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CHAPTER 4
SORPTION ELUTION

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4.1 INTRODUCTION

Chromatography[1-20] is a physical method of separation in which the components to be separated are distributed between two phases one of these phases constituting a stationary bed of large surface area, the other being a fluid that percolates through or along the stationary bed. The separation of the components depends upon the differential migration resulting from a resistive action, namely selective sorption of the components of the mixture. Chromatography is the most variable and in many respects the most adaptable technique in all branches of science, because it may be used for the examination ofavariety of chemical substances.

The two major subdivisions of chromatography, based on the mobile phase used, are gas chromatography and liquid chromatography, of which the former has extraordinary success. However, only about 15% of organic compounds are ameanable to gas chromatographic analysis. Insufficient volatility and thermal instability of many organic compounds are mainly responsible for this unfortunate limitation imposed on gas chromatography.

Liquid chromatography was in limited use even from ancient times though the principles underlying this remained unrecognised, for example, the utility of some earths for the purification of sea water was known even to Aristotle. The tremendous advances in researches in biochemistry, diagnostic medicine and pharmaceutical materials are mainly responsible for triggering the explosive growth of liquid chromatography,

as many of the substances falling under these heads are nonvolatile.

In liquid chromatography, the stationary phase may be solid or liquid. Liquid chromatography using solid as the stationary phase is potentially more useful branch, since it possesses certain advantages such as greater speed and separation efficiency, ease of automation and uncontrolled operation, easier quantitation and possibility of achieving preparative separations. Liquid-solid chromatography can be classified, based on the way in which the solid stationary phase is used, as column chromatography, thin layer chromatography and paper chromatography.

Liquid-solid chromatography in columns was the first form of chromatography. It was introduced by the Russian botanist Tswett (1905) during the investigation of plant pigments. However, the technique remained practically ignored for a number of years and it is only when Kuhn, Winterstein and Lederer (1931) reported the separation of carotenes and xanthophylls on the columns of alumina and calcium carbonate, that it has attracted attention of investigators who have shown that the chromatographic analysis can render greater service in many areas of enquiry.

Soon after this "rediscovery of chromatography", Adams and Holmes found that ion exchange could be performed on finely ground gramaphone records. This led to the development of synthetic ion exchange resins. An enormous development of different applications of ion exchange emerged, the most important among these being, ion exchange chromatography.

Systematic study of the phenomena of ion exchange revealed the great potential of ion exchangers in quantitative analysis and the real breakthrough of ion exchange was the release of information about the separation of rare earths demonstrating the possibilities of ion exchange chromatography in the separation of species with almost identical properties.

Ion exclusion, reported by Wheaton and Bauman (1953)[21,22], is one of the several new developments involving ion exchange resins. It is a molecular process whereby a nonionic solute is removed from the solution into the resin phase by sorption and the ionic solutes are excluded by the Donnan effect, the nonionic solutes are then can be physically displaced from the resin by washing with the proper developing agent. This process appears to have its utility in the deionization of aqueous solutions of nonpolar or slightly polar solutes such as alcohols, glycols, weak organic acids, ketones and amino acids.

The technique of ion exclusion has been extended to the separation of two or more nonionic compounds. Most ion exchange resins show pronounced differences in their affinities for various nonelectrolytes and can then be used as stationary phase for the separation of many organic compounds.[21-69] The scope of this separation method can be broadened by altering conditions in one of the phases. The replacement of water with concentrated electrolyte solution as developer has the effect of salting different solutes into the internal phase to various degrees and thus offering separations that are not possible in water. This technique is known as 'salting out

chromatography'. Similarly, the relative distribution of some pairs of compounds can be altered by using a mixture of water and organic solvent as the developer, and the technique is called 'solubilization chromatography'.

Thus the term 'ion exchange chromatography', in a broader sense, covers all chromatographic separations, ionic as well as nonionic substances carried on with ion exchange resins.

4.1-1 Techniques :

There are essentially three methods of performing column liquid chromatography. These are :

(i) Frontal analysis : In this technique the solution of the mixture of substances is introduced continuously into the column. Only a part of the least sorbed component is obtained in the pure form. The method is not attractive for preparative requirements as well as for analytical purposes.

(ii) Displacement analysis : In this technique the components of the mixture, initially sorbed in the column, are successively displaced (depending on the affinity for the stationary phase) by continuously passing the solution of a substance which is more strongly sorbed than the components present in the mixture. In this case also the separation of the components is not complete.

(iii) Elution analysis : In this technique the sample, initially sorbed on the top of the column, is washed down with an appropriate developer (eluent or solvent). The components often leave the column in pure form. One of its disadvantages

is much smaller capacity of the column and much higher consumption of the solvent. Simple elution (using a single solvent), stepwise elution (using several solvents successively with increasing elution capacity) and gradient elution (varying the nature of the solvent gradually and continuously, but not in steps, so that a mixture of uniformly changing composition is introduced into the column) are the three methods of carrying out the elution analysis.

4.1-2 Theory of Chromatography[1-100] :

The ultimate goal of theories of chromatography is to provide means for predicting from known, or independently measurable fundamental properties, performance of columns under given conditions and optimum conditions for given separations. However, the general theories of column performance are too complex to be solved by mathematical analysis unless very drastic simplifications are introduced and numerous simplifying assumptions are made. Inspite of these limitations, fundamental data can be used to derive simple useful relationships which serve as helpful guides in obtaining optimum operating conditions.

In general, three quantities of the chromatogram are of interest, the time necessary to elute a given component from the column, the width of the peak and the completeness of the separation (resolution).

The elution curve in chromatography represents the distribution of concentration with time and is therefore a probability density curve. Although these curves are generally asymmetric for ease of interpretation they are usually

considered symmetric and described mathematically by a Gaussian curve. Under these conditions it is possible to relate the rate of movement of the zone expressed in terms of the volume of eluent, from the start to the emergence from the column of the midpoint of the peak, to the distribution coefficient (Section 4.4-1).

Two parameters can be used to describe peak widths, the variance σ^{-2} and the number of theoretical plates, N, or the plate height, H. σ is the half of the width measured at the ordinate of $Cm/\sqrt{e}$, where Cm is the peak height of the elution curve. In the literature equations are given correlating σ^{-2} with the distribution coefficient and the column parameters.[70-96]

The theoretical plate concept was introduced by Martin and Synge, who recognized the similarity in the chromatographic process to that taking place in distillation columns. The concept was applied to ion exchange columns by Mayer and Tompkins and later modified by Glueckauf, who based his analysis on a continuous flow model. The theory brings out effects arising from the operating parameters, such as flow rate, feed concentration, particle size etc.. It also includes the distribution coefficient of a component being separated and all possible diffusion effects. Despite the value of this approach in characterizing the efficiency of distillation columns and extractors, its physical significance in chromatography is questionable. Nevertheless the measured quantities N and H are useful for characterizing band spreading and the efficiency of a chromatographic system.

Other factors being equal, for a given size of the column, its efficiency will be greater, the larger the number of theoretical plates or smaller the plate height. Also, in using it to compare the performance of different columns, one finds that their ability to separate substances does not increase in parallel with a decrease in plate height. This is obviously a practical disadvantage.

Thus it can be seen that the size, shape and position of the peaks on the chromatogram can be calculated with some success from fundamental data and the various compromises necessary in striving for optimal column performance.

Another important parameter used as a quantitative measure of the ability of a column to separate two given components is resolution. In mathematical terms it is a measure of the degree of separation of zones, represented as

$$R_n = \Delta Z/n(\sigma_1 + \sigma_2)$$

where ΔZ is the gap between the centres of the peaks of the two neighbouring zones, σ_1 and σ_2, the standard deviations of the two zones, n is an intiger greater than zero. The magnitude of n indicates the degree to which the gap between the two zones is filled and the crosscontamination by zone spreading. The value of n depends on the type of problem and chromatography and the degree of separation required. In liquid chromatography the generally accepted criterion of minimum resolution is to select the value of n as 3. This means that after having devided the effluent into fraction at a point $Ve = Vm_1 + 3\sigma_1$ at least 99.86% of each of the components is in its appropriate

fraction and the impurity amounts to at the most 0.14% of the peak of the contagious component.

Thus resolution can act as one bridge between theory and practice. However, it does not describe the physical and chemical factors which are the causes of the separation. It must also be emphasized that the resolution is not a complete measure of practical success. It provides no inkling of the time which may be consumed in achieving the separation nor does it guarantee that the zones are readily detectable. Nonetheless, it is a practical measure of some worth, if its limitations are kept in mind.

4.2 EXPERIMENTAL

For preparing a resin column, definite quantity of the air dried resin, Dowex 50W-X4 (100/200), hydrogen form (Chapter 3), was weighed, soaked in distilled water in a beaker and then transferred carefully into a pyrex glass column. The length (L), bed volume (Vb), void volume (Vi), and disc volume (Vd) were measured. The column capacity (C) was calculated from the amount of air dried resin taken in the column and its air dried capacity (c) per gram of the resin. All these quantities are reported in Table 4.2-1 for various columns L_1, L_2 and L_3 used in this study.

The solutions of benzoic acids and the solvents were prepared as described in Section 3.2.

The resin column was conditioned prior to performing the elution run by washing it with several bed volumes of the

solvent to be used as eluent, back washed by passing the solvent upwards with sufficient speed to loosen the resin and to remove air bubbles. The resin was then allowed to settle under gravity to achieve a size classification within the column.

