LIQUID CHROMATOGRAPHY OF ASPIRIN, SALICYLIC ACID AND ACETIC ACID ON CATION EXCHANGE RESIN

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A 8% cross-linked sulfonated polystyrene cation exchanger in the hydrogen or sodium form was used as stationary phase for the chromatography of aspirin, salicylic acid and acetic acid. The eluent is 0.02 M mineral acids or their sodium salts in aqueous ethanol. Phosphoric acid, nitric acid or sulfuric acid added in aqueous ethanol eluent made successful separation of aspirin, salicylic acid and acetic acid on hydrogen form cation exchanger.

INTRODUCTION

For the micro analysis of a mixture of aspirin and salicylic acid is still a troublesome problem nowadays. In the previous work in our laboratory, the ion exchange separation of aspirin, salicylic acid and acetic acid showed that aspirin and salicylic acid could not separate successfully by hydrogen form and ammonia form of polystyrene type cation exchange resin with aqueous ethanol elution[1]. Scrutinizing the elution volumes of aspirin and salicylic acid from the previous report[1]. they had slightly different value but emerged as broad bands. These peaks overlapped together and resulted in a broad band as shown in Fig. 1.[1] It might be the reason why they can not be separated. If we can get the peaks of aspirin and salicylic acid sharper we may obtain a good separation. This is our basic idea of investigating this experiment.

Dr. Walton[2] has reported that the nature of the counterion affects the elution volumes and has a marked effect on the sharpness of the elution bands. It is

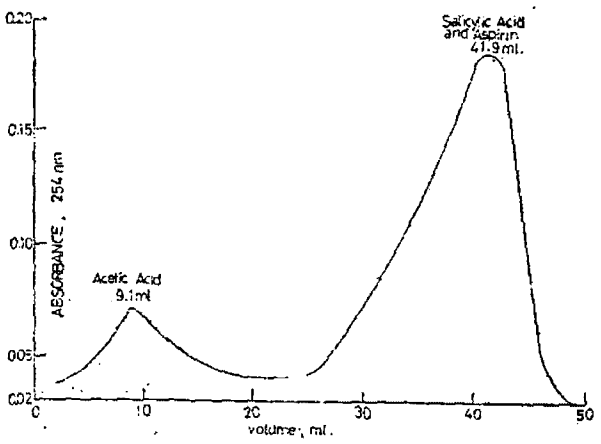

Fig. 1 Separation of aspirin, salicylic acid and acetic acid with H-form cation exchange resin, Solvent:25% ethanol Flow rate: 15 ml/hr.

common knowledge that ion exchange resins of the polystyrene type act as solid solvents for organic compound, especially those whose molecules have aromatic character[3]. Nomura *et al.*[4] showed carboxylic acids were retained much more strongly by hydrogen form resins than by sodium form resins.

In this investigation we tried to use hydrogen form and sodium form of polystyrene type cation exchange resin as stationary phase, ethanol aqueous solution as mobile

phase expecting to sharpen the peak bands and separate aspirin and salicylic acid completely. Nitric acid, sulfuric acid, phosphoric acid, boric acid, hydrofluoric acid, hydrochloric acid, hydrobromic acid, hydroiodic acid and their sodium salts were added in ethanol aqueous eluent.

EXPERIMENTAL

Apparatus

The apparatus used in this investigation was illustrated in previous report.[1] Column and fitting were obtained from Chromatronix Inc., Berkeley, Calif. U.S.A. A glass column 9 *mm* in diameter and 74 *cm* in length was mounted with a 0.5 *ml* sample injection value R6031SV. The eluent solution was filled in a stainless steel cylinder and driven under moderate pressure of nitrogen gas. 0.5 *ml* samples were injected by syringe and effluent were collected with a fraction collector. UV absorption of the effluent at 254 *nm.* was detected with Shimadzu UV-210 digital double-beam spectrophotomer.

Resin

Ion exchange resin AG 50 *W*-×8, 100-200 mesh, which is the polystyrensulfonic acid resin of Dowex-50*W*, was refined and sold by Bio-rad Corp., Richmond, Calif. U.S. Resin was converted to hydrogen form by stirring with 5% hydrochloric acid, or converted to sodium form by stirring with 10% sodium chloride solution, then transferred to a wide column and washed with more hydrochloric acid or sodium chloride solution then water. The resin was packed into column and it's bed depth was 60 *cm.*

Chemicals

Aspirin, mp. 135°, salicylic acid, mp. 159° and extra pure reagent grade of acetic acid were used in this experiment.

