

음이온 교환크로마토그래피에 의한 벤조산 및 그 유도체들의 분리에 관한 연구

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A Study on Separation of Benzoic Acid and Its Derivatives by Anion Exchange Chromatography

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요 약. 벤조산과 그 유도체들에 대한 음이온 교환분리를 여러 농도의 염화니켈—메탄올 용매내에서 연구하였다. Amberlite CG—400, Cl⁻ form에 대한 유기산들의 부피분배계수를 측정하고 이 분배계수로부터 제시된 적절한 농도의 NiCl₂—MeOH 용액을 용리액으로 사용하여 몇개의 혼합유기산을 정량적으로 분리하였다. 모든 유기산들의 농도는 자외선 분광광도계를 사용하여 정량하였다.

Abstract. The anion exchange separation of a number of benzoic acid and its derivatives has been studied in various concentrations of NiCl₂—methanol solvent medium. The volume distribution coefficients with Amberlite CG—400, Cl⁻ form have been measured in these medium. Several synthetic mixtures of organic acids were separated quantitatively by elution with a suitable concentration of NiCl₂—MeOH solution suggested by the distribution data. The concentration of organic acids were determined by UV spectrophotometry.

Introduction

Anion exchange chromatography technique has been extensively applied to the separation and determination of organic acids. C. Davies and his coworkers¹ have investigated the ion exchange behavior of aliphatic and aromatic organic acids toward strongly anion exchange Dowex 1—X8 in the formic acid medium. N. E.

Skelly and W. B. Crummett² separated benzoic acid and three isomers of hydroxybenzoic acid with Dowex 2—X8, acetate form. Rudolph H. Stehl³ used lithium chloride—mixed solvent (1:1:1 water: acetonitrile: methanol) as an eluent in the separation of aromatic sulfonates with Bio—Rex 5, chloride form. In these papers, however, it has been shown that aromatic acids, such as aromatic sulfonic and hydroxybenzoic

acid are retained strongly by the anion exchange resin and a longer time is consumed to elute such acids.

On the other hand, in the separation of organic acids by chromatography on anion exchange resin, advantage can be taken of the complex formation between metal ions present in the eluent and the species to be separated. In the previous papers⁴⁻⁶, zinc acetate, copper acetate and magnesium acetate have been known as an useful eluent which can form complexes in the separation of a number of dibasic organic acids. K. S. Lee and D. W. Lee⁷ suggested that a ferric chloride-organic solvent solution which reacts with some aromatic organic acids such as salicylic and benzohydroxamic acid to form colored stable nonadsorbable complexes was very useful as the eluent for the separation of organic acids.

The aim of the present work was to study the application of nickel chloride-methanol solvent solution as an eluent in the separation of benzoic acid and its derivatives.

The volume distribution coefficients of these acids in the various media were measured to determine the elution behavior and to find out optimum conditions of separation. The compound which has small dissociation constant such as aminobenzoic acids was adsorbed weakly on the resin, while hydroxybenzoic acids were adsorbed strongly because of their large ionization. Although phthalic and 3-nitrophthalic acid are strong acids, these acids are eluted more rapidly than others. Because they can form complex with Ni(II) ion in the eluent.

Experimental

Reagents and Apparatus. The ion exchange column of 1.5 cm internal diameter was filled with Amberlite CG-400(100 to 200 mesh, Cl⁻ form) and the resin was compacted by pour-

ing with water-methanol solvent at 1 ml per minute flow rate. When packed in this manner, resin bed was adjusted to 23.5 cm. Stock solutions of organic acids(2 mg per ml) were prepared by dissolving analytical reagent grade chemicals in water-methanol solvent. NiCl₂-MeOH solution were prepared so that the amount of methanol is expressed as percentage by volume and nickel chloride as molarity.

Volume Distribution Coefficient. The peak elution volumes(\bar{v}) were determined from experiments with single acids at various eluent concentrations. From these values, the volume distribution coefficients (Dv) were calculated from the equation⁸

$$\bar{v}/X' = Dv + \epsilon$$

Where X' is the corrected column volume and ϵ the void fraction of the column.