For carrying out the elution run, the liquid level was brought to the resin bed level. Then 25 ml of benzoic acid solution to be analysed were added carefully from the top of the resin bed so that the surface layer of the resin was disturbed as little as possible. The solution was allowed to sink into the resin by opening the pinch cock at the lower end of the column. The effluent was collected in a 25 ml measuring flask and it was marked as sample number 1. When the liquid level was again at the resin bed level, 5 ml of the eluent were added to rinse the inside of the column above the resin bed, and it was allowed to sink into the resin. Then the column was connected to an overhead reservoir of the eluent and the continuous elution was carried out at the rate of 2 ml per minute. The effluent was collected in 25 ml measuring flasks and numbered as 2, 3 and so on.

The solute content in each sample was estimated by the ultraviolet absorption. W and Ws denote the solute content in millimoles in the feed solution (25 ml) initially sorbed on the resin bed and in the effluent sample (25 ml) respectively.

After the run was completed the column was washed with distilled water and conditioned with the solvent to be used for the next run.

The same procedure was followed for the column elution of the mixtures of benzoic acids.

Table 4.2-1 : Column Parameters :

Column	L cm	10^3C eq	10^3Vb liter	10^3Vi liter	10^3Vd liter	10^3Vf liter	10^3Vo liter	$10^2\sqrt{C}$	$10^2\alpha_1$	$10^3\alpha_2$
L_1	56	170	140	55	15	25	95	41	2.06	1.0
L_2	28	88	70	30	15	25	70	30	1.50	0.8
L_3	15	46	40	15	15	25	55	22	1.10	0.6

L = Column length

C = Column capacity

Vb = Bed volume

Vi = Void volume

Vd = Disc volume (Dead volume)

Vf = Feed volume

Vo = Vi + Vd + Vf

α_1 = Column constant

α_2 = Column constant

4.3 RESULTS

Table 4.3-1 : Column elution of p-OH benzoic acid on column L_1 and benzoic acid, p-OH and 2,3-diOH benzoic acids on column L_2 with water as solvent and eluent.

Benzoic acid	p-OH	H	p-OH	2,3-diOH
Column	L_1	L_2	L_2	L_2
10^2W	1.68	1.08	1.48	1.03
Sample No.		10^2Ws		
1	-	-	-	-
2	-	-	-	0.02
3	-	-	-	0.17
4	-	-	-	0.40
5	-	-	-	0.44
6	-	0.02	0.01	0.01
7	-	0.07	0.03	-
8	-	0.17	0.11	-
9	-	0.35	0.26	-
10	-	0.44	0.60	-
11	-	0.05	0.46	-
12	-	-	0.01	-
13	0.01	-	-	-
14	0.02	-	-	-
15	0.04	-	-	-
16	0.08	-	-	-
17	0.14	-	-	-
18	0.28	-	-	-
19	0.47	-	-	-
20	0.47	-	-	-
21	0.13	-	-	-
22	0.01	-	-	-
23	-	-	-	-

Table 4.3-2 : Column elution of monohydroxybenzoic acids on column L_1 with 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	o-OH	m-OH	p-OH
10^2W	2.15	7.15	3.64
Sample No.		10^2Ws	
1-15	-	-	-
16	-	0.04	-
17	-	0.27	-
18	-	1.34	-
19	-	2.55	0.02
20	-	1.96	0.12
21	-	0.82	0.56
22	-	0.21	1.11
23	-	0.02	0.99
24	0.03	-	0.51
25	0.09	-	0.19
26	0.27	-	0.06
27	0.51	-	0.02
28	0.56	-	0.01
29	0.40	-	-
30	0.20	-	-
31	0.07	-	-
32	0.02	-	-
33	-	-	-

Table 4.3-3 : Column elution of monomethoxybenzoic acids on column L, with 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	o-OMe	m-OMe	p-OMe
10^2W	8.20	4.65	1.09
Sample No.		10^2Ws	
1-25	-	-	-
26	0.03	-	-
27	0.09	-	-
28	0.28	-	-
29	0.76	-	-
30	1.53	-	-
31	1.98	-	-
32	1.69	-	-
33	0.99	-	-
34	0.49	-	-
35	0.19	-	-
36	0.07	-	-
37	0.02	-	-
38	-	-	-
39	-	0.03	-
40	-	0.07	-
41	-	0.16	-
42	-	0.32	0.01
43	-	0.52	0.03
44	-	0.69	0.06
45	-	0.74	0.09
46	-	0.66	0.14
47	-	0.54	0.17
48	-	0.38	0.17
49	-	0.25	0.14
50	-	0.16	0.11
51	-	0.09	0.07
52	-	0.05	0.04
53	-	*	0.02
54	-	-	0.01
55	-	-	-

Table 4.3-4 : Column elution of 2,3-, 2,4- and 2,5-diOH benzoic acids on column L, with 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	2,3-diOH	2,4-diOH	2,5-diOH
10^2W	10.20	6.21	1.81
Sample No.		10^2Ws	
1-16	-	-	-
17	0.01	-	-
18	0.22	-	0.01
19	1.52	-	0.14
20	3.22	-	0.47
21	2.90	-	0.58
22	1.53	-	0.38
23	0.58	-	0.17
24	0.15	-	0.10
25	0.04	-	0.02
26	-	0.05	-
27	-	0.28	-
28	-	0.83	-
29	-	1.34	-
30	-	1.34	-
31	-	1.02	-
32	-	0.66	-
33	-	0.39	-
34	-	0.21	-
35	-	0.10	-
36	-	0.05	-
37	-	-	-

Table 4.3-5 : Column elution of 2,6-, 3,4- and 3,5-diOH benzoic acids on column L_1 with 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	2,6-diOH	3,4-diOH	3,5-diOH
10^2W	2.35	2.01	2.56
Sample No.		10^2Ws	
1-4	-	-	-
5	0.01	-	-
6	0.27	-	-
7	1.20	-	-
8	0.80	-	-
9	0.08	-	-
10	0.02	-	-
11	0.01	-	-
12	-	-	-
13	-	-	0.01
14	-	0.02	0.06
15	-	0.21	0.51
16	-	0.68	1.14
17	-	0.71	0.61
18	-	0.30	0.21
19	-	0.06	0.04
20	-	0.01	-
21	-	-	-

Table 4.3-6 : Column elution of monohydroxybenzoic acids on column L_1 with 10% dioxan in 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	o-OH	m-OH	p-OH
10^2W	11.10	8.62	4.28
Sample No.		10^2Ws	
1-9	-	-	-
10	-	0.36	0.02
11	-	3.22	0.32
12	-	3.92	1.70
13	-	0.96	1.79
14	0.08	0.07	0.45
15	1.05	-	0.03
16	3.82	-	-
17	4.05	-	-
18	1.61	-	-
19	0.29	-	-
20	-	-	-

Table 4.3-7 : Column elution of monomethoxybenzoic acids on column L, with 10% dioxan in 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	o-OMe	m-OMe	p-OMe
10^2W	10.93	9.85	2.35
Sample No.		10^2Ws	
1-13	-	-	-
14	0.15	-	-
15	1.37	-	-
16	4.07	-	-
17	3.74	-	-
18	1.27	0.03	0.01
19	0.18	0.20	0.07
20	-	0.88	0.25
21	-	2.19	0.55
22	-	2.95	0.70
23	-	2.11	0.50
24	-	1.09	0.20
25	-	0.39	0.06
26	-	0.12	0.01
27	-	-	-

Table 4.3-8 : Column elution of p-Cl, p-OEt, p-n-OPr and 2-OH-4-OEt benzoic acids on column L; with 10% dioxan in 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	p-Cl	p-OEt	p-n-OPr	2-OH-4-OEt
10^2W	1.49	2.29	0.88	2.44
Sample No.		10^2Ws		
1-19	-	-	-	-
20	0.02	-	-	-
21	0.09	-	-	-
22	0.27	-	-	-
23	0.47	-	-	-
24	0.41	0.01	-	-
25	0.19	0.06	-	-
26	0.06	0.17	-	-
27	0.01	0.37	-	-
28	-	0.52	-	-
29	-	0.52	-	-
30	-	0.37	-	-
31	-	0.20	-	-
32	-	0.08	0.01	0.01
33	-	0.02	0.02	0.03
34	-	-	0.03	0.07
35	-	-	0.06	0.14
36	-	-	0.10	0.23
37	-	-	0.13	0.32
38	-	-	0.14	0.38
39	-	-	0.14	0.38
40	-	-	0.12	0.32
41	-	-	0.08	0.25
42	-	-	0.05	0.17
43	-	-	0.02	0.11
44	-	-	0.01	0.06
45	-	-	-	0.03
46	-	-	-	-

Table 4.3-9 :Column elution of dihydroxybenzoic acids on column L, with 10% dioxan in 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	2,3-diOH	2,4-diOH	2,5-diOH	2,6-diOH	3,4-diOH	3,5-diOH
10^2W	1.18	3.51	4.00	3.04	1.00	1.99
Sample No.			10^2Ws			
1-3	-	-	-	-	-	-
4	-	-	-	0.14	-	-
5	-	-	-	1.72	-	-
6	-	-	-	0.95	-	-
7	-	-	-	0.08	-	-
8	-	-	-	0.05	0.07	0.12
9	-	-	-	0.03	0.54	1.07
10	-	-	0.19	0.02	0.36	0.73
11	0.07	-	2.72	-	0.05	0.08
12	0.47	0.01	0.98	-	-	-
13	0.51	0.04	0.12	-	-	-
14	0.12	0.48	-	-	-	-
15	0.01	1.44	-	-	-	-
16	-	1.16	-	-	-	-
17	-	0.36	-	-	-	-
18	-	0.04	-	-	-	-
19	-	-	-	-	-	-