RESULTS AND DISCUSSION

Separations on Hydrogen form Cation-Exchange Resin:

The first experiments were made on the column of hydrogen form resin AG 50*W*-×8, 100-200 mesh, resin bed depth was 60 *cm.* Because of the wide range of particle sizes in this resin the bands were broad, but separation was good, and it was possible to study the effects of solvent and additives on the elution volumes and band widths. 1 *mg/ml* of aspirin, salicylic acid and acetic acid solution and mixture of aspirin, salicylic acid and acetic acid, containing 1 *mg/ml* each, were introduced by injection respectively, and eluted with

Table 1 Effect of Different Acids in Eluent on Hydrogen Form Resin

Resin form	Eluent	Mineral acids		Retention volume (ml) HOAc	Sal. acid	Aspirin
Hydrogen	25% Ethanol	None[1]	ind.	22.4	53.6	48.0
			mix.	18.9	41.9	41.9
		HNO_3 0.02M	ind.	22.5	79.6	43.6
			mix.	22.5	92.2	47.4
		H_2SO_4 0.02M	ind.	14.5	96.0	49.1
			mix.	14.5	101.0	49.2
		H_3PO_4 0.02M	ind.	28.4	97.7	47.3
			mix.	28.4	88.1	43.5
		H_3BO_3 0.02M	ind.	16.2	60.2	49.5
			mix.	16.2	53.0	53.0
		HF 0.02M	ind.	12.2	82.7	83.9
			mix.	13.2	83.6	83.6
		HCl 0.02M	ind.	10.6	84.0	87.6
			mix.	12.9	87.3	87.3
		HBr 0.02M	ind.	24.3	119.7	108.8
			mix.	18.9	112.6	112.6
		HI 0.02M	ind.	38.0	60.2	59.1
			mix.	38.7	59.5	59.5
	Dist. Water[1]	None	ind.	25.0	85.0	81.0
			mix.	25.0	73.3	73.3

ind=individual
mix=mixture

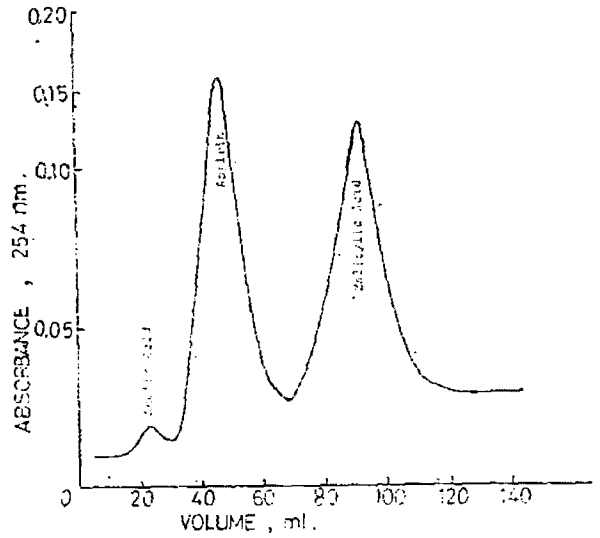

Fig. 2 Chromatogram of mixture of Acetic acid, Aspirin and Salicylic acid. Concentration: Each 1 mg/ml. Flow Rate: 15 ml/hr, Eluent: 25% Ethanol+0.02 M HNO_3

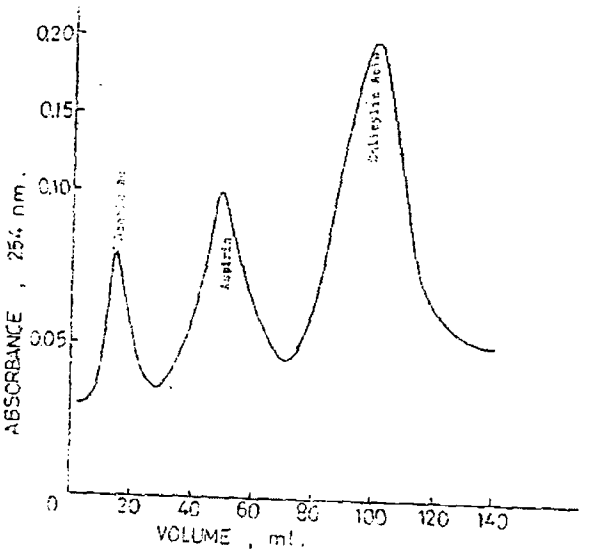

Fig. 3 Chromatogram of mixture of Acetic acid, Aspirin and Salicylic acid. Concentration: Each 1 mg/ml. Flow Rate: 15 ml/hr. Eluent: 25% Ethanol+0.02 M H_2SO_4

25% ethanol aqueous solution. The flow rate was 15 *ml/hr*. In each experiment, nitric acid, sulfuric acid, phosphoric acid, boric acid, hydrofluoric acid, hydrochloric acid, hydrobromic acid or hydroiodic acid was added individually into 25% ethanol

