SOME CONSIDERATIONS ON THE "CHARGE" ON A METAL ION IN ION-EXCHANGE CHROMATOGRAPHY

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SUMMARY

Published work on the determination of the "charge" on an ion by means of ion-exchange equilibria is reviewed and a number of examples are discussed in which such determinations led to erroneous results. The effect of outer-sphere complexing (or ion pairing) in solution and with exchange groups is discussed.

INTRODUCTION

Throughout the development of the theory and practice of ion-exchange chromatography, a certain picture of electrostatic attraction between ions in solution and fixed groups on the resin was taken for granted. The best summary of early work on the topic was given by Walton[1].

The basic structure of a sulphonic ion exchanger such as Dowex 50 was pictured as shown in Fig. 1.
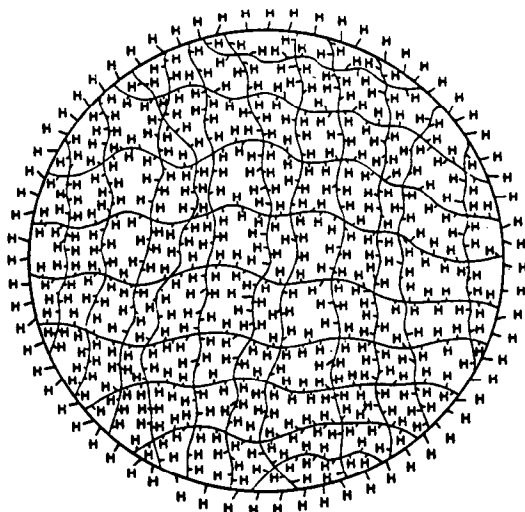

Fig. 1. Basic structure of Dowex 50. (From ref. 15).

The application of equations based on the law of mass action and similar[1] created the general belief that in such a network two or more species would swim around like fish and, depending on their attraction to the negatively charged sulphonic groups present throughout the resin particle, they would be more or less retarded with respect to the flowing eluent. This picture led to the determination of the "charge" on a metal ion using chromatographic or equilibrium data with ion exchangers.

Our laboratory was interested in such correlations in connection with the development of ion-exchange papers and several papers were published on this topic. The best summary of this work up to 1965 can be found in a review by Carunchio and Grassini-Strazza (ref. 2, pp. 282–284):

Total charge on the complex

To determine this quantity, most authors have used methods very similar to or the same as that developed by NELSON AND KRAUS[139] for investigating alkaline-earth metal citrates on anion-exchangers. In this method, the ion-exchange equilibrium is expressed as:

$$aX^{x-} + x(A^{a-})_r \rightleftarrows a(X^{x-})_r + xA^{a-} \tag{4}$$
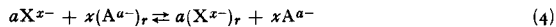
where X^{x-} and A^{a-} denote respectively the complex ion and the eluting anion, the resin phase being indicated by r. The exchange constant can then be written as:

$$K = G \frac{[X^{x-}]_r^a [A^{a-}]^x}{[X^{x-}]^a [A^{a-}]_r^x} \tag{5}$$

where G is the ratio of the activity coefficients and the quantities in brackets are concentrations. Assuming that G and the term $[A^{a-}]_r$ are constant, i.e. that $[X^{x-}]_r$ is small in comparison with $[A^{a-}]_r$, we can write eqn. (5) in the form:

$$\text{const.} = \frac{K}{G}[A^{a-}]_r^x = D^a[A^{a-}]^x \tag{6}$$

since:

$$D = \frac{[X^{x-}]_r}{[X^{x-}]} \tag{7}$$

Differentiating eqn. (6), we obtain:

$$\frac{d \log D}{d \log (A^{a-})} = S = -\frac{x}{a} \tag{8}$$

The slope S of the curve obtained by plotting $\log D$ against $\log [A^{a-}]$ is numerically equal to the ratio between the charge on the complex ion (x) and that on the eluting anion (a).

LI AND WHITE[127] used this method to determine the charge on the citrates of Co(II), Zn(II), and Th(IV) and verified the result for the Co(II) citrate by a somewhat different technique, in which the resin was not in the citrate but in the Cl⁻ form. However, the attempts to determine x for a uranyl citrate complex failed, since the very strong affinity of the uranyl ion for the resin citrate makes it impossible to obtain reasonable distribution coefficients for this system. The very stable uranyl citrate complex, which is evidently formed, may serve as a basis for the separation of uranium from metals on anion-exchangers.

