ADSORPTION PAPER CHROMATOGRAPHY OF INORGANIC ANIONS IN ACETATE BUFFERS

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Separations of inorganic substances by paper chromatography with aqueous solvents have been investigated by various authors since the early work of SCHÖNBEIN and GOPPELSROEDER. The movement of cations depends largely on the pH of the developing solution as has been summarised by PICKERING[1] and HAYEK[2]. Separations of cations that can be utilised in analytical chemistry are rare[3] unless the substances to be separated are carrier-free tracers[4,5].

MILIČEVIĆ[6] investigated not only cations but also anions and LEDERER AND WARD[7] showed by paper chromatography with N KCl that anions move essentially near the liquid front, thus ruling out adsorption on the paper in the interpretation of paper electrophoretic results. RIPAN *et al.*[8] have shown recently that polyanions, namely molybdate and its heteropolyacids and even dichromate, are retained on the paper when developed with saturated KCl solution and that this adsorption depends on the pH value of the solvent. No adsorption was recorded in alkaline solvents. These workers utilise their results in explaining problems of complex chemistry but do not concern themselves with the mechanism of the adsorption.

In recent years paper chromatography with aqueous solvents is also being used extensively for the separation of organic substances. To mention a few examples: phenols by BOSCOTT *et al.*[9] and KEITH *et al.*[10], alkaloids by DE MOERLOOSE[11] and CASINOVI *et al.*[12], anthoxanthins and anthocyanins by GAGE *et al.*[13].

The most useful solvents for organic substances were acetate buffers or aqueous solutions of acetic acid.

Since so far no general study dealing with inorganic anions has been reported, it was decided to carry out an investigation of these anions, as hydrolysis and cation exchange, which seem to govern the behaviour of cations, does not occur with them. It was thus hoped that further information on the mechanism of "adsorption" paper chromatography could be obtained.

EXPERIMENTAL

Several inorganic anions (as their alkali salts) were chromatographed in an aqueous solution containing 10 % sodium acetate and 10 % acetic acid on Whatman No. 3 MM paper, as these conditions seem to be the most favourable for numerous separations of

phenols and alkaloids. As shown in Table I, quite a number of anions are retained by the paper and move slower than the liquid front.

TABLE I

R_F VALUES OF INORGANIC ANIONS

Solvent: 10 g sodium acetate and 10 ml glacial acetic acid made up to 100 ml with distilled water.
Paper: Whatman No. 3 MM. Method: ascending development (at about 20°).

Anion	R_F value	Anion	R_F value
WO_4^-	0.95	AsO_4^{-3}	0.98
CrO_4^{-2}	0.64	AsO_3^{-3}	0.86
MoO_4^{-2}	0.66 + comet	Cl^-	0.87
SeO_3^{-2}	0.87	Br^-	0.86
TeO_3^{-2}	0.66	I^-	0.83
VO_3^-	0.54	CNS^-	0.77
TeO_4^{-2}	0.89	Ferrocyanide	1.0
BO_3^-	0.87	Ferricyanide	1.0
		$Co(NO_2)_6^{-3}$	1.0
		PO_4^{-3}	1.0

The acids that are unstable in acetic acid such as NO_2^- or BrO_3^- could, of course, not be chromatographed. In aqueous 10 % sodium acetate the following R_F values were observed for the halogen oxyacids: $BrO_3^- = 0.91$; $IO_3^- = 0.84$ and $IO_4^- = 0.83$ (with a comet to 0).

From Table I it is already obvious that there is absolutely no relationship between the movement and the charge of the anion. Tri- and tetravalent anions seem to move much faster as a rule than mono- and divalent ones.

Several cations were also chromatographed in the same solvent as in Table I and their R_F values are given in Table II.

TABLE II

R_F VALUES OF SOME CATIONS
(Conditions as in Table I)

Cation	R_F value	Cation	R_F value
Co^{+2}	1.0	$[CoNO_2(NH_3)_5]^{+2}$	0.95
Ni^{+2}	1.0	$[Co(NO_2)_2(NH_3)_4]^+$	0.87
Cu^{+2}	1.0	$[Co(NO_2)_3(NH_3)_3]^0$	0.77
UO_2^{+2}	1.0		

The series of cobaltammines shows that also for the cations the "adsorption" does not follow the ionic charge of the complex as has also been shown previously with other aqueous solvents[14].

It was thus considered that some non-ionic adsorption process is responsible for the different movement of various anions and the following experiments were carried out to investigate its properties.

