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Note

Derivatization at the liquid–solid interface

Application to the trace analysis of organic acids in aqueous samples

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Organic trace analysis is frequently used in environmental monitoring and quality control, as well as in biomedical and many other problems. Substances at very low concentrations are usually enriched by solvent extraction and subsequent evaporation of the solvent. In recent years, macroreticular resins have been employed to a great extent to enrich the trace organics^{1,2}. However, in this method large amounts of solvent are needed to wash the resin, leading to lengthy periods of evaporation. In view of the strong sorbent–sorbate interaction, derivatization of the substance to be analysed directly on the surface of the resin seems an attractive notion. Preparation of fatty acid esters on ion-exchange resins was initiated by Hornstein *et al.*³, but little attention has been paid to this method since. It consists of dissolving the unsaponified fat containing natural occurring free fatty acids in petroleum ether, the latter then being selectively adsorbed on a strong anion-exchange resin. After the solution was filtered, the resin is washed and then esterified with anhydrous methanol–HCl. After subsequent filtering, washing, extraction and concentration, a petroleum ether solution of the esters is obtained. The trifluoroacetylation of amines on a cation exchanger has been reported, but neither details of the trace analytical technique nor any data were given⁴.

The present work refers to the adsorption of organic acids from aqueous solution on an anion-exchange resin and the transformation of the acids into their ethyl esters directly on the surface of the resin. This method works satisfactorily in the low ppm concentration range studied. The optimal conditions and the site of reaction have been studied.

EXPERIMENTAL

Reagents and chemicals

Boron trifluoride–diethyl ether (BF₃ 47–48%), sodium hydroxide, absolute ethanol, dichloromethane, all the dibasic acids and phenylacetic acid were obtained from Beijing Chemical Plant (reagent grade); absolute ethanol and dichloromethane were redistilled. The fatty acids, their ethyl esters, and *m*-methoxybenzoic acid were obtained from Sigma (St. Louis, MO, U.S.A.), OV-17 and Chromosorb W AW DMCS support were from Chrompack (Middelburg, the Netherlands). Anion-ex-

change resin #717 was purchased from Shanghai Resin Works (Shanghai, China). Double-distilled water was prepared in this laboratory.

Gas chromatography

The instrument used was a Perkin-Elmer F-17 gas chromatograph equipped with a flame ionization detector. The glass column, 2 m × 3 mm I.D., was packed with 5% OV-17 on Chromosorb W AW DMCS (60–80 mesh). The column temperature was programmed from 170°C to 210°C at 5°C/min for dibasic acids, and isothermal at 200°C and 160°C for fatty acids and aromatic acids, respectively. Nitrogen was the carrier gas at 35 p.s.i.g. head pressure.

Treatment of the ion-exchange resin

The resin was immersed in 1 *N* sodium hydroxide solution, stirred for 10 min and left to stand for 30 min. The alkaline solution was decanted off and the resin washed with water until the wash water became neutral; it was then stored in water until use.

Calibration

Dichloromethane solutions of the ethyl esters of lauric (12:0), myristic (14:0) and palmitic (16:0) acids were used for calibration with palmitic ester as the internal standard. Because the ethyl esters of dibasic and aromatic acids were not commercially available, the method proposed by Davis⁵, which should be valid at least on a relative basis, was used. In this method, the standards were prepared in two ways under the same conditions as for the actual analysis. In the first, method 2 μg of each acid to be studied were derivatized and diluted successively to five different concentrations: 8, 2.7, 0.8, 0.27 and 0.08 ng/ μl . In the second method, 20, 60, 200, 600 and 2000 ng of the acids were derivatized and diluted to give the same range of concentrations. If the calibration curves (log peak area vs. log picograms injected) obtained from the two series of solutions were superimposable, the derivatization reaction was considered quantitative and the resultant solutions were used as standards. In the present work, the acids were derivatized and the resultant ester solution of each acid was then adjusted to an identical concentration (see Table IV), supposing 100% transformation. Four or five different concentrations of each acid were tried, which varied by at least an order of magnitude. A 1- μl volume of each of these solutions was subjected to gas chromatographic analysis, and the peak heights were compared. The reaction was considered practically complete if the peak heights were close to one another for a certain acid derivatized at different concentrations. To these solutions was added the internal standard ($\text{C}_{14:0}$ ethyl ester for dibasic and $\text{C}_{10:0}$ ethyl ester for aromatic acids), and the mixture was further diluted to give the calibration standards. Peak heights relative to the internal standard were used for calibration.

