2248 © 1982 The Chemical Society of Japan Bull. Chem. Soc. Jpn., 55, 2248—2253 (1982) [Vol. 55, No. 7

High Performance Liquid Chromatography Using Polymer Gels for Direct Analyses and Characterizations of Soluble Organic Pollutants in Water

Kohei URANO,* Hiroyuki FURUUNE, and Koji HAYASHI

Department of Safety and Environmental Engineering, Yokohama National University,
156 Tokiwadai, Hodogaya-ku, Yokohama 240

(Received October 9, 1981)

The optimum conditions in HPLC separations using columns packed with polymer gels were investigated for analyzing and characterizing directly various organic pollutants in water without complicated pretreatment. Experimental results showed that polystyrene gel column was unsuitable for direct analyses of water samples. Direct analyses of various organic compounds in water could be achieved on polyacrylester and polyhydroxystyrene columns by using 0.02 mol L^{-1} phosphoric acid and acetonitrile (85:15) as a mobile phase. At 40 °C, these systems of column and mobile phase gave values of height equivalent to a theoretical plate ranging from approximately 0.10 to 0.25 mm and retention times ranging from approximately 8 to 60 min for various compounds in water. Separation characteristics of these polymer column systems were discussed by taking into account solubilities, functional groups, aromaticities, or molecular weights of organic compounds in water. Furthermore, several wastewaters and their treated waters were directly analyzed for examples.

The conventionally used indices of organic pollution of water are biochemical oxygen demand (BOD), chemical oxygen demand (COD), and concentrations of several toxic pollutants. However, fractionations, characterizations, and analyses of soluble organic pollutants by chromatographic methods such as gel chromatography (Gel C), gas chromatography (GC), and high performance liquid chromatography (HPLC) have recently been used for evaluating organic pollutions and treatment processes of organic wastewater.[1] Since actual water samples contain miscellaneous compounds, there are many cases that the compounds in water need not necessarily to be separated and analyzed completely and need only to be classified and characterized by molecular weight, aromaticity, functional group, or solubility in water. Furthermore, it is expected especially that direct analytical methods without complicated pretreatments such as extraction and reaction are developed for evaluation of water qualities. Columns and operating conditions for direct analyses of water samples should be applicable in the analysis of a wide range of compounds. In other words, there is necessary to select the optimum columns and operating conditions so that analyses of water samples can be achieved within a short time by a very simple operation.

In previous papers, the authors reported about the characteristics of Gel C using Sephadex gel[2] and a specific GC using steam as carrier gas[3,4] for direct analyses and/or characterizations of organic pollutants in water. The characteristics of UV detector for Gel C and HPLC were also reported in another paper.[5] In this study, the optimum conditions together with applicability of HPLC using columns packed with typical polymer gels were investigated for analyses and characterizations of soluble organic pollutants in water without complicated pretreatment. By this series of studies about chromatographic methods for direct analyses and/or characterizations of organic pollutants in water, water pollution with organic matter and water treatment processes will be estimated more exactly than by the conventional indices only.

Materials and Methods

Aqueous solutions of 48 organic compounds whose properties are shown in Table 1, wastewaters from an oil refinery and a dye factory, and their treated waters by adsorption with activated carbon were used as samples in this study. The equipment used in this study consisted of Nipponbunko HPLC Model A-1, two ultraviolet (UV) absorption detectors, a refractive index (RI) detector, and a three pen recorder. Three kinds of commercial packed columns of synthetic polymer gels, whose properties are shown in Table 2, were tested for direct analyses of the water samples. The water samples of 5—50 μL were injected directly, and the organic compounds were separated on the polymer gels by using various mobile phases and were detected with the UV detectors at wavelengthes of 220 nm and 275 nm and the RI detector. Retention time (t_R) and time of peak width (W_t) were obtained for the aqueous solution of organic compound, and height equivalent to a theoretical plate (HETP) was calculated by the following equation:

$$\mathrm{HETP} = L\,W_t^2/16t_R^2.$$

Here, L is the length of packed column. Better resolutions can be achieved by using systems of column and mobile phase showing smaller values of HETP. In this study, the optimum conditions which can be used for a wide range of organic compounds without complicated operations will be selected, and applicability of the column systems for analyses and/or characterizations of various organic pollutants in actual water samples will be discussed.

