Evaluation of Chitin and Chitosan as a Sorbent for the Preconcentration of Phenol and Chlorophenols in Water

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The evaluation of the applicability of chitin and chitosan as a sorbent material to solid phase extraction of phenol and chlorophenols was made by using high-performance liquid chromatography (HPLC). For the measurement of the adsorptive capabilities, adsorption isotherms as well as breakthrough curves were obtained by means of batch experiments or column experiments respectively. The increasing tendency of the amount of adsorption with the number of chlorine atom in phenol molecules was observed for both chitin and chitosan. The driving force of the interaction could be attributed to hydrogen bonding among electro-negative atoms in chitin, chitosan or chlorophenols.

Keywords Chitin, chitosan, sorbent, solid phase extraction, phenol, chlorophenol, adsorptive capability

Chitin, poly- β -(1 $\rightarrow$ 4)-*N*-acetyl-D-glucosamine, the main component of shells of Crustacea such as crabs, shrimps and various insects is the most widely occurring natural biopolymer along with cellulose. Chitin is clustered with calcium carbonate and proteins whereas chitosan is prepared from chitin by deacetylating its acetamide groups with a strong alkaline solution.^{1,2} Though chitosan is found naturally in the cell walls of certain fungi, these sources are not yet utilized for industrial use.³ At the present, chitosan is obtained predominantly through alkaline deacetylation of chitin.

Since chitin and chitosan have various bioactivities and functionalities, they attract much attention and are studied in various fields of application.⁴⁻⁸ While chitin is insoluble in most solvents, chitosan is readily soluble in acidic solutions and easily available for industrial purposes.⁹ Many applications are due to the secondary amino groups of chitosan which show polycationic, chelating and film-forming properties along with high solubility in dilute acetic acid.¹⁰

For the chromatographic determination of low concentrations of environmental organic pollutants, especially in complex matrices, the preconcentration of samples is necessary. Considerable attention is being paid to solid phase extraction (SPE) as an alternative over the laborious and time-consuming liquid-liquid extraction (LLE). Various types of sorbents, such as octadecyl-bonded silica (C₁₈)¹¹, porous polymeric resins¹², carbon-based sorbents¹³, ion-exchange resins¹⁴ are being used for that purpose.

At the present study, the applicability of chitin and chitosan as a sorbent material in solid phase extraction of environmental organic contaminants like chlorophenols

was evaluated by using a high-performance liquid chromatograph equipped with a diode-array detector (DAD).

Experimental

Chemicals and reagents

Phenol (Ph), 2-chlorophenol (2-CP), 4-chlorophenol (4-CP), 2,4-dichloro-phenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), pentachlorophenol (PCP) were obtained from Fluka (Ronkonkoma, NY, USA). Stock solutions of 2000 mg/l of each compound were prepared with methanol and stored in a refrigerator. Working solutions were prepared by diluting the stock solutions with methanol just prior to use. HPLC-grade acetonitrile was obtained from J. T. Baker (Phillipsburg, NJ, USA). Distilled water was further purified with an ultrapure cartridge system (Barnstead/Thermolyne, USA). All other reagents were of analytical grade. For the preparation of the mobile phase, acetonitrile or water were filtered through a 0.2 μ m nylon filter (Whatman, Maldstone, England) or 0.45 μ m cellulose nitrate filter (Whatman) respectively.

Apparatus

Chromatographic experiments were performed using a Hewlett-Packard (Waldbronn, Germany) 1050 Series ternary gradient liquid chromatograph with a diode-array detector. The system control and data analysis were executed by a HPLC3D Chemstation (DOS series). Separation was carried out using a Mightysil RP-18 GP C18 column (250 $\times$ 4.6 mm i.d., 5 μ m) from Kanto Chemical Co., Inc. (Tokyo, Japan). A Rheodyne Model 7125 injector having a 20 μ l loop was used.

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Chromatographic conditions and detection

The chromatographic separations were carried out by a gradient of acetonitrile and 10^{-3} M phosphoric acid mixing from 38:62 to 95:5 within 20 min. The mobile phase was degassed by helium (99.99%) purging during the gradient elution. In order to determine the optimum wavelengths for detection, UV spectra were recorded from 190 to 500 nm for each phenol. The detection wavelength was set at 225 nm in case of monitoring all phenols simultaneously.

Sorbents

Chitin and chitosan were obtained from Sigma (St. Louis, MO, USA). They were ground and sieved to 100 – 150 mesh, followed by purifying with a Soxhlet extraction and sonication in the media of organic solvents sequentially. The structures of chitin and chitosan are shown in Fig. 1.

Batch type determination

For the determination of the relationship between the amount of sorbent and the amount adsorbed, 25, 50, 100 mg of sorbents with aliquots of standard solution in water (pH 3 adjusted with hydrochloric acid) were mixed in a glass vial followed by shaking for 30 min with a Fisher Vortex-Genie 2 Mixer (Fisher Scientific, Pittsburgh, PA, USA).

In order to obtain the adsorption isotherms, 100 mg of each sorbent, standard solution and water (pH 3 with HCl) were mixed in a glass vial to give the desired concentration of each compound. It was also shaken for 30 min in order to reach equilibrium. Twenty microliters of aliquot, after filtering through a $0.45\ \mu\text{m}$ PVDF syringe filter (Whatman), were injected to the HPLC.

Experiment with column

For the measurements of breakthrough volume, a stainless-steel column ($20 \times 2\ \text{mm}$ i.d.) was introduced. The sorbent was slurry-packed with methanol under

vacuum and the top and bottom of each sorbent bed were fitted with stainless-steel frits ($10\ \mu\text{m}$ pore size).