Table 4.3-10 : Column elution of dimethoxybenzoic acids on column L_1 with 10% dioxan in 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	2,3-diOMe	2,4-diOMe	2,5-diOMe	2,6-diOMe	3,4-diOMe
10^2W	2.86	1.48	0.63	1.73	1.72
Sample No.			10^2Ws		
1-10	-	-	-	-	-
11	-	-	-	0.11	-
12	-	-	-	0.62	-
13	0.07	-	-	0.73	-
14	0.49	-	-	0.23	-
15	1.18	-	-	0.02	-
16	0.84	-	-	-	-
17	0.25	-	-	-	-
18	0.03	-	-	-	-
19	-	-	-	-	-
20	-	-	-	-	-
21	-	-	-	-	0.02
22	-	-	-	-	0.09
23	-	-	0.01	-	0.23
24	-	0.02	0.04	-	0.36
25	-	0.07	0.09	-	0.39
26	-	0.18	0.14	-	0.31
27	-	0.29	0.15	-	0.19
28	-	0.33	0.11	-	0.08
29	-	0.27	0.06	-	0.03
30	-	0.17	0.02	-	-
31	-	0.08	-	-	-
32	-	0.03	-	-	-
33	-	0.01	-	-	-
34	-	-	-	-	-

Table 4.3-11 : Column elution of hydroxy-methoxybenzoic acids on column L_1 with 10% dioxan in 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	2-OH-3-OMe	2-OH-4-OMe	2-OH-5-OMe	4-OH-3-OMe
10^2W	6.12	2.06	6.28	3.93
Sample No.	10^2Ws			
1-11	-	-	-	-
12	-	-	-	0.05
13	-	-	-	0.31
14	-	-	-	1.17
15	-	-	-	1.42
16	-	-	-	0.76
17	-	-	-	0.21
18	0.06	-	-	0.02
19	0.35	-	-	-
20	1.08	-	-	-
21	1.71	-	0.10	-
22	1.54	-	0.48	-
23	0.92	-	1.13	-
24	0.38	-	1.62	-
25	0.11	0.02	1.52	-
26	0.02	0.07	0.95	-
27	-	0.18	0.41	-
28	-	0.33	0.11	-
29	-	0.42	-	-
30	-	0.42	-	-
31	-	0.30	-	-
32	-	0.20	-	-
33	-	0.10	-	-
34	-	0.05	-	-
35	-	0.01	-	-
36	-	-	-	-

Table 4.3-12 : Column elution of benzoic acid and monohydroxy benzoic acids on column L_2 with 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	H	o-OH	m-OH	p-OH
10^2W	0.39	0.92	3.55	1.28
Sample No.		10^2Ws		
1-8	-	-	-	-
9	-	-	0.50	-
10	-	-	1.64	0.01
11	0.01	-	1.12	0.10
12	0.13	-	0.25	0.41
13	0.18	0.07	0.03	0.50
14	0.08	0.25	-	0.21
15	0.01	0.35	-	0.04
16	-	0.21	-	-
17	-	0.07	-	-
18	-	-	-	-

Table 4.3-13 : Column elution of dihydroxybenzoic acids on column L_2 with 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	2,3-diOH	2,4-diOH	2,5-diOH	2,6-diOH	3,4-diOH	3,5-diOH
10^2W	2.44	2.48	3.01	1.75	1.21	2.12
Sample No.	10^2Ws					
1-2	-	-	-	-	-	-
3	-	-	-	0.25	-	-
4	-	-	-	1.28	-	-
5	-	-	-	0.23	-	-
6	-	-	-	0.02	-	-
7	-	-	-	-	0.01	0.01
8	-	-	0.04	-	0.15	0.34
9	0.03	-	0.33	-	0.59	1.04
10	0.21	-	1.25	-	0.39	0.61
11	0.81	-	1.02	-	0.07	0.10
12	0.87	-	0.30	-	-	-
13	0.38	0.05	0.03	-	-	-
14	0.10	0.26	-	-	-	-
15	0.04	0.72	-	-	-	-
16	0.02	0.78	-	-	-	-
17	-	0.47	-	-	-	-
18	-	0.19	-	-	-	-
19	-	0.05	-	-	-	-
20	-	-	-	-	-	-

Table 4.3-14 : Column elution of benzoic acid and 2,3-, 2,6- and 3,5-dimethoxybenzoic acids on column L_2 with 10% dioxan in 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	H	2,3-diOMe	2,6-diOMe	3,5-diOMe
10^2W	0.94	1.67	1.56	0.58
Sample No.		10^2Ws		
1-4	-	-	-	-
5	-	-	0.03	-
6	-	0.02	0.35	-
7	0.07	0.36	0.92	-
8	0.50	0.91	0.24	-
9	0.35	0.36	0.03	-
10	0.03	0.02	-	-
11-17	-	-	-	-
18	-	-	-	0.03
19	-	-	-	0.07
20	-	-	-	0.11
21	-	-	-	0.13
22	-	-	-	0.11
23	-	-	-	0.07
24	-	-	-	0.05
25	-	-	-	0.03
26	-	-	-	0.01
27	-	-	-	-

Table 4.3-15 : Column elution of dihydroxybenzoic acids on column L_3 with 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	2,3-diOH	2,4-diOH	2,5-diOH	2,6-diOH	3,4-diOH	3,5-diOH
10^2W	3.10	3.56	1.73	2.66	2.28	2.44
Sample No.			10^2Ws			
1	-	-	-	-	-	-
2	-	-	-	0.72	-	-
3	-	-	-	1.85	-	-
4	-	-	-	0.10	0.19	0.21
5	0.12	-	0.13	-	1.13	1.26
6	1.14	0.02	0.88	-	0.82	0.87
7	1.36	0.35	0.63	-	0.15	0.11
8	0.42	1.48	0.09	-	-	-
9	0.05	1.23	-	-	-	-
10	-	0.39	-	-	-	-
11	-	0.07	-	-	-	-
12	-	0.01	-	-	-	-
13	-	-	-	-	-	-

Table 4.3-16 : Column elution of benzoic acid, m-OH, 2-OH-3-OMe and 4-OH-3-OMe on column L_3 with 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	H	m-OH	2-OH-3-OMe	4-OH-3-OMe
10^2W	0.60	3.38	0.57	0.82
Sample No.		10^2Ws		
1-4	-	-	-	-
5	-	0.50	-	-
6	0.05	1.81	-	-
7	0.29	0.94	-	0.02
8	0.23	0.14	-	0.15
9	0.03	-	-	0.31
10	-	-	0.02	0.25
11	-	-	0.10	0.09
12	-	-	0.17	0.02
13	-	-	0.17	-
14	-	-	0.09	-
15	-	-	0.03	-
16	-	-	-	-

Table 4.3-17 : Column elution of benzoic acid, 3,4-diOH, 3,5-diOH and 2,3-diOMe benzoic acids on column L_3 with 10% dioxan in 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	H	3,4-diOH	3,5-diOH	2,3-diOMe
10^2W	4.45	0.92	0.55	0.53
Sample No.		10^2Ws		
1	-	-	-	-
2	-	0.02	0.02	-
3	0.12	0.38	0.25	0.03
4	1.26	0.47	0.24	0.17
5	2.68	0.04	0.03	0.30
6	0.36	0.01	-	0.05
7	0.07	-	-	-
8	-	-	-	-

Table 4.3-18 : Column elution of p-alkoxybenzoic acids on column L_3 with 10% dioxan in 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	p-OMe	p-OEt	p-n-OPr	p-n-OBu
10^2W	0.64	0.90	0.60	0.31
Sample No.		10^2Ws		
1-5	-	-	-	-
6	0.13	-	-	-
7	0.33	0.07	-	-
8	0.16	0.30	-	-
9	0.02	0.34	0.03	-
10	-	0.15	0.11	-
11	-	0.03	0.18	-
12	-	-	0.17	0.01
13	-	-	0.09	0.02
14	-	-	0.01	0.05
15	-	-	-	0.07
16	-	-	-	0.07
17	-	-	-	0.05
18	-	-	-	0.03
19	-	-	-	0.02
20	-	-	-	-

Table 4.3-19 : Column elution of p-halobenzoic acids on column L_3 with 10% dioxan in 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	p-Cl	p-Br	p-I
10^2W	0.83	0.68	0.62
Sample No.		10^2Ws	
1-4	-	-	-
5	0.01	-	-
6	0.15	-	-
7	0.40	0.04	-
8	0.23	0.17	-
9	0.03	0.30	-
10	-	0.18	0.01
11	-	0.03	0.05
12	-	-	0.12
13	-	-	0.17
14	-	-	0.15
15	-	-	0.09
16	-	-	0.04
17	-	-	0.01
18	-	-	-

Table 4.3-20 : Column elution of mixtures of (A) m-OH and o-OH and (B) 2,3-diOH and 2,4-diOH benzoic acids on column L_1 with 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	m-OH +	o-OH	2,3-diOH +	2,4-diOH
10^2W	3.77	9.98	0.72	1.70
Sample No.		10^2Ws		
1-16	-	-	-	-
17	0.06	-	-	-
18	0.45	-	0.01	-
19	1.23	-	0.08	-
20	1.23	-	0.21	-
21	0.63	-	0.24	-
22	0.17	-	0.15	-
23	0.01	-	0.05	-
24	-	0.01	0.01	-
25	-	0.22	-	-
26	-	0.86	-	0.04
27	-	2.08	-	0.14
28	-	2.73	-	0.30
29	-	2.26	-	0.43
30	-	1.20	-	0.41
31	-	0.49	-	0.25
32	-	0.16	-	0.10
33	-	0.05	-	0.04
34	-	-	-	0.01
35	-	-	-	-