Results and Discussion

Characteristics of Polystyrene Gel Column. *Mobile Phase*: The most important factor in the direct analyses of organic compounds in water is selection of the optimum system of column and mobile phase liquid. The column of polystyrene gel (Jascopack HP 01) can be used with mobile phases of organic

Table 1. Organic compounds used

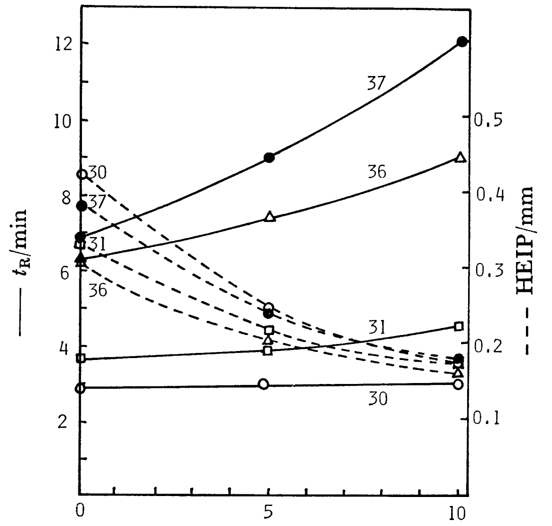
Sample number	Compound name	Molecular weight	Solubility mol L^{-1}
1	Ethyl alcohol	46	∞
2	Propyl alcohol	60	∞
3	Hexyl alcohol	102	6.1×10^{-2}
4	Acetaldehyde	44	∞
5	Propionaldehyde	58	2.8
6	Butyraldehyde	72	5.0×10^{-1}
7	Valeraldehyde	86	3.8×10^{-1}
8	Formic acid	46	∞
9	Acetic acid	60	∞
10	Butyric acid	88	∞
11	Hexanoic acid	116	8.6×10^{-2}
12	Acetone	58	∞
13	Diethyl ketone	86	5.5×10^{-1}
14	Isobutyl methyl ketone	100	2.0×10^{-1}
15	Methyl acetate	74	4.4
16	Methylamine	31	—
17	Trimethylamine	59	6.9
18	2-Amino ethanol	61	∞
19	Malonic acid	104	5.8
20	Adipic acid	146	9.9×10^{-2}
21	Lactic acid	90	∞
22	Malic acid	134	4.2×10^{-1}
23	Sodium citrate	236	—
24	Glycine	75	3.0
25	Glycylglycine	132	—
26	Glutamic acid	147	—
27	D-Glucose	180	4.6
28	Lactose	342	4.2×10^{-1}
29	Raffinose	596	2.0×10^{-1}
30	Benzenesulfonic acid (B.S.)	158	v.s.[a]
31	Phenol	94	8.8×10^{-1}
32	Benzoic acid	122	2.4×10^{-2}
33	Benzaldehyde	106	2.8×10^{-2}
34	Aniline	93	3.9×10^{-1}
35	Benzonitrile	103	2.1×10^{-2}
36	Nitrobenzene	123	1.6×10^{-2}
37	Chlorobenzene	113	4.4×10^{-3}
38	Resorcinol	110	5.3
39	*m*-Cresol	108	2.1×10^{-1}
40	*p*-Cresol	108	1.8×10^{-1}
41	Salicylic acid	138	1.3×10^{-2}
42	*m*-Toluic acid	136	7.2×10^{-3}
43	*m*-Toluidine	107	3.0×10^{-2}
44	*d*-Phenyl alanine	166	7.9×10^{-2}
45	*p*-Ethyl B.S.	186	v.s.
46	*p*-Alkyl(C_{10}—C_{14}) B.S.[b]	—	v.s.
47	2-Naphthalenesulfonic acid	208	v.s.
48	2-Naphthol	144	5.1×10^{-3}

a) v.s.: Very soluble. b) Surfactant for detergent.