Before measurements, sorbents were flushed with water (pH 3 with HCl) in order to remove the remaining methanol until the baseline stability was obtained. To obtain breakthrough curves, a standard solution of 0.1 mg/l of phenols dissolved in water at pH 3 (with HCl) was pumped through the column and directly introduced to the detector at a flow rate of 2.5 ml/min.

Results and Discussion

Purification of chitin and chitosan

The impurities in the sorbent should be removed until satisfactory background was obtained because the presence of trace impurities has been shown to exert a vital effect on the analysis. Each sorbent was treated with Soxhlet extractor for 6 h following sonication for 30 min in methanol and acetonitrile.

The extracts of each solvent were concentrated with a rotary evaporator to a volume of 1 ml for examination of purity by HPLC under the same condition as for analytes with gradient elution. The purification step was repeated until the blank chromatogram reached a clear response beyond the range of interferences.

Breakthrough volumes

Breakthrough measurements were carried out only for chitin because chitosan swelled in aqueous phase. The breakthrough curves for selected phenols on chitin are shown in Fig. 2.

If the breakthrough volume is taken as the volume at which the signal reaches 5% of the total signal, the breakthrough volumes obtained for phenol, 2-chlorophenol, 2,4,6-trichlorophenol, pentachlorophenol were measured as 2.6, 2.8, 3.9 and 11.3 ml respectively.

The breakthrough volume was found to be dependent

Fig. 1 Chemical structure of chitin and chitosan.

Fig. 2 Breakthrough curves of Ph (●), 2-CP (▲), 2,4,6-TCP (■) and PCP (◆) recorded with a $20 \times 2\ \text{mm}$ i.d. precolumn packed with chitin.

Fig. 3 Freundlich plots of individual adsorption isotherms for chitin. (●) Ph; (▲) 2-CP; (■) 4-CP; (○) 2,4-DCP; (△) 2,4,6-TCP; (□) PCP.

Fig. 4 Freundlich plots of individual adsorption isotherms for chitosan. (●) Ph; (▲) 2-CP; (■) 4-CP; (○) 2,4-DCP; (△) 2,4,6-TCP; (□) PCP.

on the number of substituents in phenol molecules. As the number of substituents was increased, the breakthrough volume was also increased. It can be predicted that the main interaction in adsorption between chlorophenols and chitin is attributed to hydrogen bonding between chlorine, oxygen atoms in phenol molecules and hydrogen atoms in chitin showing more affinity as the number of chlorine atoms substituted increased.

Adsorption isotherms

In general, the adsorption isotherm defines the distribution of a solute between the ligand and solid phases after the adsorptive reaction has reached an equilibrium condition. Two of the most commonly used isotherms are the Langmuir and the Freundlich equations. Since the Langmuir equation gave a relatively poor fit, the applicability of Freundlich equation was examined.

The Freundlich equation represents an empirical relationship of the amount of adsorbate in the adsorbed phase to that in solution:

$$q_e = K_F c_e^{1/n}$$

where q_e is the amount adsorbed per unit mass of

adsorbent, c_e is the remaining concentration in the aqueous solution after adsorption, K_F is the Freundlich constant and $1/n$ is an arbitrary parameter. A linearized form of this equation can be obtained as follows:

$$\log q_e = \log K_F + (1/n) \log c_e$$

A plot of $\log q_e$ vs. $\log c_e$ would give $1/n$ and K_F from the slope and intercept. The results are shown in Figs. 3 and 4. The intercept, K_F , is an indicator of adsorption ability and the slope, $1/n$, is an indicator of concentration dependence of adsorption. The adsorption ability is higher when the value of K_F is larger.

Freundlich parameters and correlation coefficients obtained from Figs. 3 and 4 are listed in Table 1. As shown in Table 1, the K_F values increases as the degree of chlorination increases for both sorbents. This tendency is the same as that of breakthrough volume measured. Comparing chitin and chitosan, the K_F values of chitosan are larger than those of chitin. This result may be due to the swelling of chitosan. Chitosan is more hydrophilic than chitin because of polar amine group; thus it swells in aqueous media and the active sites for hydrogen bonding are well developed. The similar slopes of the isotherm lines for each sorbent indicate

Table 1 Parameters and correlation coefficients for the Freundlich equation

	Chitin			Chitosan		
	K_F	$1/n$	r	K_F	$1/n$	r
Ph	0.00045	1.879	0.942	0.0094	0.797	0.995
2-CP	0.00062	1.976	0.984	0.0103	0.884	0.918
4-CP	0.00088	1.865	0.996	0.0100	0.874	0.894
2,4-DCP	0.00243	1.501	0.983	0.0151	0.785	0.989
2,4,6-TCP	0.00203	1.594	0.992	0.0213	0.967	0.991
PCP	0.01282	1.200	0.998	0.0426	1.118	0.974

that the concentration dependence of phenols is similar without regard to the number of the substituents.

The present study has suggested the adsorption mechanism of phenols and chlorophenols for chitin and chitosan and the possibility of applying these sorbent materials to solid phase extraction. From the results of breakthrough volumes and adsorption isotherms, the main interaction in adsorption between the sorbents and phenols may be predicted to hydrogen bonding among electronegative atoms in chitin, chitosan or chlorophenols including chlorine, hydroxide or amine groups. Both chitin and chitosan may be useful in preconcentration of chlorophenols around environmental samples in the future.

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