Table 4.3-21 : Column elution of mixtures of (A) m-OH and m-OMe and (B) 4-OH-3-OMe and 2-OH-5-OMe benzoic acids on column L_1 with 10% dioxan in 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	m-OH +	m-OMe	4-OH-3-OMe +	2-OH-5-OMe
10^2W	2.78	2.18	3.29	2.72
Sample No.		10^2Ws		
1-9	-	-	-	-
10	0.34	-	-	-
11	1.25	-	-	-
12	0.97	-	0.05	-
13	0.21	-	0.26	-
14	0.02	-	0.90	-
15	-	-	1.08	-
16	-	-	0.69	-
17	-	-	0.25	-
18	-	-	0.05	-
19	-	0.02	-	-
20	-	0.12	-	0.02
21	-	0.40	-	0.23
22	-	0.64	-	0.62
23	-	0.59	-	0.78
24	-	0.33	-	0.68
25	-	0.12	-	0.30
26	-	0.01	-	0.08
27	-	-	-	0.01
28	-	-	-	-

Table 4.3-22 : Column elution of mixtures of (A) 3,5-diOH and 2,4-diOH and (B) 3,4-diOH and 4-OH-3-OMe benzoic acids on column L, with 10% dioxan in 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	3,5-diOH + 2,4-diOH		3,4-diOH + 4-OH-3-OMe	
10^2W	1.43	3.13	1.18	1.83
Sample No.	10^2Ws			
1-7	-	-	-	-
8	0.12	-	0.07	-
9	0.79	-	0.62	-
10	0.49	-	0.42	-
11	0.04	-	0.04	-
12	-	0.01	-	0.02
13	-	0.08	-	0.19
14	-	0.69	-	0.54
15	-	1.31	-	0.66
16	-	0.77	-	0.34
17	-	0.20	-	0.09
18	-	0.03	-	-
19	-	-	-	-

Table 4.3-23 : Column elution of mixtures of (A) 4-OH-3-OMe and 3,4-diOMe and (B) 2,3-diOMe and 3,4-diOMe benzoic acids on column L, with 10% dioxan in 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	4-OH-3-OMe + 3,4-diOMe		2,3-diOMe + 3,4-diOMe	
10^2W	3.28	1.59	1.42	1.59
Sample No.	10^2Ws			
1-11	-	-	-	-
12	0.04	-	0.01	-
13	0.38	-	0.04	-
14	0.99	-	0.25	-
15	1.03	-	0.59	-
16	0.59	-	0.41	-
17	0.17	-	0.12	-
18	0.02	-	0.02	-
19	-	-	0.01	-
20	-	-	-	-
21	-	-	-	-
22	-	0.01	-	0.01
23	-	0.07	-	0.08
24	-	0.21	-	0.21
25	-	0.37	-	0.38
26	-	0.39	-	0.39
27	-	0.29	-	0.29
28	-	0.17	-	0.16
29	-	0.03	-	0.04
30	-	0.01	-	0.01
31	-	-	-	-

Table 4.3-24 : Column elution of a mixture of 2,6-diOH, 3,5-diOH and 2,4-diOH benzoic acids on column L_2 with 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	2,6-diOH +	3,5-diOH +	2,4-diOH
10^2W	0.53	1.02	1.48
Sample No.		10^2Ws	
1-2	-	-	-
3	0.08	-	-
4	0.39	-	-
5	0.08	-	-
6	0.01	-	-
7	-	0.02	-
8	-	0.25	-
9	-	0.48	-
10	-	0.21	-
11	-	0.03	-
12	-	-	-
13	-	-	0.09
14	-	-	0.32
15	-	-	0.48
16	-	-	0.34
17	-	-	0.16
18	-	-	0.05
19	-	-	0.01
20	-	-	-

Table 4.3-25 : Column elution of mixtures of (A) 2,6-diOH and 2,4-diOH and (B) 2,3-diOH and 2-OH-3-OMe benzoic acids on column L_3 with 0.01N aqueous hydrochloric acid as solvent.

Benzoic acid	2,6-diOH + 2,4-diOH		2,3-diOH + 2-OH-3-OMe	
10^2W	1.92	2.47	1.10	0.29
Sample No.	10^2Ws			
1	-	-	-	-
2	0.55	-	-	-
3	1.27	-	-	-
4	0.08	-	-	-
5	-	-	0.04	-
6	-	0.01	0.36	-
7	-	0.22	0.49	-
8	-	0.89	0.17	-
9	-	0.91	0.02	-
10	-	0.36	-	0.02
11	-	0.08	-	0.06
12	-	0.01	-	0.10
13	-	-	-	0.08
14	-	-	-	0.04
15	-	-	-	0.01
16	-	-	-	-

Table 4.3-26 : Column elution of mixtures of (A) p-Cl and p-I and (B) p-OEt and p-n-OBu benzoic acids on column L_3 with 10% dioxan in 0.01N aqueous hydrochloric acid as solvent and eluent.

Benzoic acid	p-Cl	+	p-I	p-OEt	+	p-n-OBu
10^2W	0.72		0.43	0.70		0.19
Sample No.			10^2Ws			
1-4	-		-	-		-
5	0.01		-	-		-
6	0.13		-	-		-
7	0.36		-	0.05		-
8	0.21		-	0.24		-
9	0.04		-	0.26		-
10	-		-	0.14		-
11	-		0.02	0.02		-
12	-		0.06	-		0.01
13	-		0.12	-		0.01
14	-		0.13	-		0.04
15	-		0.07	-		0.06
16	-		0.03	-		0.05
17	-		0.01	-		0.03
18	-		-	-		0.01
19	-		-	-		-

4.4 DISCUSSION

The uptake of the solute by the resin is characterized by the distribution coefficient which can be expressed in various ways such as volume distribution coefficient, Kv; weight distribution coefficnent, Kw. In chromatography distribution coefficient is the important factor as it determines the rate of movement of the solute down the column. It is related to the peak elution volume, the width of the elution curve and the resolution.

In the present study the distribution coefficient is expressed in terms of the sorption coefficient, B (defined in Chapter 3). This is related to the volume distribution coefficient Kv (per ml of the resin) and weight distribution coefficient Kw (per gm of the resin) as,

$$B = Kv/c' = Kw/c$$

where c' denotes capacity of the resin in equivalents per ml of the resin and c, the capacity in equivalents per gm of the resin. Therefore, it follows that it would be possible to correlate the sorption coefficient, B, with

(i) the peak elution volume,

(ii) the width and peak height of the elution curve,

(iii) the number of theoretical plates and

(iv) the resolution

by simple equations and these equations may be compared with the equations given in the literature,[70-96] where the above quantities are related to either volume distribution coefficient or weight distribution coefficient.

Generally the equations are based on the assumption that the elution curves are symmetric described mathematically by a Gaussian curve. However, when certain components are eluted from the chromatographic column they are seen as asymmetric peaks - customarily called bands with a diffuse or sharp trailing or leading front. In such cases calculations based on Gaussian distribution may lead to considerable errors.

Preliminary studies of the column behaviour of some benzoic acids using water as the solvent and the eluent have shown that these acids emerge out of the column as asymmetric bands with a smeared leading front and a sharp trailing front indicating that some parts of the zone move faster than the others (Table 4.3-1). This is due to the partial exclusion of these acids from the resin phase. Hence, for the acids with lower pK values the departure from the Gaussian distribution will be more. In separation studies this is undesirable because this leads to overlapping and therefore, to a smeared separation of adjacent bands. Further, the chromatographic equations become less valid and may give erroneous results, if applied.

However, this problem can be overcome by using 0.01N aqueous hydrochloric acid as solvent and eluent, in which the ion exclusion mechanism becomes less or inoperative and the elution curves become fairly symmetric. In consistance with this observation the column elution runs of all the benzoic acids were carried out in 0.01N aqueous hydrochloric acid and/or 10% dioxan in 0.01N aqueous hydrochloric acid. Even in acidic solvents, solutes with low sorption coefficients, e.g. 2,6-dihydroxybenzoic acid, may show some tailing i.e. the bands with

a diffuse trailing front which may be due to the overloading of the column. Tailing becomes more apparent with longer columns.

4.4-1 : Peak Elution Volume :

The peak elution volume, Vm, denotes the volume of the eluent from the start of the elution run to the mid-point of the peak. It can be expressed in terms of B as:

$$Vm = B.C \qquad (1)$$

where C is the total capacity of the resin in equivalents in the given column. From equation (1) it follows that

(i) Vm is directly proportional to B

(ii) Vm is directly proportional to C and for the given column C is directly proportional to the length of the resin bed, L, hence, Vm is also directly proportional to L.

However, equation (1) is valid only if the void volume, Vi; disc volume (dead volume), Vd; and feed volume, Vf are negligible, and if they are not negligible we may express the peak elution volume as :

$$Vmo = B.C + Vo \qquad (2)$$

where Vo is the sum of Vi and Vd, and in the present study, since Vf is sufficiently large it is also included in Vo. Thus

$$Vo = Vi + Vd + Vf \qquad (3)$$

The values of Vmo are calculated for various benzoic acids from their B values (Chapter 3) for different values of C (i.e. for different values of L) and these are compared with the experimental values of Vmo obtained by carrying out the actual

column elution runs of these acids on different columns (Table 4.3-1 to 4.3-19). The calculated and experimental values of Vmo are reported in Table 4.4-5-1.

4.4-2 : Width and Peak Height of an Elution Curve :

As mentioned in Section 4.1, the width of an elution curve is a measure of column performance or column efficiency and is generally expressed in terms of σ or β. For a Gaussian curve these two quantities are related as :

$$\beta = 2\sqrt{2}.\sigma \qquad (4)$$

The width of an elution curve depends generally on the distribution coefficient of the solute and the column parameters such as resin particle size, area of cross section, flow rate, feed volume and temperature. In the literature equations are given correlating σ^{-2} (variance) with the distribution coefficient and the column parameters. By considering one variable at a time, holding other parameters constant, its effect on the width of elution curve can be studied.