Table 2. Packed columns of polymer gels employed[a)]

Polymer	Commercial name	Column size	Limit pressure
Polystyrene	Jascopack HP-01	$4.6\phi\times250$ L	50 kg cm^{-2}
Polyacrylester	Shodex OH pak M614	$6.0\phi\times250$ L	15 kg cm^{-2}
Polyhydroxy-styrene	Shodex Ion pak C811	$8.0\phi\times500$ L	30 kg cm^{-2}

a) These columns can be used at a temperature lower than 60 °C and a flow rate lower than 1.5 mL min^{-1}.



Water content/%

Fig. 1. Changes of retention time and HETP with water content in mobile phase for Jascopack HP 01.
1.0 mL min^{-1}, 40 °C.

solvents or mixtures of polar organic solvents containing less than 10% water. Retention times and the values of HETP of several organic compounds were obtained at 40 °C with mixtures of methanol and water as mobile phases. The changes of t_R and HETP of several benzene substitutes with water contents of the mobile phases are shown in Fig. 1, representatively. With methanol only, the retention times were too short for separation of various compounds, and the values of HETP were slightly large. The mixtures of methanol and water were better than methanol only, but the variations of t_R among compounds was not large enough to separate various compounds. In the case of using mixtures of phosphoric acid solution and methanol or acetonitrile as the mobile phases, the values of HETP were improved, but the variation of t_R among compounds was also not large enough. Thus, it was concluded that direct analyses of various organic compounds in water could not be achieved by using the column packed with polystyrene gel, and other polymer columns were studied.

Characteristics of Polyacrylester Gel Column. *Mobile Phase*: The column of polyacrylester gel (Shodex OH pak M614) can be used with the mobile phases of water or aqueous solutions containing less than 30% polar organic solvents. Retention times and the values of HETP for several typical organic compounds at 40 °C were obtained with various mobile phases of water, aqueous solution of phosphoric acid, or mixtures of aqueous solution of phosphoric acid and methanol or acetonitrile. The peaks showed tailing or leading, and both values of t_R and HETP were large in the case of using water only. Though the values of HETP decreased by addition of phosphoric acid, t_R were still very long for several organic compounds. In the case of using mixture of aqueous solution of phosphoric acid and methanol, t_R was not satisfactorily decreased. The mixtures of 0.02 mol L^{-1} phosphoric acid with different contents of acetonitrile were tested as mobile phases at a flowrate of 0.57 mL min^{-1}. The changes of t_R for several benzene substitutes with the content of acetonitrile are shown in Fig. 2, representatively. From these results, the mixture of phosphoric acid solution and acetonitrile of 15% was thought to be better than the other mobile phases, because t_R was not too long, and variation of t_R among compounds was large enough to separate various compounds. Consequently, the mixture of 0.02 mol L^{-1} phosphoric acid and acetonitrile (85:15) was selected as a standard mobile phase of this column for the analyses of various organic compounds in water.