Trace analysis of organic acids in aqueous solutions

The pH of the solution was adjusted to ca. 11 with 1 *N* sodium hydroxide, and aliquots from 20 to 100 ml (depending on the concentration) were put in a beaker. Then 0.8–2 g of the resin (see following text) were added, and the mixture was stirred for 15–20 min. The solution was filtered off and the resin washed five times with 2 ml of ethanol, to remove the water. The resin was transferred to a 20-ml test-tube,

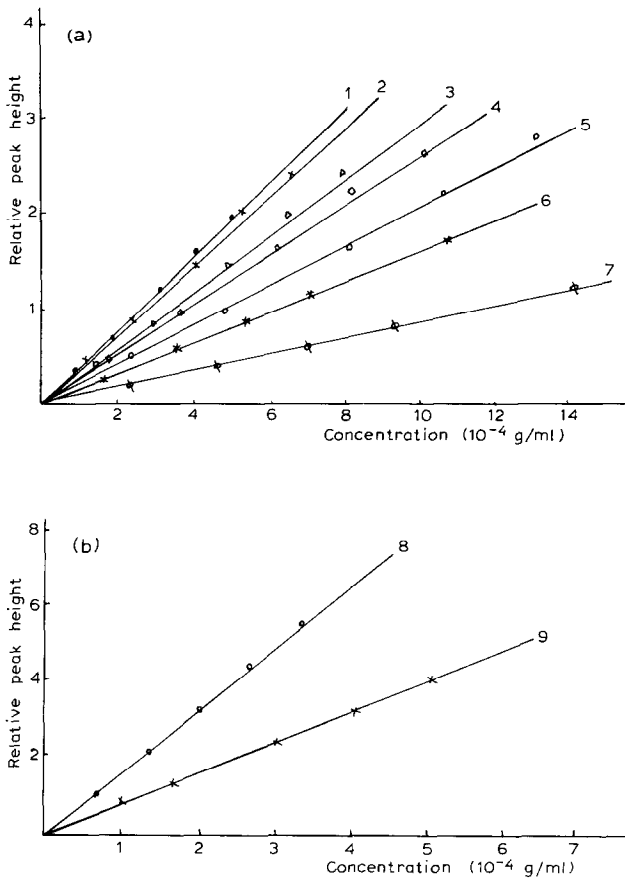


Fig. 1. Calibration curves for various acids. 1 = Glutaric; 2 = adipic; 3 = pimelic; 4 = suberic; 5 = sebacic; 6 = phenylacetic; 7 = *m*-methoxybenzoic; 8 = $C_{12:0}$; 9 = $C_{14:0}$.

and 2 ml of absolute ethanol and 0.5 ml of BF_3 -diethyl ether were added. The test-tube was heated in a $95^\circ C$ water-bath for 20, 80 and 110 min for the analysis of fatty, aromatic and dibasic acids, respectively. The contents were cooled, 5 ml of dichloromethane were added, and the mixture was shaken for a short time and filtered. The filtrate was washed with 2×5 ml of water in a 25-ml separating funnel, the organic layer was led through an anhydrous sodium sulphate column to remove the water, and the collected solution was evaporated in a graduated centrifuge tube (calibrated beforehand) to a fixed volume with a stream of pure argon.

RESULTS AND DISCUSSION

Calibration curve

Fig. 1 shows plots of relative peak heights against concentration for various acids. The concentration range covered was greater than that of the aqueous samples because of the enriching effect of this method.

Recovery

Recoveries (R) are listed in Table I. They were calculated for the acids contained in the aqueous solutions using the equation

$$R (\%) = \frac{c_i V_i f}{C V} \cdot 100$$

where C and V are the concentration and volume of the aqueous sample, C_i and V_i the corresponding values of the solution resulting from the trace analysis procedure, C_i being read from the calibration curve, and f is the correction factor. The latter is

TABLE I
RECOVERIES OF ORGANIC ACIDS AT VARIOUS CONCENTRATIONS

Volume of the aqueous solutions, 20 ml; weight of resin added, 0.8 g.