In this work the effect of distribution coefficient and column length on σ have been studied, the other parameters were held constant. Under these conditions it would be possible to give an equation correlating σ with B and C as :

$$\sigma = a_1\sqrt{B} + a_2 B \qquad (5)$$

where

$$a_1 = k_1\sqrt{C} \qquad (6)$$

and

$$a_2 = k_2\sqrt{C} \qquad (7)$$

k_1 and k_2 being constants.

The values of a_1 and a_2 could be calculated by plotting experimentally determined values of $\sigma/\sqrt{B}$ versus $\sqrt{B}$ for the elution runs on different columns (Table 4.2-1). Hence, from the values of a_1, a_2 and C the constants k_1 and k_2 can be obtained from equations (6) and (7) respectively. The values of k_1 and k_2 thus computed are 0.05 and 0.0025 respectively and remain constant for all the values of C studied in this work.

It may be suggested empirically that k_1 and k_2 may be expressed in terms of feed volume, Vf, by

$$k_1 = \sqrt{Vf}/\pi \qquad (8)$$

and

$$k_2 = Vf/\pi^2 = k_1^2 \qquad (9)$$

Now combination of equations (5), (6) and (7) gives σ in terms of B and C as :

$$\sigma = k_1 \sqrt{B.C} + k_2.B\sqrt{C}$$

$$= k_1 \sqrt{B.C}\ (1 + \sqrt{B}.k_2/k_1) \qquad (10)$$

Since σ and β are related (equation 3) we can also express β in terms of B and C as :

$$\beta = 2k_1 \sqrt{2B.C}\ (1 + \sqrt{B}.k_2/k_1) \qquad (11)$$

Another important quantity of the elution curve is the peak height which refers to the concentration at the peak elution volume, denoted by Cm. This depends upon (i) the amount of the solute loaded (i.e. initially sorbed on the column), W, which refers to the area under the elution curve and (ii) the width of the elution curve. For a Gaussian curve these are related as:

$$Cm = W/\sqrt{2\pi}\,\sigma \qquad (12)$$

From equations (10) and (12) it follows that

$$Cm = \frac{W}{k_1 \sqrt{2\pi BC}\ (1+\sqrt{B}\, k_2/k_1)} \qquad (13)$$

The illustrative calculations of σ and Cm for the given values of B, C and W and their experimental determination are shown in Section 4.4-5. The calculated and experimental values of σ and Cm are reported in Table 4.4-5-1.

4.4-3 Number of Theoretical Plates :

The number of theoretical plates N or the plate height H is an indirect measure of the width of an elution curve and hence the column performance. The experimental determination of the number of theoretical plates in the given column, for the particular compound, involves the experimental determination of peak elution volume and either the width (σ or β) or peak height of the elution curve. These are related to the number of theoretical plates by the following equations :

$$N_1 = (Vm/\sigma)^2 \qquad (14)$$

$$= 8(Vm/\beta)^2 \qquad (15)$$

$$N_2 = 2\pi \left[\frac{Vm \cdot Cm}{W}\right]^2 \qquad (16)$$

Theoretically $N_1 = N_2$

In the present study, the number of theoretical plates for the column elution runs of various benzoic acids on different columns have been calculated using the experimentally determined values of Vm, σ , Cm and W. These values are reported in Table 4.4-5-1. For a particular compound, N_1 and N_2 are comparable and hence the average of these is taken as N experimental denoted by N_{exp}.

Further from equations (1), (10) and (14) it follows that

$$N = \left[\frac{B.C}{k_1\sqrt{BC}\ (1 + \sqrt{B}\ k_2/k_1)} \right]^2 \qquad (17)$$

on simplification, and putting $k_1^2 = k_2$, we get

$$N = \frac{B.C}{k_2\ (1 + k_1\sqrt{B})^2} \qquad (18)$$

The equation (18) expresses N in terms of B and C and the values of N calculated using this equation are reported in Table 4.4-5-1.

4.4-4 Resolution (Separation Study) :

The quantitative measure of separation is the Resolution given by the equation :

$$R_n = \frac{\Delta Z}{3(\sigma_1 + \sigma_2)} = \frac{Vm_2 - Vm_1}{3(\sigma_1 + \sigma_2)} \qquad (19)$$

where $\Delta Z = Vm_2 - Vm_1$, the gap between the neighbouring peaks. The subscripts 1 and 2 denote solutes 1 and 2.

Combining equations (1), (10) and (19) and on simplification, we get

$$R_n = \frac{\sqrt{C}\ (\sqrt{B_2} - \sqrt{B_1})}{3k_1 \left[1 + \frac{k_2}{k_1} \times \frac{B_2 + B_1}{\sqrt{B_2} + \sqrt{B_1}} \right]} \qquad (20)$$

From equation (20) it follows that for the given pair of solutes having sorption coefficients B_1 and B_2, the resolution, Rn for the particular column (i.e. particular value of C) can be calculated and thus the extent of separation can be predicted

before carrying out the actual column runs.

An illustrative calculations of Rn for 3,5-dihydroxybenzoic acid (B = 1.68) and 2,4-dihydroxybenzoic acid (B = 3.70) on column L_2 is shown in Section 4.4-5. Rn is also calculated from the experimentally determined values of Vm and σ for these two acids from their individual column elution runs on the same column, using equation (19). The two values of Rn thus obtained are in fair agreement. Then the actual separation was tried by taking the mixture of these acids, the two components separated in accordance with the resolution computed from their B values and from their individual runs.

For some other pairs of benzoic acids of interest, the values of Rn (calculated from B values and also from the individual column elution runs) are reported in Table 4.4-5-2. The column elution runs of ~~some~~ mixtures of some illustrative pairs have been carried out (Table 4.3-20 to 4.3-26).

As seen earlier, Vm is proportional to C and σ is proportional to $\sqrt{C}$. Therefore, Rn increases with $\sqrt{C}$ thus always reaching unity-indicating a satisfactory separation - if a sufficiently long column can be used. By setting Rn = 1 in equation (20), the minimum number of equivalents of the resin, Cmin required for the separation of the desired pairs of benzoic acids can be calculated. The equation of Cmin can be written as :

$$C_{min} = \left[\frac{1 + A_2}{A_1}\right]^2 \qquad (21)$$

where

$$A_1 = \frac{\sqrt{B_2} - \sqrt{B_1}}{3k_1} \qquad (21a)$$

and

$$A_2 = \frac{k_2}{k_1} \times \frac{B_2 + B_1}{\sqrt{B_2} + \sqrt{B_1}} \qquad (21b)$$

Table 4.4-5-2 gives the calculated values of Cmin for some pairs of benzoic acids.

Depending on the value of Rn, separations may be classified as :

(1) Quantitative and efficient separations : These have Rn value as unity or almost unity. This in fact, is the object of any chromatographic separation.

(2) Quantitative but inefficient separations : In these Rn value is considerably greater than unity, thus resulting in a waste of time and eluent.

(3) Incomplete separations : These have Rn value less than unity and peaks overlap considerably. However, the peaks can be easily identified. Thus for identification purposes resolution need not always be unity.

4.4-5 : Calculations :

The calculation and experimental determination of various chromatographic quantities for the column elution runs reported in Tables 4.3-1 to 4.3-19 are illustrated in this Section by considering a pair of benzoic acids.

Calculation I

Benzoic acid : 3,5-diOH

Solvent and eluent : 0.01N aqueous HCl

Column : L_2 ; C = 0.088 equivalents

W = 2.12×10^{-2} millimoles ; B = 1.68

The graph of the elution run is obtained by plotting $10^2 W_s$ versus sample number (Table 4.3-13), where, W_s denotes the amount of the solute in millimoles in 25 ml effluent sample. The circles represent the analyzed fraction of the eluent. The continuous graph is drawn through the circles and the resulting plot is the experimental elution curve, Fig. 1.

Now

$$V_o = V_i + V_d + V_f$$
$$= 0.030 + 0.015 + 0.025 = \underline{0.070} \text{ liter (Table 4.2-1)}$$

$$V_{m(cal)} = B.C$$
$$= 1.68 \times 0.088 = \underline{0.148} \text{ liter}$$

$$V_{mo(cal)} = V_{m(cal)} + V_o$$
$$= 0.148 + 0.070 = \underline{0.218} \text{ liter}$$

$$V_{mo(exp)} = OX = 9.2 \text{ samples (Fig.1)}$$
$$= 9.2 \times 0.025 = \underline{0.230} \text{ liter}$$

$$V_{m(exp)} = V_{mo(exp)} - V_o$$
$$= 0.230 - 0.070 = \underline{0.160} \text{ liter}$$

$$k_1 = \sqrt{V_f/\pi}$$
$$= \sqrt{0.025}/3.14 = \underline{0.050}$$

$$k_2 = V_f/\pi^2 = k_1^2 = \underline{0.0025}$$

$$a_1 = k_1 \sqrt{C}$$
$$= 0.05 \sqrt{0.088} = \underline{1.50 \times 10^{-2}}$$

$$a_2 = k_2 \sqrt{C}$$
$$= 0.0025 \sqrt{0.088} = \underline{0.8 \times 10^{-3}}$$

$$C_{m(cal)} = \frac{W}{k_1 \sqrt{2\pi BC}(1 + k_1 \sqrt{B})} = \underline{4.0 \times 10^{-1}}$$

$$C_m' = XY = \underline{1.06 \times 10^{-2}} \quad (Fig.1)$$

$$C_{m(exp)} = 40 C_m' = 40 \times 1.06 \times 10^{-2} = \underline{4.2 \times 10^{-1}}$$

$$\sigma_{(cal)} = a_1 \sqrt{B} + a_2 B = 1.50 \times 10^{-2} \sqrt{1.68} + 0.8 \times 10^{-3} \times 1.68 = \underline{0.021} \text{ liter}$$

$$\sigma_{(exp)} = 1/2 \times PZ \ (Fig.1) = 1/2 \times 0.04 = \underline{0.020} \text{ liter}$$

$$N_1 = \left[V_{m(exp)}/\sigma_{(exp)}\right]^2 = (0.160/0.020)^2 = \underline{64}$$

$$N_2 = 2\pi \left[\frac{V_{m(exp)} \cdot C_{m(exp)}}{W}\right]^2 = 2 \times 3.14 \left[\frac{0.160 \times 4.2 \times 10^{-1}}{2.12 \times 10^{-2}}\right]^2 = \underline{63}$$

$$N_{exp} = \frac{N_1 + N_2}{2} = \frac{64 + 63}{2} = \underline{64}$$

$$N_{cal} = \frac{B.C}{k_2(1 + k_1 \sqrt{B})^2} = \frac{1.68 \times 0.088}{2.5 \times 10^{-3}(1 + 0.05\sqrt{1.68})^2} = \underline{50}$$