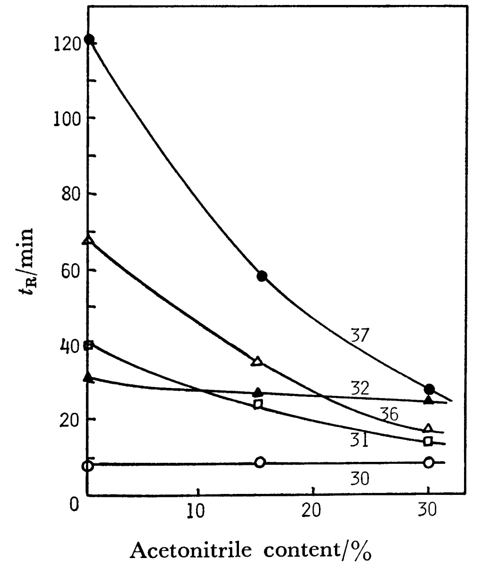


Fig. 2. Change of retention time with acetonitrile content in mobile phase for Shodex OH pak M614. 0.57 mL min^{-1}, 40 °C.

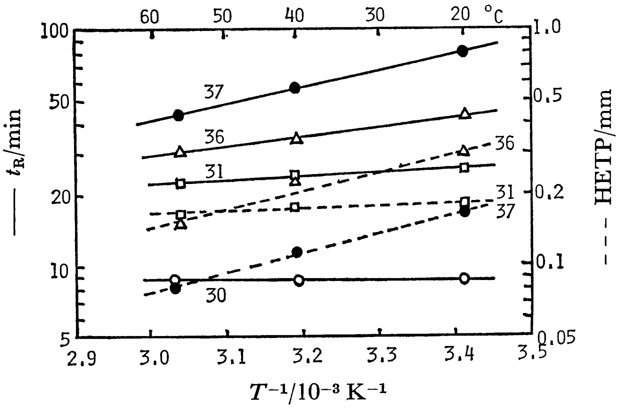


Fig. 3. Changes of retention time and HETP with temperature for Shodex OH pak M614. 0.02 mol L^{-1} H_3PO_4 and acetonitrile 15:85, 0.57 mL min^{-1}.

Influence of Column Temperature: Change of t_R and HETP with column temperature in the case of using the above standard mobile phase are representatively shown in Fig. 3 for several organic compounds. The values of HETP decreased with temperature, but the influence of temperature on the separation performance was small, because the apparent activation energies of separation were approximately 4 to 20 kJ mol^{-1}. Since the temperature higher than 40 °C made operation troublesome, 40 °C was chosen as a standard temperature in the following experiments.

Relation between Retention Times and Properties of Organic Compounds: Values of t_R and HETP for various organic compounds obtained using the standard mobile phase at a flowrate of 0.57 mL min^{-1} and 40 °C are shown in Table 3. The values of HETP for all the organic compounds were small ranging from approximately 0.13 mm to 0.25 mm.

The retention times of benzenesulfonate, ethylbenzenesulfonate, and naphthalenesulfonate were short, approximately 10 min, but those of alkylbenzenesulfonates in a synthetic detergent could be classified into 5 groups ranging from 20 to 60 min according to the number of alkyl carbons which ranged from 10 to 14 as shown in Fig. 4. The retention times of the aromatic compounds except the low molecular weight sulfonates exceeded 20 min. It was found that the retention times for hydrophobic aromatic compounds tend to be longer than those of hydrophilic compounds. The logarithmic relationships between the retention times and solubilities in water are shown in Fig. 5 for the aromatic compounds. These relationships could be shown by two lines for the two groups of the carboxylic acids and the others. The

TABLE 3. VALUES OF t_R AND HETP FOR VARIOUS COMPOUNDS WITH SHODEX OH PAK M614

Compound number	t_R/min	HETP/mm	Compound number	t_R/min	HETP/mm
4	13.0	0.25	30	8.7	0.20
5	18.3	0.22	31	23.9	0.18
6	15.8	0.25	32	27.6	0.19
8	9.7	0.14	33	24.1	0.18
9	13.3	0.13	34	26.0	0.22
10	14.5	0.17	35	25.3	0.24
12	11.8	0.20	36	36.0	0.21
14	16.1	0.17	37	58.0	0.12
16	13.3	0.18	38	20.0	0.16
17	13.8	0.17	39	28.5	0.20
18	13.8	0.17	40	28.7	0.20
19	10.3	0.16	41	32.7	0.18
20	12.8	0.19	42	35.9	0.16
22	10.9	0.24	43	31.8	0.20
23	13.0	0.16	44	23.1	0.22
24	13.5	0.18	45	9.1	0.25
25	15.7	0.18	47	9.9	0.24
26	11.7	0.15	48	84.0	0.13

Flow rate: 0.57 mL min^{-1}, mobile phase: 0.02 mol L^{-1} phosphoric acid and acetonitrile (85:15), temp: 40 °C.