<i>Acid</i>	<i>Concentration in water (10⁻⁶ g/ml)</i>	<i>R (%)</i>	<i>S.D.</i>	<i>n</i>
C _{12:0}	10.0	94	8.0	9
	7.7	92	7.3	10
	0.8	77	4.6	8
C _{14:0}	11.5	99	4.8	9
	8.8	96	7.9	10
	0.9	85	6.8	8
Glutaric	3.1	90	8.0	8
	0.6	90	7.2	7
Adipic	4.1	87	4.5	8
	0.8	83	10.2	7
Pimelic	5.0	86	3.2	8
	1.0	85	8.6	8
	0.5	91	7.1	6
Suberic	6.3	86	4.5	8
	1.3	87	10.9	8
	0.6	92	8.2	6
Sebacic	8.0	84	3.6	8
	1.6	93	9.2	8
	0.8	97	10.0	6
Phenyl- acetic	3.7	87	6.4	7
	0.7	84	6.3	6
	0.3	108, 103	—	2
<i>m</i> -Methoxy- benzoic	9.9	104	6.9	7

TABLE II

RECOVERIES OF ORGANIC ACIDS FROM THE ETHANOL-TREATED RESIN AND THE ETHANOL FILTRATE

Acid	Concentration in water (10^{-6} g/ml)	Mean recovery (%)		Time of heating (min)	Number of tests
		Resin	Filtrate		
C _{12:0}	7.7	99	0	20	3
C _{14:0}	8.8	84	0	20	3
Glutaric	3.1	97	0	110	3
Adipic	4.1	89	0	110	3
Pimelic	5.0	87	0	110	3
Suberic	6.3	87	0	110	3
Sebacic	8.0	84	0	110	3
Phenylacetic	3.7	102	0	80	2
<i>m</i> -Methoxy- benzoic	9.9	84	0	80	2

unity for aromatic and dibasic acids, and for fatty acids is given by the ratio

$$\frac{\text{molecular weight of the acid}}{\text{molecular weight of the corresponding ester}}$$

Blank tests were run with water, resin and the reagent, and no interfering peak appeared at the sensitivity setting used in the analysis.

The reaction site

To investigate if the acids were esterified right on the surface of the resin, the following experiment was carried out. Resin with the adsorbed acids was heated in a 95°C water-bath together with 2 ml of ethanol but without BF₃ ether. After being heated for the same length of time as that for derivatization, the mixture was filtered.

TABLE III

RECOVERY OF DIBASIC ACIDS WITH DIFFERENT AMOUNTS OF RESIN USED FOR DIFFERENT VOLUMES OF WATER SAMPLE

Sample volume (ml)	Amount of resin (g)	Recovery of acid (%)				
		Glutaric, 3.14*	Adipic, 4.14*	Pimelic, 5.02*	Suberic, 6.34*	Sebacic, 8.2*
20	0.2	40	37	37	35	37
20	0.4	44	40	40	41	38
20	0.6	41	39	37	38	38
20	0.85	82	80	84	89	86
50	1.5	88	82	80	81	78
100	0.8	55	64	59	63	58
100	1.5	69	70	72	70	66
100	1.8	104	102	102	101	93

* Concentration of the acid in water, 10^{-6} g/ml.

TABLE IV

RELATIVE PEAK HEIGHTS OF THE ESTERS OF DIBASIC AND AROMATIC ACIDS AFTER ADJUSTMENT TO A THEORETICALLY IDENTICAL CONCENTRATION

Concentrations in 10^{-6} g/ml; RPH = relative peak height, with the data in sixth column as 1.00.

Acid	Concn. before derivatization						Adjusted concn.
	Conc.	13	26	260	520		
Glutaric	RPH	0.82	0.98	1.05	1.00		310
	Conc.	17	34	340	680		410
Adipic	RPH	0.90	1.03	1.04	1.00		
	Conc.	20	40	400	800		500
Pimelic	RPH	0.94	1.00	1.10	1.00		
	Conc.	25	50	500	1000		630
Suberic	RPH	0.89	1.07	1.00	1.00		
	Conc.	33	66	660	1320		820
Sebacic	RPH	1.11	1.16	1.09	1.00		
	Conc.	28	70	140	210	280	350
Phenylacetic	RPH*	0.92	1.02	1.20	1.00	1.05	
	Conc.	44	110	220	330	440	560
<i>m</i> -Methoxybenzoic	RPH*	0.90	0.95	0.87	1.00	1.05	

* Peak heights relative to the internal standard were compared for better quantitation.