Variation of the R_F value with the amount chromatographed

One way of examining whether the adsorption isotherm is linear is by chromatographing varying amounts. As shown in Fig. 1 there is no appreciable change for the R_F value of CrO_4^{-2} when 10 μl of solutions containing from 0.5 % to 3 % of K_2CrO_4
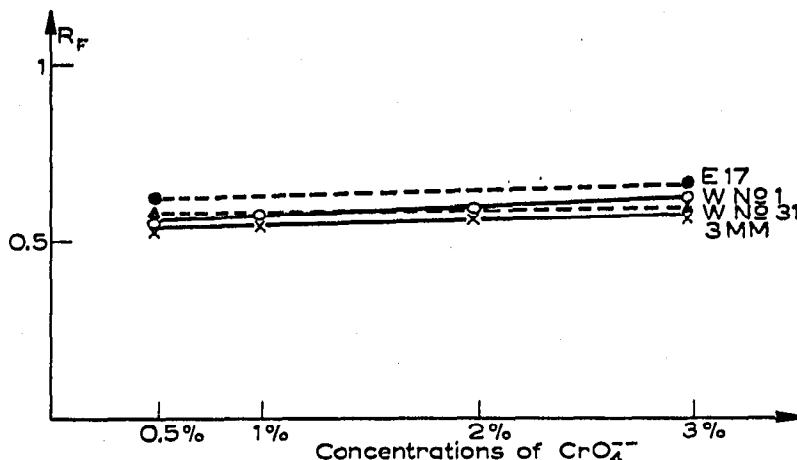

Fig. 1. The R_F values of chromate solutions of varying concentration (0.5 % to 3 %) are plotted for four different brands of Whatman paper (No. 1, No. 31, No. 3 MM and E 17). Solvent: 10 % acetic acid + 10 % sodium acetate.

are chromatographed side by side on the same sheet. For molybdate the change in R_F value is greater (Fig. 2); however the behaviour of molybdate in acid solution is governed by polymerisation equilibria in addition to the adsorption equilibrium. Hence the results with molybdate serve only to show that with certain ions the R_F value may be found to vary with the concentration.

The influence of the solvent to paper ratio on the R_F value

If an adsorption mechanism is to be ascribed to the retention of anions then the R_F value should vary with the solvent–paper ratio, which would be identical to the factor A_L/A_S of the equation of MARTIN[15]. The solvent–paper ratios were determined for four papers of different thickness by weighing the dry paper and then the developed chromatogram and deducing the liquid weight from that. The results obtained, which are in good agreement with those of other workers[16], are given in Table III.

TABLE III

Paper	Liquid–paper ratios
Whatman No. 1	1.03; 1.01
Whatman No. 3 MM	1.32
Whatman E 17	1.7; 1.69
Whatman No. 31	1.41; 1.3

The order of the increasing R_F values due to the solvent–paper ratio should thus be: Whatman No. 1 (lowest R_F) No. 3 MM, No. 31, E 17 (highest R_F). However, as shown in Figs. 1 and 2, this is certainly not the case.
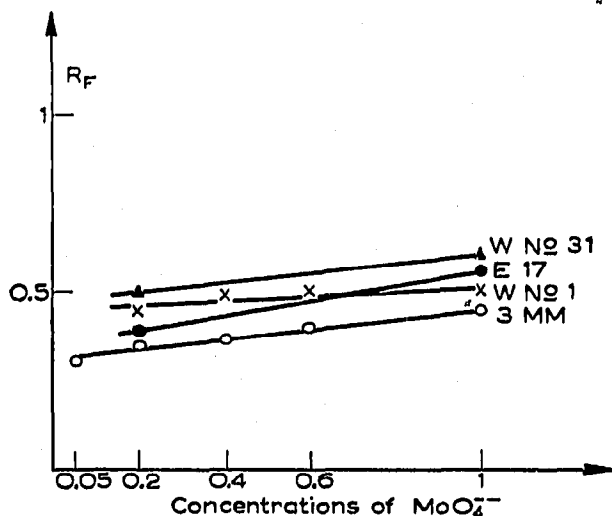
Fig. 2. The R_F values of ammonium molybdate in concentrations from 0.05 % to 1 % on Whatman No. 1, No. 31, No. 3 MM and E 17 papers. Solvent: 10 % acetic acid + 10 % sodium acetate.
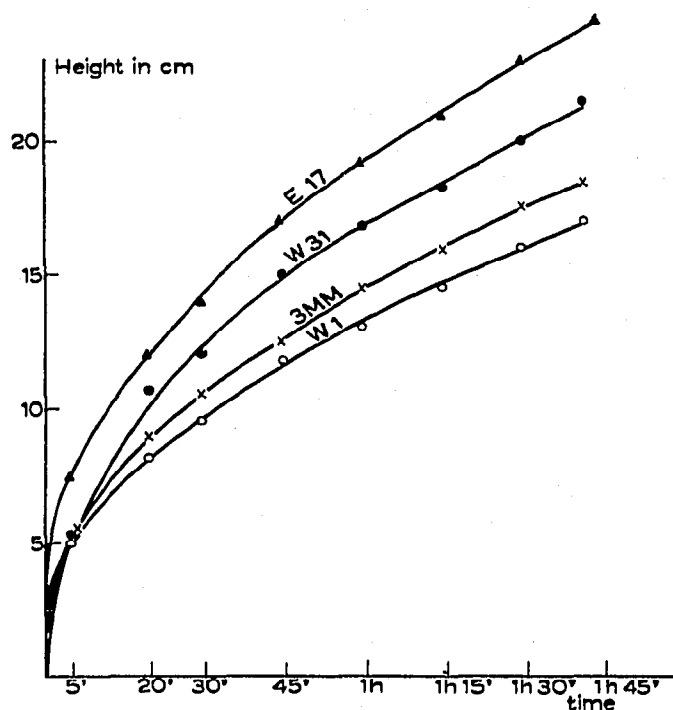
Fig. 3. Liquid movement in ascending development plotted against time on Whatman No. 1, No. 31, No. 3 MM and E 17 papers.