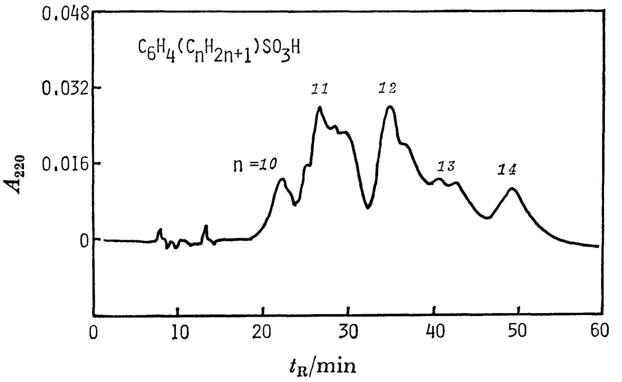


Fig. 4. Chromatogram of alkylbenzenesulfonates in synthetic detergent with Shodex OH pak M614. 0.02 mol L^{-1} H_3PO_4 and acetonitrile 15:85, 0.57 mL min^{-1}, 40 °C.

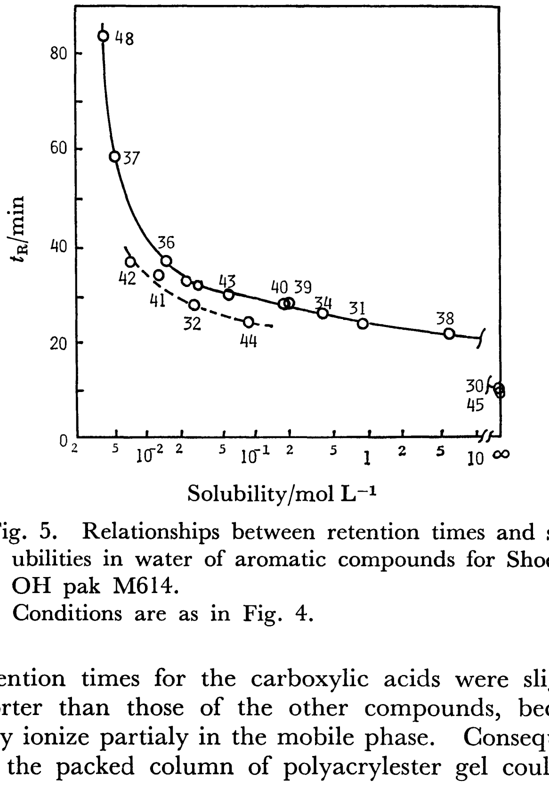


Fig. 5. Relationships between retention times and solubilities in water of aromatic compounds for Shodex OH pak M614.
Conditions are as in Fig. 4.

retention times for the carboxylic acids were slightly shorter than those of the other compounds, because they ionize partialy in the mobile phase. Consequently, the packed column of polyacrylester gel could be used for the direct analyses of various aromatic compounds in water. The retention time of an aromatic compound can, therefore, be predicted from its chemical structure and solubility, and conversely, the compound in an unknown sample can be characterized or identified from its retention time. Since the retention times of all the used aliphatic compounds, whose solubilities in water are larger than 10^{-1} mol L^{-1} were in the range from 10 to 18 min, separation was difficult when many kinds of aliphatic compounds coexist in water. However, the peaks for an unknown sample within 20 min could be characterized as the peaks of aliphatic compounds or the low molecular weight aromatic sulfonates.