Calculation II :

Benzoic acid : 2,4-diOH

Solvent and eluent : 0.01N aqueous HCl

Column : L_2; C = 0.088 equivalent

W = 2.48×10^{-2} millimoles; B = 3.70

From Tables 4.2-1 and 4.3-13 and Fig.2, on calculation, the following results are obtained :

$Vm_{(cal)} = \underline{0.326}$ liter $Vm_{(exp)} = \underline{0.323}$ liter

$Vmo_{(cal)} = \underline{0.396}$ liter $Vmo_{(exp)} = \underline{0.393}$ liter

$Cm_{(cal)} = \underline{3.1 \times 10^{-1}}$ $Cm_{(exp)} = \underline{3.2 \times 10^{-1}}$

$\sigma_{(cal)} = \underline{0.032}$ liter $\sigma_{(exp)} = \underline{0.031}$ liter

$N_{cal} = \underline{104}$ $N_{exp} = \underline{109}$

Calculation III :

$$Rn_{(cal)} = \frac{\sqrt{C}\ (\sqrt{B_2} - \sqrt{B_1})}{3k_1 \left[1 + \frac{k_2}{k_1} \cdot \frac{B_2 + B_1}{\sqrt{B_2} + \sqrt{B_1}}\right]}$$

$$= \frac{Vm_{(cal)_2} - Vm_{(cal)_1}}{3\left[\sigma_{(cal)_2} + \sigma_{(cal)_1}\right]}$$

$$= \frac{0.326 - 0.148}{3(0.032 + 0.021)} = \underline{1.12}$$

$$Rn_{(exp)} = \frac{Vm_{(exp)_2} - Vm_{(exp)_1}}{3\left[\sigma_{(exp)_2} + \sigma_{(exp)_1}\right]}$$

$$= \frac{0.323 - 0.160}{3(0.031 + 0.020)} = \underline{1.07}$$

$$C_{min} = \left[\frac{1 + A_2}{A_1}\right]^2$$

where $$A_1 = \frac{\sqrt{B_2} - \sqrt{B_1}}{3k_1} = \frac{\sqrt{3.70} - \sqrt{1.68}}{3 \times 0.05} = \underline{4.20}$$

and $$A_2 = \frac{k_2}{k_1} \cdot \frac{B_2 + B_1}{\sqrt{B_2} + \sqrt{B_1}}$$

$$= \frac{0.0025}{0.05} \cdot \frac{3.70 + 1.68}{\sqrt{3.70} + \sqrt{1.68}} = \underline{0.084}$$

therefore,

$$C_{min} = \left[\frac{1 + 0.084}{4.20}\right]^2 = 0.067 \text{ equivalents}$$

Fig. 3 gives the experimental elution curve for the column elution of the mixture of 3,5-diOH and 2,4-diOH benzoic acids on column L_2 (C = 0.088 equivalents) with 0.01N aqueous hydrochloric acid as solvent and eluent (Table 4.3-24).

Tables 4.4-5-1 and 4.4-5-2 give the calculated and experimental values of the chromatographic quantities for the substituted benzoic acids of interest.

Fig 1 Plot of Ws versus sample number for the column elution run of 3,5-diOH benzoic acid on column L_2 with 0.01N aqueous hydrochloric acid as solvent and eluent.

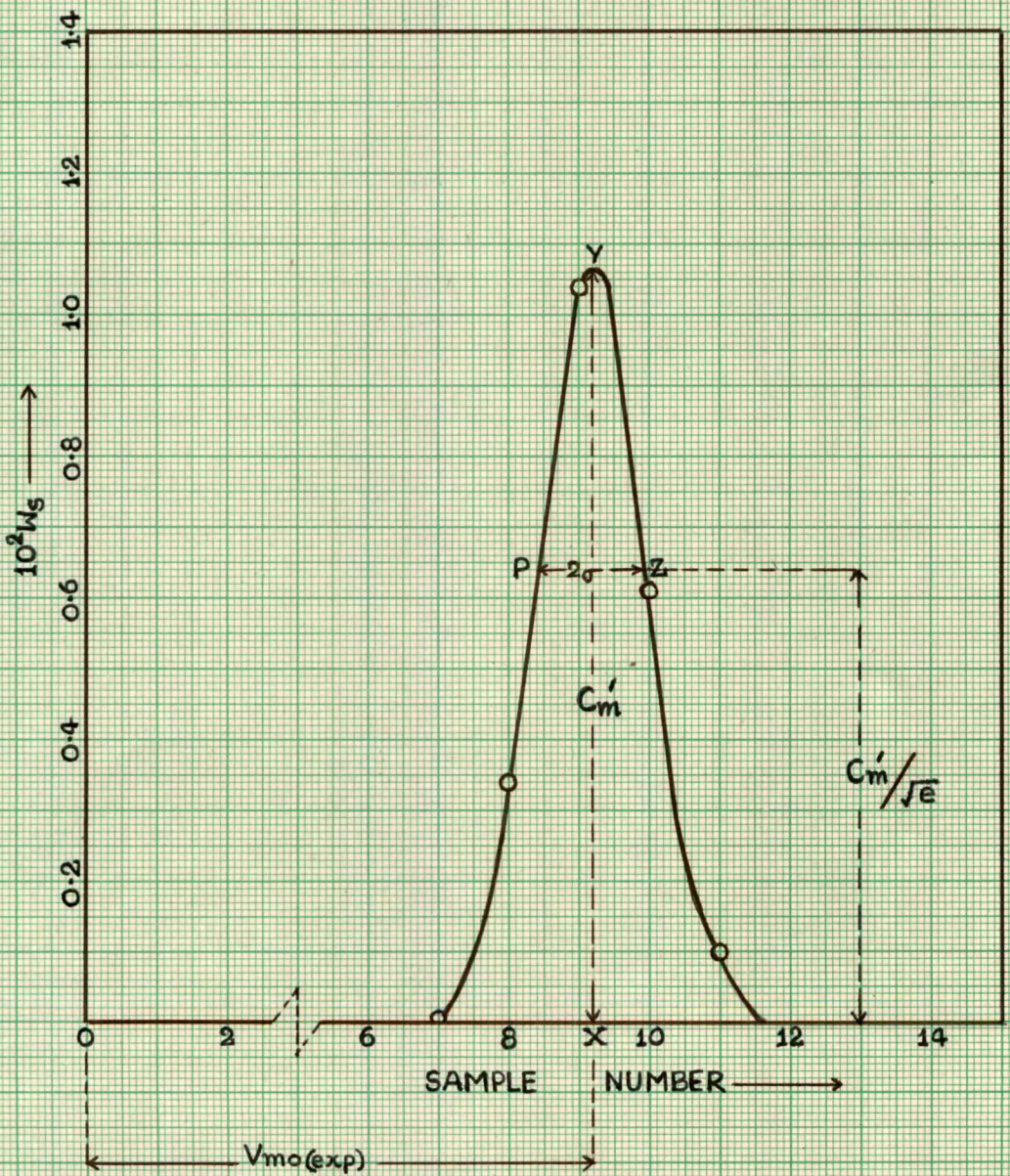

Fig 2 Plot of Ws versus sample number for the column elution run of 2,4-diOH benzoic acid on column L_2 with 0.01N aqueous hydrochloric acid as solvent and eluent.