The speed of development should also be a factor, since for a fast liquid flow with a slow equilibrium rate higher R_F values might be obtained than with a liquid flow permitting attainment of equilibrium between the cellulose surface and the solvent. As can be seen from Fig. 3 the speed of development follows the order of the solvent–paper ratios. Thus both factors seem either negligible or overruled by another property of the paper.

Effect of pH on the R_F value

Fig. 4 shows the variation of the R_F values of selenite, chromate, molybdate and metavanadate with the pH value. The pH value was varied by adding increasing amounts of acetic acid to 10 % sodium acetate. Whatman No. 3 MM paper was used only. The "true" anions selenite and chromate do not change their R_F values from pH 5.8 to 4.2, vanadate and molybdate, which undergo polymerisation in this pH range, do vary somewhat.

Effect of salt concentration on the R_F value

We tried to measure the effect of salt concentration without change of the pH by developing with various dilutions of mixtures of sodium acetate and acetic acid at constant ratio (1:1). There is a considerable change for CrO_4^{-2}, MoO_4^{-2} and VO_3^- but little change for SeO_3^{-2} as shown in Fig. 5. The small change in R_F value for the

selenite may be due to its rather high R_F which does not easily register differences in the distribution coefficient. Molybdate increases its R_F with an increase in the salt concentration. In general, however, it seems that R_F values are depressed by the ionic concentration. This definitely rules out the possibility of anion exchange and strongly suggests a reversible salting-out (precipitation-like) process. It must not be forgotten,
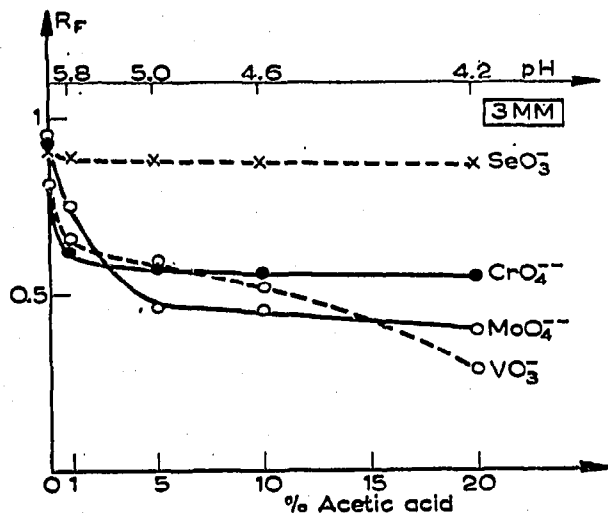