Characteristics of Polyhydroxystyrene Gel Column.
Mobile Phase: Similar to the polyacrylester column, the column of poly(*p*-hydroxystyrene) gel (Shodex Ion pak C811) can be used with water or aqueous solutions containing less than 30% polar organic solvents. Further, the retention times with water or phosphoric acid as mobile phase were also very long for several hydrophobic compounds. Thus as with the case of the polyacrylester column, the mixture of 0.02 mol L^{-1} phosphoric acid and acetonitrile (85:15) was used for mobile phase, and the results are shown in Table 4. By this mobile phase, most organic compounds eluted within 60 min at a flow rate of 1.0 mL min^{-1}. The values of HETP were in the range from 0.10 mm to 0.21 mm for many organic compounds. This values were slightly better than those of the polyacrylester column. But amines and amino acids were not eluted within 120 min at a flow rate of 1.0 mL min^{-1}, because these cationic compounds combined strongly with the anionic group of the gel. Conversely, the retention times of all the sulfonates including alkylbenzenesulfonates in detergent were short and approximately 8.5 min, because they completely ionize to anions in mobile phase. Therefore, the anionic resin could not be used for separation of cationic compounds such as amines and amino acids and strong anionic compounds such as sulfonates.

Table 4. VALUES OF t_R AND HETP FOR VARIOUS COMPOUNDS WITH SHODEX ION PAK C-811

Compound number	t_R/min	HETP/mm	Compound number	t_R/min	HETP/mm
1	20.2	0.15	27	11.4	0.10
2	23.4	0.13	28	9.8	0.18
3	45.6	0.11	29	9.4	0.18
4	18.2	0.18	30	8.2	0.20
5	20.5	0.16	31	32.0	0.16
6	24.5	0.13	32	37.5	0.15
7	27.4	0.12	33	50.3	0.17
8	14.3	0.09	35	53.2	0.17
9	15.0	0.14	36	66.8	0.18
10	17.1	0.16	37	102.3	0.19
11	19.0	0.12	38	21.4	0.15
12	19.3	0.10	39	41.3	0.18
13	22.9	0.10	40	41.5	0.18
14	24.5	0.12	41	37.5	0.15
15	20.7	0.11	42	53.0	0.18
19	11.1	0.18	45	8.4	0.21
20	12.7	0.17	46	8.5	0.20
21	11.7	0.14	47	8.5	0.21
22	11.4	0.16			

Flow rate: 1.0 mL min^{-1}, mobile phase: 0.02 mol L^{-1} phosphoric acid and acetonitrile (85:15), temp: 40 °C.

Relation between Retention Times and Properties of Organic Compounds: The values of t_R of the aromatic compounds except sulfonates exceeded 20 min, and it was found that t_R tend to decrease with solubilities in water similar to the case of the polyacrylester column. Changes of t_R for the aromatic compounds with their solubilities are shown in Fig. 6. These relationships could be shown by two lines for carboxylic acids and the other compounds as in the case of the polyacrylester column. The retention times of the carboxylic acids were shorter than those of the other compounds for a same solubility, because they ionize partialy to anions in mobile phase. On the other hand, there were no clear relationships between t_R and solubilities in water for aliphatic compounds. Variations of t_R with molecular weight are shown in Fig. 7 for each group of aliphatic compounds. The values of t_R for all the aliphatic compounds increased with their molecular weight in the similar series of compounds, and t_R increased in the following order for the compounds which have the same number of alkyl carbons: alcohols > aldehydes > ketones $\simeq$ esters > monocarboxylic acids > dicarboxylic acids. However, the retention times of sugars decreased with molecular weight by gel permeation function as shown in also Fig. 7, and raffinose was eluted at a very short time. Consequently, this column could not be used for separation of nonaromatic compounds with molecular weights exceeding 600.