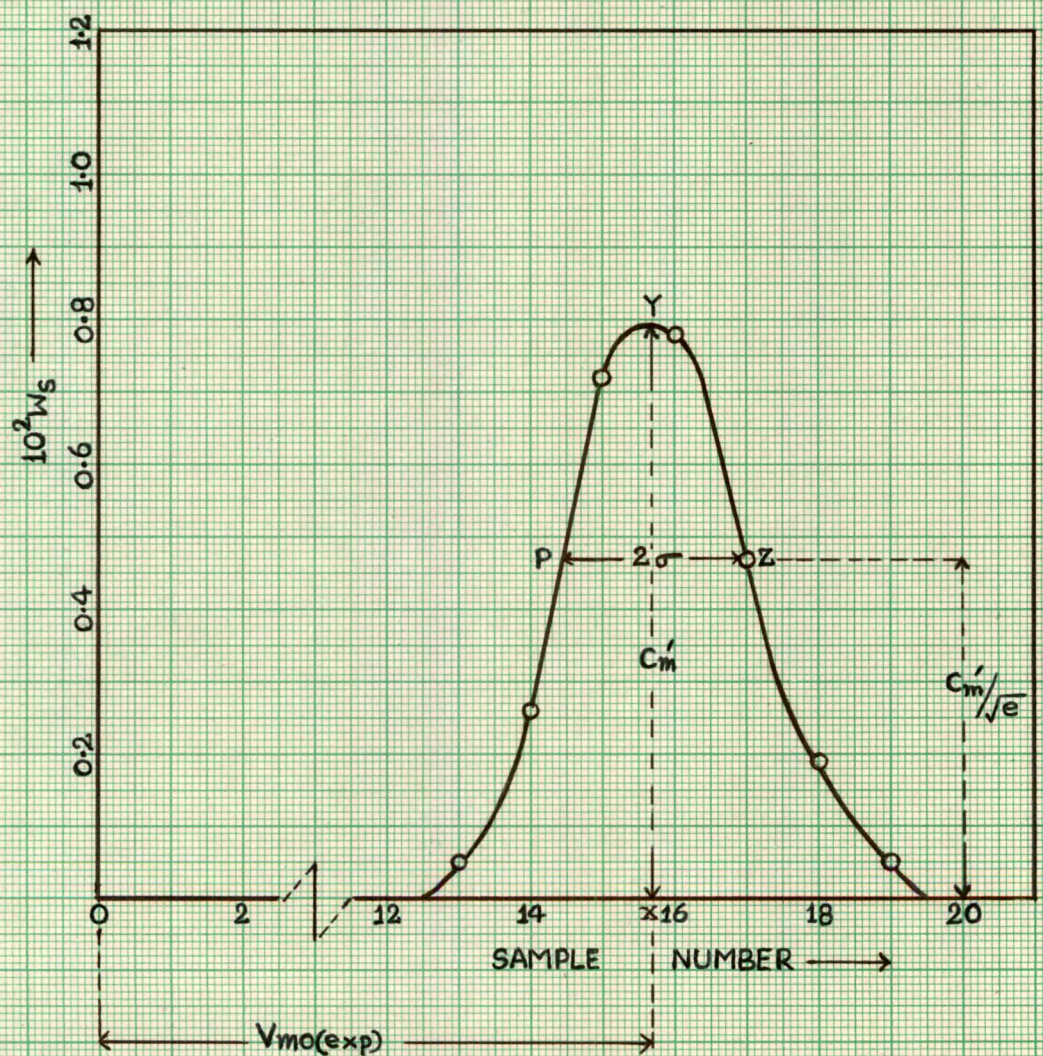

Fig 3 Plot of Ws versus sample number for the column elution run of the mixture of (a) 3,5-diOH and (b) 2,4-diOH benzoic acids on column L_2 with 0.01N aqueous hydrochloric acid as solvent and eluent.

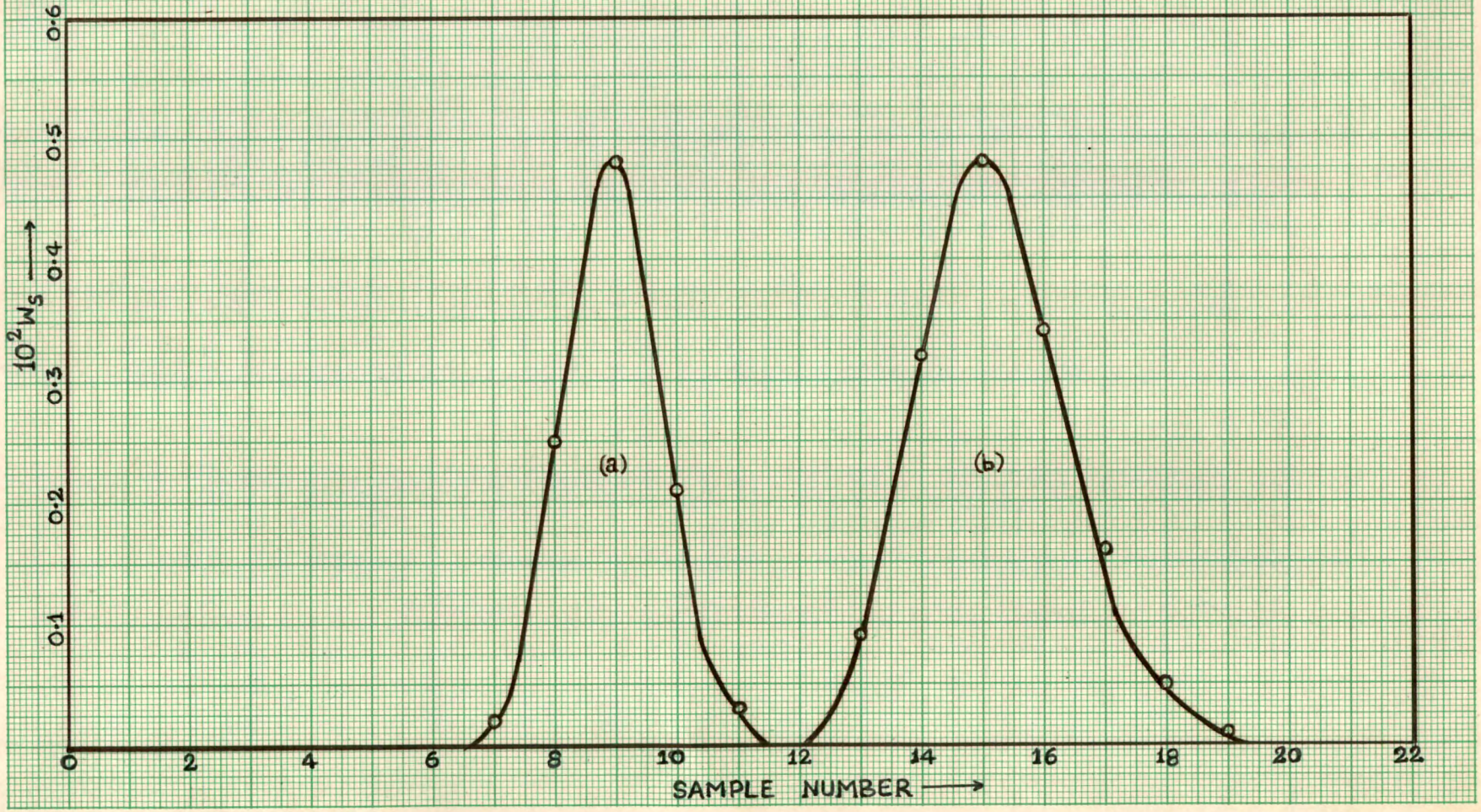

Table 4.4-5-1 : Calculated and experimental quantities for the column elution runs of benzoic acids. (WH = 0.01N aq. HCl, DWH = 10% dioxan in 0.01N aq. HCl).

Benzoic acid	Solvent	B	Column	10^2W millimoles	10^3Vmo liter		$10^3\sigma$ liter		10^2Cm		N_1	N_2	N_{Exp}	N_{cal}
					cal.	exp.	cal.	exp.	cal.	exp.				
H	WH	2.82	L_2	0.39	318	320	27	25	5	7	100	125	113	84
			L_3	0.60	185	183	20	21	12	12	37	41	39	42
	DWH	1.45	L_2	0.94	198	208	19	20	20	20	48	54	51	45
			L_3	4.45	122	120	15	18	117	108	13	16	15	20
o-OH	WH	3.40	L_1	2.15	673	693	40	38	22	23	247	257	252	209
			L_2	0.92	369	370	30	28	12	14	115	130	123	99
	DWH	1.75	L_1	11.10	393	418	29	28	152	164	133	143	138	106
m-OH	WH	2.15	L_1	7.15	460	483	31	28	92	103	192	196	194	139
			L_2	3.55	259	260	24	23	59	66	68	78	73	62
			L_3	3.38	154	155	18	19	75	74	28	30	29	30
	DWH	1.00	L_1	8.62	265	294	22	21	156	160	90	86	88	60
p-OH	WH	2.62	L_1	3.64	540	560	35	31	41	46	225	217	221	162
			L_2	1.28	301	318	26	26	20	20	91	94	93	79
	DWH	1.28	L_1	4.28	313	315	25	24	68	73	84	89	87	76
o-OMe	WH	4.00	L_1	8.20	775	780	45	41	76	79	279	274	277	228
	DWH	1.74	L_1	10.93	391	410	29	26	150	156	147	144	146	104

contd...

m-OMe	WH	6.00	L_1	4.65	1115	1125	56	61	33	30	285	277	281	313
	DWH	2.40	L_1	9.85	503	548	34	34	116	116	177	179	178	144
p-OMe	WH	6.44	L_1	1.09	1190	1193	56	62	8	7	313	312	313	382
	DWH	2.65	L_1	2.35	546	548	36	34	26	28	177	183	180	157
			L_3	0.64	177	178	19	19	13	13	42	39	41	41
p-OEt	DWH	3.48	L_1	2.29	687	713	42	43	22	21	207	202	205	199
			L_3	0.90	215	218	23	26	16	14	39	40	40	48
p-n-OPr	DWH	5.05	L_1	0.88	954	958	51	60	7	6	207	218	213	284
			L_3	0.60	287	285	28	34	9	8	46	59	53	69
p-n-OBu	DWH	7.40	L_3	0.31	395	388	34	44	4	3	57	65	61	100
p-Cl	DWH	2.82	L_1	1.49	574	583	37	33	16	19	219	243	231	168
			L_3	0.83	185	180	20	19	17	16	43	37	40	42
p-Br	DWH	3.87	L_3	0.68	233	225	24	24	11	12	50	57	54	55
p-I	DWH	6.00	L_3	0.62	331	333	31	38	8	7	54	62	58	79
2,3-diOH	WH	2.45	L_1	10.20	512	513	34	33	120	130	160	178	169	150
			L_2	2.44	286	290	26	26	38	36	72	66	69	69
			L_3	3.10	168	165	19	23	65	56	25	25	25	36
	DWH	1.15	L_1	1.18	291	315	23	23	20	21	92	96	94	73
2,4-diOH	WH	3.70	L_1	6.21	724	735	43	43	58	56	221	209	215	214
			L_2	2.48	396	393	32	31	31	32	109	109	109	104
			L_3	3.56	225	210	23	24	61	60	42	43	43	55
	DWH	1.64	L_1	3.51	374	383	28	26	50	58	123	142	133	99

contd...