Flow rate elution of eluent from column was maintained to 0.4 ml to 0.7 ml per minute. The organic acids in eluates were determined by UV spectrophotometry.

Results and Discussion

Volume Distribution Coefficients (Dv). Volume distribution coefficients using column method were measured to investigate the elution behavior and to find out the optimum condition of separation. The results are given in Table 1. As indicated in Table 1, the order of Dv values is the same as the order of strength of acids in all concentrations. The magnitude of Dv values generally increases with decreasing the pK_a value of acids, except for phthalic and 3-nitrophthalic acid. Therefore, it can be seen that *p*-aminobenzoic acid having large pK_a value (4.84) is adsorbed weakly by the anion resin, while *o*-hydroxybenzoic acid($pK_a=2.97$) is adsorbed much stronger than the other acids under study. However, although *p*-hydroxy-

Table 1. *D_v* values of benzoic acid and its derivatives in various concentrations of $\text{NiCl}_2\text{--MeOH}$, with Amberlite CG-400, Cl^- form, 100 to 200 mesh resin. Flow rate; 0.4~0.7 ml/min. Resin bed; 1.5×23.5 cm

Acid	Eluent					
	0.05 M NiCl_2		0.1 M NiCl_2		0.2 M NiCl_2	
	20 % MeOH	40 % MeOH	20 % MeOH	40 % MeOH	20 % MeOH	40 % MeOH
Benzoic	19.5	13.9	13.2	7.48	7.15	3.02
<i>o</i> -Aminobenzoic	13.6	8.69	8.31	3.02	3.41	1.88
<i>p</i> -Aminobenzoic	17.7	12.5	9.83	4.54	4.16	2.27
<i>p</i> -Nitrobenzoic	51.4	15.9	24.6	4.16	12.8	2.27
<i>o</i> -Hydroxybenzoic	>80	>80	>80	64.2	53.7	23.8
<i>p</i> -Hydroxybenzoic	57.1	28.7	29.1	11.3	14.4	5.68
Phthalic	13.2	8.31	7.86	3.77	3.02	1.88
3-Nitrophthalic	10.9	5.67	6.80	2.65	2.27	1.51

benzoic acid has large pK_a value(4.48) compared with benzoic acid ($pK_a=4.20$) and *p*-nitrobenzoic acid ($pK_a=3.40$), it is bound strongly by the resin and emerges much later from the ion exchange column than either of these compounds. In order to explain the phenomena, it is assumed that other factors² such as molecular configuration, adsorption, solubility, etc, besides ionic strength probably have some effect on the distribution coefficient.

On the other hand, in spite of large ionization constant, 3-nitrophthalic and phthalic acid ($pK_{a1}=3.00$ $pK_{a2}=5.28$) have small *D_v* values. This is because the formation of non-adsorbable complexes between Ni(II) ion and dibasic carboxylic acid has a predominant influence on distribution coefficient, as one would expect.

Another important factor which has influence on the distribution coefficient is the methanol percentage of the eluent. It was observed that the distribution coefficients of all compounds decreased when there were increases in methanol percentage. Especially, 3-nitrobenzoic acid was greatly decreased and eluted more quickly with increasing methanol percentage. The results

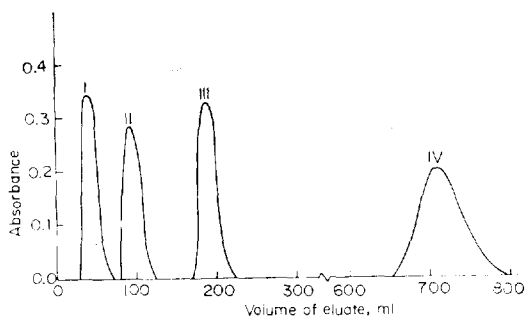


Fig. 1. Separation of 2.0 mg phthalic(I), 2.0 mg benzoic(II), 1.0 mg *p*-hydroxybenzoic(III), and 2.0 mg *o*-hydroxybenzoic(IV). Eluent: 0.2 M $\text{NiCl}_2\text{--}20\%$ MeOH solution, Flow rate: 0.5~0.7 ml/min.

suggest that the decrease of distribution coefficients by addition of methanol is related to the decrease of dielectric constant of the eluent, dissociation of organic acids and the interaction of resin and solvent⁹.