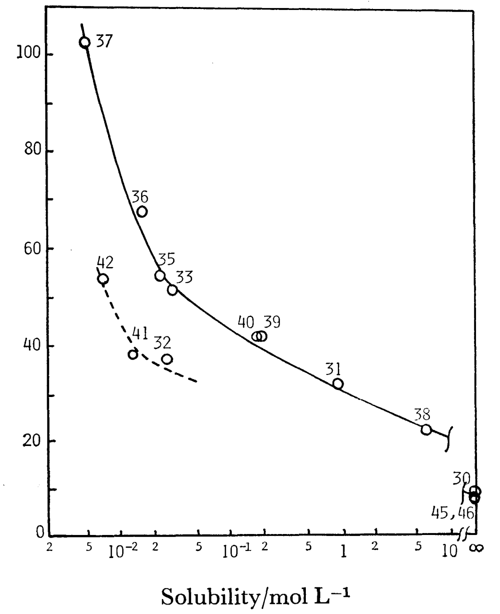


Fig. 6. Relationships between retention times and solubilities in water of aromatic compounds for Shodex Ion pak C811.
Flow rate; 1.0 mL min^{-1}, other conditions are as in Fig. 4.

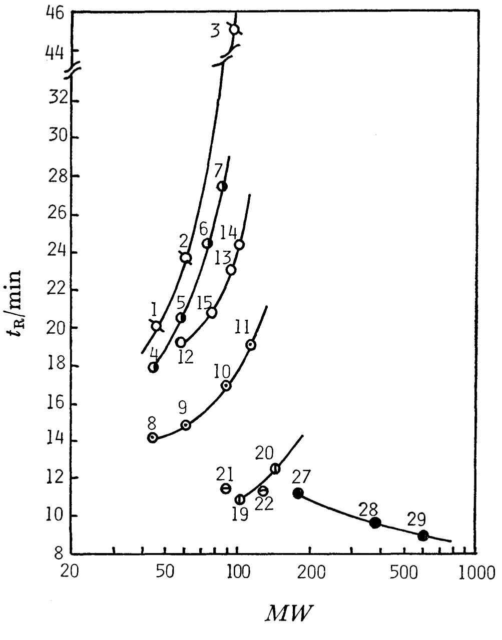


Fig. 7. Relationships between retention times and molecular weights of aliphatic compounds for Shodex Ion pak C811.
Conditions are as in Fig. 6.

From the foregoing results, the following conclusions were obtained:

(1) The column of polystyrene gel was unsuitable for direct analyses of water samples containing various organic compounds.

(2) The column of polyacrylester gel could be used for direct analyses of various organic compounds in water, but it was unsuitable for analyses of aliphatic compounds.

(3) The column of polyhydroxystyrene gel could be used for direct analyses of various aliphatic and aromatic compounds in water, but it could not be used for analyses of strong anionic compounds such as sulfonates, cationic compounds such as amines and amino acids, and nonaromatic compounds whose molecular weights exceed 600.

Application to Wastewater Analyses. For examples, raw and treated wastewaters from an oil refinery and a dye factory were directly analyzed after filtration with 0.45 μm membrane by the HPLC with Shodex OH pak M614 using the standard mobile phase at 40 °C. The chromatograms are representatively shown in Figs. 8 and 9. Eight peaks were found in the chromatogram of the raw wastewater from the oil refinery. The peaks 1—6 were characterized to be low molecular weight aliphatic compounds, and the peaks 7 and 8 were identified to be phenol, and *o*- and *m*-cresols respectively, the main pollutants in this wastewater, by their retention times, UV absorbances at two wave lengths,[5] and coloration with 4-aminoantipyrine. The chromatogram of its treated