Then 2 ml of ethanol and 0.5 ml of BF_3 ether were added to the resin, and only 0.5 ml of BF_3 ether was added to the ethanol filtrate. The procedure was repeated, and the ester contents were determined separately for the resin and the filtrate. Table II summarizes the results.

These results show that the acids stay on the resin until they are transformed into their esters, and that the derivatizing reaction proceeds at the liquid-solid interface. It is likely that a relatively high concentration of the acids at the surface leads to good results obtained.

The amount of resin to be used

In view of the low concentrations of the acids tested, the resin used in this work is in large excess. However, the use of a much smaller amount will render handling difficult. Table III lists the recoveries obtained with different amounts of resin.

It can be seen from the above results that the larger the sample volume is, the more resin is needed for a good recovery. Approximately 0.8, 1.5 and 2.0 g of (wet) resin were therefore used for sample volumes of 20, 50 and 100 ml, respectively, irrespective of the acid concentration.

The yields of esterification of dibasic and aromatic acids

Aromatic and dibasic acids in the concentration range 10^{-5} – 10^{-4} g/ml ethanol

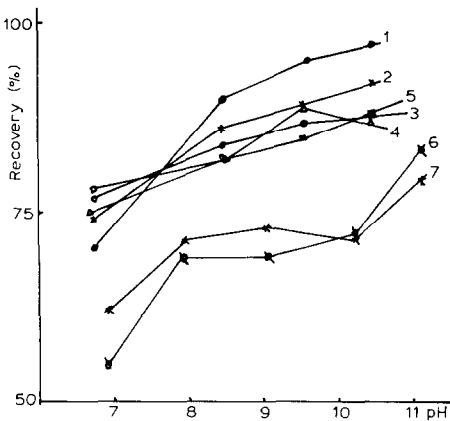


Fig. 2. Effect of pH on the recovery. 1 = Glutaric; 2 = adipic; 3 = pimelic; 4 = suberic; 5 = sebacic; 6 = $C_{12:0}$; 7 = $C_{14:0}$.

were derivatized and then adjusted to an identical concentration (assuming 100% transformation) for each acid. The relative peak heights of the esters were similar for each acid (Table IV). This illustrates the consistency of the reaction yields and the validity of Davis' method⁵.

Effect of pH on adsorption

The pHs of aqueous samples were adjusted to the basic side prior to adsorption to enhance the ionization of the acid molecules. The effect of pH on recovery is shown in Fig. 2. It is evident that the more weakly ionizing (fatty) acids need higher pH. A pH of 11 is sufficient for the adsorption of all the different kinds of the acid tested in this experiment.

Reaction time

The time needed for a complete reaction varies with different acids (Fig. 3). For the weakly ionizing fatty acids 20 min is sufficient, whereas for the aromatic and dibasic acids a much longer time is required.

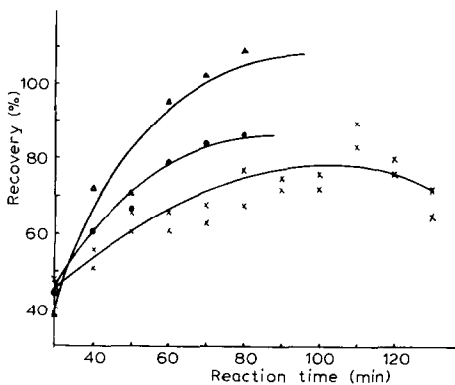


Fig. 3. Variation of recovery of the acids with reaction time. ▲ = *m*-Methoxybenzoic; ● = phenylacetic; × = all the dibasic.

CONCLUSION

Adsorption of organic acids from water and subsequent derivatization directly on the surface of the adsorbent resin is a promising method of trace analysis. The derivatization is an interfacial reaction, capable of yielding good and reproducible results.

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