capacity for tetrachloroethylene and was of the S type, that is, affinity of the solute to the adsorbent is quite small.

Table 2 summarizes experimental affinity values of PE-CF and NPE-CF obtained by Freundlich's equation.

Endotoxin removal from tap water by various adsorbents

In order to compare the endotoxin removal efficiency, tap water which contains various natural sources of endotoxin was used. As a result, PE-CF treated water contained the lowest concentration of endotoxin activity when compared with the water samples treated with the other adsorbents, charcoal and Amberlite XAD-2 (Table 3).

DISCUSSION

Polyethylene, a hydrophobic material, can be wetted in the presence of some organic solvents or detergents, it is stable against acid, alkali, various organic solvents and detergents. This paper reports the following adsorption characteristics for endotoxin in water.

Adsorbents must have a large area of binding sites, and many modifications of three dimensional structure designed to increase the surface area can be considered. The most popular conformation is of microfibres like PE-MF but these have a low water flux during practical use. Therefore, to get sufficient water flux, a beady or granular shape is preferable. The PE-CF is beady and has approximately 63 % surface porosity, giving it an approximately 300-fold larger surface area than that of NPE-CF and a 4-fold larger surface area than that of PE-MF. As shown in Table 1, PE-CF adsorbed 4·3 mg of LPS/g, whilst PE-MF adsorbed 1·3 mg/g. This result indicates that the polymer's surface area and its adsorption capacity are nearly proportional. However, this correlation between PE-CF and NPE-CF was in disagreement. The reasons for this contradiction are unclear at the present time.

In addition the pore size distribution of PE-CF is important. As shown in Fig. 1, PE-CF has a microfibril structure possessing various pore sizes on the surface. It has been reported that endotoxins in water have a size distribution from 10^4 Da

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to 0·1 μm in size (Hannecart-Pokorni, Dekegel & Dupuydt, 1973). Therefore, it seems that the microfibrile structure of the PE-CF and its fine conformation of varying pore sizes contributes to the binding of various sizes of endotoxin molecules at a good rate of efficiency. This speculation is supported by the fact that the correlation of the adsorption capacities between PE-CF and PE-MF is contingent upon the polymer's surface areas.

Thirdly, the adsorption manner of LPS for PE-CF was studied, being quite similar to the Ln type of adsorption isotherms at 25 °C. When the temperature increased, the adsorption isotherm was bent and increased when compared to the control temperature. PE-CF pretreated by heating at 70 °C for 1 h and then allowed to cool had the same binding capacity for LPS as PE-CF with no treatment (data not shown). These results indicate that interaction between PE-CF and LPS at an increased temperature is not due to a change of the polymer's nature. In general, increased binding of solute to an adsorbent is recognized to be a hydrophobic interaction (Schneider, Kresheck & Scheraga, 1965). In contrast, both orange II and 4MQNO showed a decrease in their binding capacity to PE-CF when temperature rose, indicating that interactions other than a hydrophobic interaction were involved between PE-CF and these organic compounds. For PE-CF, these two organic compounds showed a typical Ln type of adsorption isotherm. Tetrachloroethylene showed an S type adsorption isotherm. The presence of salt in LPS solution resulted in a little increase in binding capacity of PE-CF as compared to the control without salt. We may speculate that addition of salt changes the LPS to a more fitting conformation for a hydrophobic binding. The binding capacity of PE-CF over a range of pH 5·1 to 9·1 did not change significantly. LPS between these pH ranges would not be altered in terms of its conformational features. Negative electrokinetic (zeta) potential of polyethylene is very close over a range of pH 5–9 (Beneš & Paulenová, 1973). The LPS molecule also has a negative charge (Nolan, McDewitt & Goldman, 1975; Hou *et al.* 1980). Therefore ionic interaction of endotoxin with PE-CF can be eliminated. Recently, immobilized histamine has been reported to have a binding affinity to LPS in hydrophobic and ionic interactions (Minobe *et al.* 1983). In aqueous solution millions of micells of amphipathic LPS molecules aggregate (Ribi *et al.* 1966). The LPS solution used in this paper seemed to be of the same nature, the LPS micells comprising hydrophilic group outside and hydrophobic group inside in aqueous solution. However, PE-CF binds efficiently to this type of LPS.

The adsorption profile of LPS was examined by a model experiment using a fluorescein isothiocyanate-LPS (*E. coli* 055: B5) conjugate (Skelly, Munkenbeck & Morrison, 1979). The fluorescein-LPS was passed through the membrane and observed under fluorescent microscopy. The EHF membrane was stained through the texture with fluorescein-LPS (Sawada *et al.* 1986).

Fourthly, an attempt was made to regenerate the used PE-CF. Approximately 60 % of the LPS (0113) and 95 % of both orange II and 4NQNO were removed from the adsorbent by a simple rinse with 70 (v/v) % ethanol (data not shown).

A number of commercially available adsorbents for endotoxin removal are available, polypropylene beads, Shell 5820 (Shell Chemical Co., Houston, Texas) and immobilized gel. Detoxi-gel™ (Pierce Chemical Co., Rockford, IL), in addition to conventional adsorbents such as charcoal and Amberlite XAD-2. The former

two are specifically for the removal of endotoxin from, for example protein solution, serum, and solutions containing pharmacologically important components. PE-CF is useful for treating large samples of water. The PE-CF has a better capability to bind endotoxin in tap water than other commercially available adsorbents such as charcoal and Amberlite XAD-2. PE-CF therefore has the potential to be utilized as an adsorbent for various fields; e.g. pharmaceutical, food, clinical, biotechnological, environmental and laboratory purposes.

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