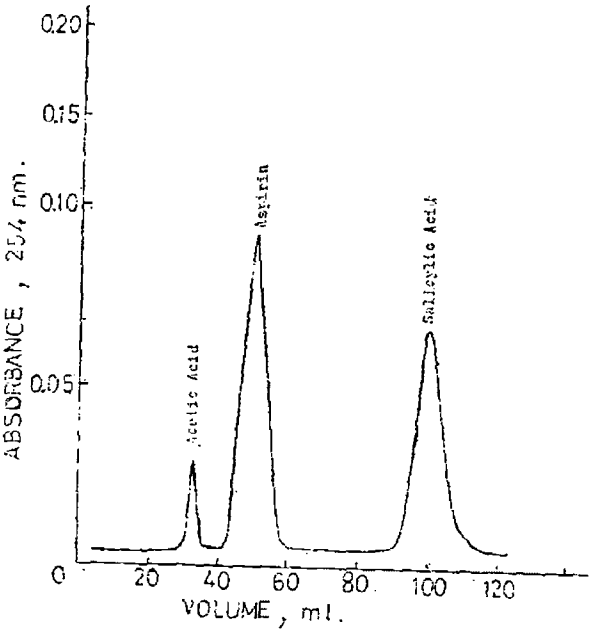

Fig. 4 Chromatogram of mixture of Acetic acid, Aspirin and Salicylic acid. Concentration: Each 1 mg/ml. Flow Rate: 15 ml/hr. Eluent: 25% Ethanol+0.02 M H_3PO_4

aqueous eluent and make the final concentration 0.02 *M*. The retention volumes are summarized in Table 1. Their effluents were monitored by the absorption of UV light at 254 *nm*. Only eluents containing few quantities of nitric acid, sulfuric acid and phosphoric acid can successfully separate aspirin and salicylic acid. The chromatogram of mixture of acetic acid, aspirin and salicylic acid are shown in Figure 2, Figure 3 and Figure 4. The results showed that adding few quantities of phosphoric acid got the best resolution.

From the results of above experiments, only adding 0.02 *M* of phosphoric acid, sulfuric acid or nitric acid in 25% ethanol aqueous eluent can separate aspirin and salicylic acid. Therefore, the separation must not be affected by hydrogen ion concentration. It might be the effect of phosphate, sulfate or nitrate anions. Therefore we

used the sodium form of cation exchange resin for the further investigation.

Separations on Sodium form Cation-Exchange Resin:

These experiments were made on the column of sodium form resin AG 50*W*-×8, resin bed depth was shrank to 50*cm*. 1*mg/ml* of aspirin, salicylic acid and acetic acid solution and the mixture of aspirin, salicylic acid and acetic acid, containing 1 *mg/ml* each, were introduced by injection respectively, and eluted with 25% ethanol aqueous solution. The flow rate was 15 *ml/hr*. In each experiment sodium nitrate, sodium sulfate, sodium phosphate, sodium borate, sodium fluoride, sodium chloride, sodium bromide or sodium iodide was added individually into eluent and make the final concentration 0.02 *M*. The effluents were monitored by the absorption of UV light at 254 *nm*. The retention volumes were summarized in Table 2. The results showed none of them could separate the mixture of aspirin and salicylic acid.

Table 2 Effect of Different Salts in Eluent on Sodium Form Resin

Resin form	Eluent	Salts		Retention volume (ml)		
				HOAc	Sal. acid	Aspirin
Sodium	25% Ethanol	None	ind.	17.4	15.7	17.3
			mix.	13.4	13.4	13.4
		$NaNO_3$ 0.02M	ind.	24.8	25.3	38.1
			mix.	22.5	22.5	39.1
		Na_2SO_4 0.02M	ind.	27.1	37.9	23.5
			mix.	17.8	45.0	17.8
		Na_3PO_4 0.02M	ind.	17.8	14.3	11.6
			mix.	13.9	13.9	13.9
		$Na_2B_4O_7$ 0.02M	ind.	9.8	14.5	13.8
			mix.	17.0	17.0	17.0
		NaF 0.02M	ind.	15.3	15.8	13.5
			mix.	13.6	13.6	13.6
		NaCl 0.02M	ind.	11.1	15.6	24.9
			mix.	27.0	27.0	17.0
		NaBr 0.02M	ind.	15.1	14.4	15.8
			mix.	14.9	14.9	14.9
		NaI 0.02M	ind.	13.6	26.0	23.7
			mix.	12.9	22.9	22.9

From the results of above experiments, none of any sodium salts that we added in the eluent can get a good resolution. It might be caused by the shrinkage of resins, the porous in the resin were shrank 1/5 from hydrogen form to sodium form but we can not make a clear explanation for these experiments. According to the above experiments, the following conclusions can be made.

CONCLUSION

1. The mixture of aspirin, salicylic acid and acetic acid can be separated successfully through hydrogen form resin AG 50*W*-×8, eluted with 25% ethanol aqueous solution containing 0.02 *M* phosphoric acid, nitric acid or sulfuric acid. Adding phosphoric acid into 25% ethanol aqueous eluent gave the best resolution.

2. The separation of aspirin, salicylic acid and acetic acid could not achieve through sodium form resin AG 50 *W*-×8 eluted with 25% ethanol aqueous solution containing 0.02 *M* sodium nitrate, sodium sulfate, sodium phosphate, sodium borate, sodium fluoride, sodium bromide or sodium iodide.

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(*Received August 4, 1978*)