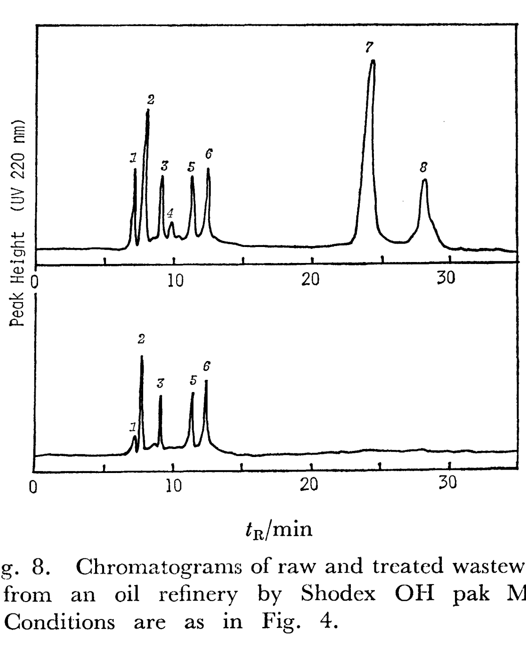


Fig. 8. Chromatograms of raw and treated wastewaters from an oil refinery by Shodex OH pak M614. Conditions are as in Fig. 4.

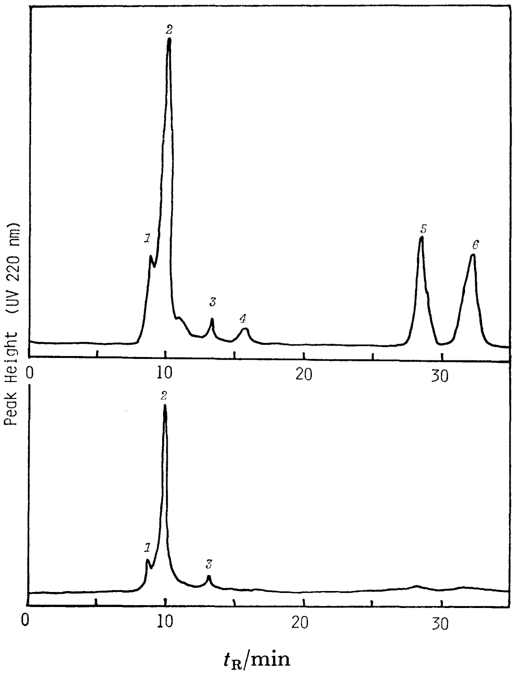


Fig. 9. Chromatograms of raw and treated wastewaters from a dye factory by Shodex OH pak M614. Conditions are as in Fig. 4.

water showed that phenol and cresols were completely removed by adsorption with activated carbon, but aliphatic compounds were only partially removed. On the other hand, six clear peaks were found in the chromatogram of the raw wastewater from the dye factory. The peaks 2—6 were identified to be naphthylaminedisulfonate, acetic acid, produced dye, cresols, and *m*-toluidine, respectively, and the peak 1 was assumed to be an aromatic sulfonate. The chromatogram of its treated water showed that dye, cresols, and toluidine were effectively removed by adsorption with activated carbon, but naphthylaminedisulfonate, acetic acid, and an unknown compound (peak 1) were only partially removed. As shown in these examples, the HPLC systems could be applied in direct analyses and characterizations of water containing various organic pollutants, and they are more superior than the conventional water quality tests in the evaluation of water quality and water treatment processes.

References

1) N. Tambo and T. Kamei, *Water Res.*, **12**, 931 (1978).
2) K. Urano, K. Katagiri, and K. Kawamoto, *Water Res.*, **14**, 741 (1979).
3) K. Urano, K. Ogura, and H. Wada, *Water Res.*, **15**, 225 (1980).
4) K. Urano, H. Maeda, K. Ogura, and H. Wada, *Water Res.*, **16**, 323 (1982).
5) K. Urano, K. Kawamoto, and K. Hayashi, *Jpn. J. Water Poll. Res.*, **4**, 43 (1981).