2,5-diOH	WH	2.35	L_1	1.81	495	515	33	31	22	23	184	179	182	147
			L_2	3.01	277	260	25	23	48	51	68	65	67	69
			L_3	1.73	163	158	18	20	38	36	26	29	28	36
	DWH	1.12	L_1	4.00	285	280	22	18	73	110	106	163	135	75
2,6-diOH	WH	0.51	L_1	2.35	166	185	14	19	67	50	22	23	23	26
			L_2	1.75	115	100	11	11	63	51	8	5	7	16
			L_3	2.66	79	70	8	13	133	75	1	1	1	9
	DWH	0.42	L_1	3.04	166	130	15	17	81	70	4	4	4	22
3,4-diOH	WH	1.74	L_1	2.01	391	415	28	28	29	29	130	134	132	112
			L_2	1.21	223	230	21	21	23	24	58	62	60	53
			L_3	2.28	135	133	15	19	60	46	17	16	17	28
	DWH	0.85	L_1	1.00	240	233	19	20	21	22	48	58	53	58
			L_3	0.92	94	93	10	20	37	19	4	4	4	14
3,5-diOH	WH	1.68	L_1	2.56	381	410	28	23	37	45	188	201	195	104
			L_2	2.12	218	230	21	20	40	42	64	63	64	50
			L_3	2.44	132	133	15	19	64	52	17	17	17	26
	DWH	0.82	L_1	1.99	234	233	19	19	42	43	53	56	55	53
			L_3	0.55	93	88	10	21	22	10	3	2	3	14
2-OH-3-OMe	WH	6.66	L_3	0.57	361	313	32	34	7	7	58	63	61	91
	DWH	2.65	L_1	6.12	546	533	36	35	68	70	157	158	158	157
2-OH-4-OMe	DWH	3.76	L_1	2.06	734	735	43	47	19	17	185	175	180	220
2-OH-4-OEt	DWH	5.15	L_1	2.44	971	963	52	64	19	16	184	204	194	284
2-OH-5-OMe	DWH	3.24	L_1	6.28	646	610	40	39	63	66	174	184	179	190
4-OH-3-OMe	WH	4.00	L_3	0.82	239	233	24	26	14	12	47	43	45	55
	DWH	1.53	L_1	3.93	355	365	27	26	58	57	87	98	93	92

contd...

2,3-diOMe	DWH	1.70	L_1	2.86	384	383	28	25	41	48	133	147	140	107
			L_2	1.67	220	200	21	20	32	37	42	52	47	51
			L_3	0.53	133	123	15	18	14	12	14	15	15	27
2,4-diOMe	DWH	3.36	L_1	1.48	666	690	41	44	14	13	183	172	178	194
2,5-diOMe	DWH	3.24	L_1	0.63	646	665	40	41	6	6	193	185	189	190
2,6-diOMe	DWH	1.30	L_1	1.73	316	315	25	25	28	30	77	91	84	78
			L_2	1.56	184	175	18	18	35	37	34	39	37	40
3,4-diOMe	DWH	3.14	L_1	1.72	629	623	40	45	17	16	138	152	145	178
3,5-diOMe	DWH	5.33	L_2	0.58	539	525	39	45	6	5	102	96	99	145

Table 4.4-5-2 : Calculated and experimental quantities for the column elution runs of some mixtures of benzoic acids.

Benzoic acids	Solvent	C milli equiv	Elution range	Rn Cal.	Rn Exp	C milli equiv
m-OH + o-OH	WH	170	16-23 / 24-32	1.00	1.06	192
	DWH	170	10-14 / 14-19	0.84	0.84	246
o-OMe + m-OMe	WH	170	26-37 / 39-52	1.12	1.13	137
	DWH	170	14-19 / 18-26	0.59	0.77	489
m-OH + m-OMe	WH	170	16-23 / 39-52	2.50	2.40	29
	DWH	170	10-14 / 18-26	1.42	1.54	88
p-OMe + p-OEt	DWH	170	18-26 / 24-33	0.60	0.71	462
p-OMe + p-n-OPr	DWH	170	18-26 / 32-44	1.56	1.45	71
		46	6-9 / 9-14	0.78	0.67	71
p-OEt + p-n-OBu	DWH	46	7-11 / 12-19	1.05	0.81	38
p-Cl + p-Br	DWH	46	5-9 / 7-11	0.36	0.35	279
p-Cl + p-I	DWH	46	5-9 / 10-17	0.95	0.90	45
p-Br + p-I	DWH	46	7-11 / 10-17	0.59	0.58	126
2,3-diOH + 2,4-diOH	WH	170	17-25 / 26-36	0.92	0.97	205
		88	9-16 / 13-19	0.63	0.60	205
3,4-diOH + 2,3-diOH	WH	170	14-20 / 17-25	0.65	0.54	449
3,5-diOH + 2,4-diOH	WH	88	7-11 / 13-19	1.12	1.07	67
	DWH	170	9-11 / 12-18	0.99	1.11	174

contd...

2,3-diOH + 2-OH-3-OMe	WH	46	5-9 / 10-15	1.26	0.87	27
	DWH	170	11-15 / 18-26	1.44	1.25	82
3,4-diOH + 4-OH-3-OMe	WH	46	4-7 / 7-12	0.89	0.74	58
	DWH	170	8-11 / 12-18	0.83	0.96	260
4-OH-3-OMe + 2-OH-3-OMe	DWH	170	12-18 / 18-26	1.01	0.92	170
4-OH-3-OMe + 2-OH-5-OMe	DWH	170	12-18 / 21-28	1.45	1.26	88
2,3-diOMe + 2-OH-3-OMe	DWH	170	13-18 / 18-26	0.84	0.83	251
4-OH-3-OMe + 3,4-diOMe	DWH	170	12-18 / 21-29	1.36	1.18	90
2,3-diOMe + 3,4-diOMe	DWH	170	13-18 / 21-29	1.20	1.14	118
2,6-diOMe + 2,5-diOMe	DWH	170	11-15 / 23-30	1.69	1.77	60

WH = 0.01N aqueous hydrochloric acid

DWH = 10% dioxan in 0.01N aqueous hydrochloric acid.

4.4-6 Conclusions :

The study of the sorption-elution behaviour of substituted benzoic acids indicates that :

(i) Feed volume can affect elution volume - the larger feed volume results in an increase in the peak elution volume. The fair agreement between the values of Vmo calculated, which include the feed volume, with the experimental values of Vmo supports this.

(ii) Feed volume can also affect the column efficiency. This is evident from the dependance of σ^2 on the feed volume. If the feed volume is large there will be appreciable band spreading at the time of introduction and the peak will be broadened and the deviations of the peak shape from Gaussian increases. Thus, in the case of benzoic acids having lower B values in a given solvent the experimental values of σ^2 are considerably larger than the calculated values and this is more so with shorter columns. In the case of benzoic acids having higher B values when eluted from longer columns, the elution curves get flattened and the observed σ^2 values are thus higher than those calculated on the basis of Gaussian distribution. In the present study, the feed volume and the volume of the fractions collected are both 25 ml, in consistence with the solubility of the acids and the detectability of the components in the effluent. However, it should be possible to increase the column efficiency by taking smaller feed volume.

(iii) In liquid chromatography where the kinetics of sorption are controlled by particle diffusion, the theoretical plate height

is not a characteristic of the column alone, but depends also on the partition coefficients and on the diffusion coefficients of the substances to be separated. Therefore, we must expect different theoretical plate heights and numbers for every substance almost. Calculations have shown that the trend is as expected.

The observed difference between N_{exp} and N_{cal} may be attributed to the fact that the square terms are involved and hence the experimental error gets magnified.

(iv) Increase in length (L) and hence C would seem to provide a solution to any separation problem. This is true within some practical limits. If the column is too long, the flow rate will be very low and the process becomes time consuming. It may also happen that the zones, when separated, will be so dilute as to be undetectable.

(v) The separation of molecules that differ in the type or number of functional groups would be easier, e.g. separation of m-hydroxybenzoic acid and m-methoxybenzoic acid.

(vi) The separation of position isomers, at least partially, of the monosubstituted benzoic acids is possible, e.g. separation of o- and m-hydroxybenzoic acids.

(vii) The separation of the members of the homologous series can be achieved under suitable conditions. In the p-alkoxybenzoic acid series, it is observed that the elution curves of the adjacent members overlap to some extent. This can be overcome by decreasing the feed volume and it should be possible to achieve a complete separation.

(viii) The separation of the position isomers of the disubstituted benzoic acids is also possible. In general, sterically

hindered isomers can be separated from other isomers in which the steric effect is lesser or negligible, e.g. separation of 2,6-dihydroxybenzoic (γ-resorcylic), 3,5-dihydroxybenzoic (α-resorcylic) and 2,4-dihydroxybenzoic (β-resorcylic) acids.

Thus the technique provides a convinient and useful procedure for the separation of substituted benzoic acids and in general, should be applicable to the separation of other molecular families.

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