Fig. 4. Variation of R_F values of selenite, chromate, molybdate and vanadate with pH. Varying amounts of acetic acid (o, 1, 5, 10 and 20%) were added to 10% sodium acetate. The pH values indicated above were measured with a glass electrode. Paper: Whatman No. 3 MM.
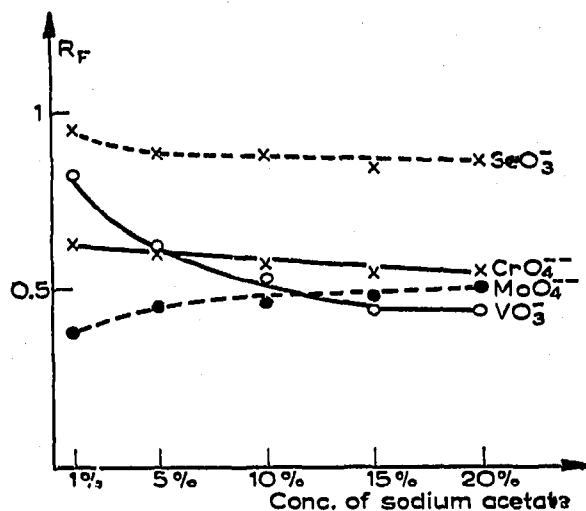
Fig. 5. Variation of R_F values with increase in salt concentration. Equal quantities of sodium acetate and acetic acid (1, 5, 10, 15 and 20%) in increasing amounts are used as solvent. Paper: Whatman No. 3 MM.

however, that in increasing the solute of the developing solvent its water content is considerably decreased and a solution of 20% sodium acetate and 20% acetic acid may have dielectric properties which are very different from more dilute solutions.

The effect of changing the cation of the salt in the developing solution

We substituted Li and K for Na acetate in the solvent which gave as shown in Table IV a higher R_F value for the solvent containing Li and a lower one for that containing K in all cases. This again suggests strongly that the mechanism is one similar to a salting-out process. Results with aluminium acetate and magnesium acetate are

TABLE IV

R_F VALUES OF ANIONS IN ACETATE BUFFERS WITH DIFFERENT CATIONS

Solvent	MoO_4^{-2}	CrO_4^{-2}	TeO_3^{-2}	SeO_3^{-2}	VO_3^-
Lithium acetate + acetic acid	0.45	0.59	0.59	0.88	0.61
Sodium acetate + acetic acid	0.44	0.56	0.61	0.86	0.47
Potassium acetate + acetic acid	0.40	0.54	0.57	0.85	0.26

inconclusive as the pH value varies with the cation. Substitution of NaCl or Na_2SO_4 for part of the sodium acetate in the buffer did not give any change in R_F values.

The amounts of Li and K acetate were equivalent to 10 g of Na acetate. The acetate was added to 10 ml glacial acetic acid and the volume made up to 100 ml with distilled water.

Some analytical separations

We considered it necessary to confirm the possibilities of separations by chromatographing several mixtures (conditions as in Table I):

(*i*) *Vanadate–tungstate*. Mixtures separated well and the spots of tungstate were detected with $SnCl_2$ + KCNS in HCl. Vanadate gives a yellow spot without a reagent.

R_F values in mixtures:

V	0.49	0.49
W	0.92	0.94

(*ii*) *Tellurite–selenite*. Both are detected with $SnCl_2$ in HCl. If tellurite is present in large amounts it may precipitate at the point of application and yield a forward comet from $R_F = 0$.

R_F values in mixtures:

TeO_3^{-2}	0.66	0.66
SeO_3^{-2}	0.87	0.88

(*iii*) *Arsenite–arsenate*. Detection is best carried out by dipping in a solution of silver nitrate.

R_F values in mixtures:

As(III)	0.86	0.86
As(V)	0.96	0.96

DISCUSSION AND CONCLUSION

Our results with the relatively few anions investigated suggest the following conclusions for chromatography on cellulose with acetate buffers:

1. Both adsorption of anions and the change in R_F values with varying ionic strength seem to exclude the possibility of anion exchange.

2. No relation between the solvent–paper ratio and the R_F value could be observed nor was there any correlation between the solvent speed and the R_F value. Thus an adsorption mechanism analogous to the partition mechanism of partition chromatography seems to be unlikely.

3. The influence of the cation of the acetate buffer and of the ionic strength seem to suggest a mechanism similar to salting-out or precipitation.

4. Among the twenty odd inorganic acids stable in acetate buffer only those with a tendency to polymerisation namely VO_3^-, MoO_4^{-2} and CrO_4^{-2} are considerably retained by the paper as has already been observed by RIPAN et al.[8]. This observation and the sequence of the cobaltammines suggest that the adsorption is favoured by large molecular size and/or low molecular charge.

SUMMARY

The adsorption paper chromatography of inorganic anions in acetate buffers was studied and the possible adsorption mechanism discussed. Some analytical separations were recorded.

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