Separation of Mixtures. From the results presented in Table 1, the separation of the synthetic organic acid has been attempted.

The chromatogram shown in Fig. 1 demonstrates the separation of phthalic, benzoic, *p*-hydroxybenzoic, and *o*-hydroxybenzoic acid. These four acids were quantitatively separated

by elution with 0.20 M NiCl_2 -20 % MeOH solution. In this condition, 3-nitrophthalic acid and *p*-nitrobenzoic acid were overlapped with phthalic and *p*-hydroxybenzoic acid each other and there were not included in Fig. 1. Ortho and para aminobenzoic acid appeared in the same elution band as suggested from D_v values. In the separation of a mixture of *p*-nitrobenzoic acid and *p*-hydroxybenzoic acid, *p*-nitrobenzoic acid was separated quantitatively from *p*-hydroxybenzoic acid by elution with 0.05 M NiCl_2

-40 % MeOH solution. This elution curve is shown in Fig. 2. As mentioned above, as D_v value of *p*-nitrobenzoic acid is greatly influenced by addition of methanol, *p*-nitrobenzoic acid is eluted more rapidly than *p*-hydroxybenzoic acid with increasing methanol percentage and decreasing the concentration of nickel chloride. Therefore, both acids could be separated each other in 0.05 M NiCl_2 -40 % MeOH media.

Fig. 3 shows the results from a run in which stepwise elution was employed in separating four component mixtures, 3-nitrophthalic, *p*-nitrobenzoic, *p*-hydroxybenzoic and *o*-hydroxybenzoic acid.

In all cases, *o*-hydroxybenzoic acid which was held very strongly on resin appeared very late and was easily separated from the other acids, whereas two aminobenzoic acids including 3-nitrophthalic and phthalic acid could not be separated completely in present studies.

Consequently, it should be emphasized that separation can be enhanced if experimental conditions such as column height, rate of flow, particle size of resin and the size of the samples are adjusted differently.

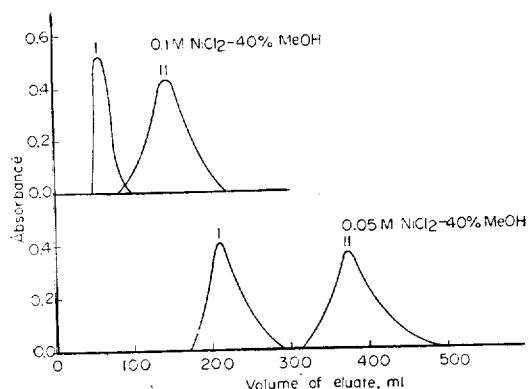


Fig. 2. Separation of 1.5 mg *p*-nitrobenzoic(I) and 1.5 mg *p*-hydroxybenzoic acid(II) by elution with NiCl_2 -MeOH solution. Flow rate: 0.4~0.6 ml/min.

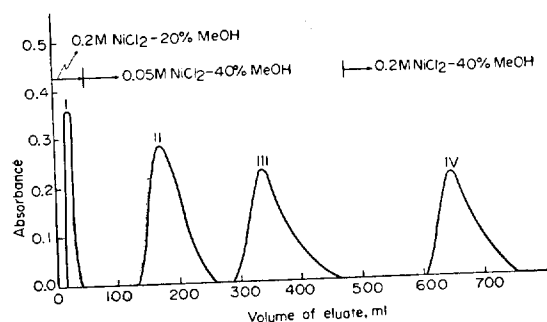


Fig. 3. Separation of 0.8 mg 3-nitrophthalic(I), 1.0 mg *p*-nitrobenzoic(II), 1.0 mg *p*-hydroxybenzoic(III), and 1.5 mg *o*-hydroxybenzoic acid(IV) by stepwise elution with NiCl_2 -MeOH solution. Flow rate: 0.4~0.6 ml/min.

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