The method of NELSON AND KRAUS[139] has been used in conjunction with other techniques to determine the charge x on several complexes such as the oxygenated cobalt glycylglycinate[140] ($x = -1$), a plutonium(IV) oxalate[124] ($x = -6$), two protactinium(V) complexes formed in H_2SO_4 solutions[141] ($x = -1$ and -3), complexes of Sr(II) with dicarboxylic acids[142] ($x = 0$), and a Pm(III)–EDTA complex formed in solutions with pH 1.8 to 9.0 ($x = -1$)[143]. The value of x has also been determined for some complexes of Fe(II) and Ti(IV) by BEUKENKAMP AND HERRINGTON[137, 138] using a similar method and by NABIVANETS[133] for the complexes of Ti(IV) formed in HCl solutions of various concentrations.

A cation exchanger in the H^+ form is used in the method of CADY AND CONNICK[48,91,134–138] for determining x of cationic complexes. This method is based on the fact that the equilibrium distribution of the complex cation depends on the concentration of H^+ (the exchangeable ion) in the aqueous phase and the resin phase. Thus the ion-exchange constant K for the reaction:

$$M^{x+} + x(H^+)_r \rightleftarrows (M^{x+})_r + xH^+ \tag{9}$$
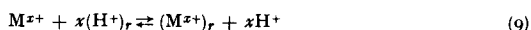
is given by:

$$K = G \frac{[M^{x+}]_r \, [H^+]^x}{[M^{x+}] \, [H^+]_r^x} \tag{10}$$

where G is the ratio of the activity coefficients. The distribution of M^{x+}, *i.e.* $[M^{x+}]_r/[M^{x+}]$, varies as the x-th power of the hydrogen ion distribution $[H^+]_r/[H^+]$. We can calculate x and K by substituting in eqn. (10) the values obtained from two equilibrations at different hydrogen-ion concentrations. Strictly accurate values are obtained only if K is the same for both equilibrations. This is not so in practice, because the activity coefficients in the aqueous phase vary from one equilibration to another. However, the method can still be used, since x is known to be a whole number of n (charge per metal atom), so the experimental values need not be very accurate.

LEDERER AND KERTES[144] correlated the charge on reversibly adsorbed cationic complexes with their R_F values on ion-exchange paper. Equations (9) and (10) are equally valid for exchange reactions occurring on paper impregnated with ion-exchangers. The logarithmic form of eqn. (10) is as follows (provided that $G = 1$):

$$\log K = \log \frac{[M^{x+}]_r}{[M^{x+}]} + x \log [H^+] - x \log [H^+]_r \tag{11}$$

To express $\log K$ as a function of R_F, the only parameter that is directly measurable on paper, we use the following relationship valid for partition chromatography:

$$R_F = \frac{A_L}{A_L + \alpha A_S} \tag{12}$$

where A_L is the cross-section of the mobile phase, A_S is the cross-section of the stationary phase, and α is the partition coefficient (ratio of the concentration in the stationary phase to that in the mobile phase). Equation (12) can also be written as:

$$\frac{1}{R_F} - 1 = \alpha \frac{A_S}{A_L} \tag{13}$$

Substitution of this into eqn. (11) gives:

$$\log K = \log \frac{A_L}{A_S} + \log \left[\frac{1}{R_F} - 1 \right] + x \log [H^+] - x \log [H^+]_r \tag{14}$$

Moreover, since $\log K$, $\log A_L/A_S$, and $x \log [H^+]_r$ are assumed to be constant, we obtain:

$$\log \left[\frac{1}{R_F} - 1 \right] = -x \log [H^+] + \text{constant} \tag{15}$$

Utilizing the relationship $\log (1/R_F - 1) = R_M$, we may simplify eqn. (15) and write:

$$R_M = x \, \text{pH} + \text{constant} \tag{16}$$

This relationship holds for each given cation eluted with hydrogen ions but the method is limited to eluent concentrations that give rise to intermediate R_F values. It has been used with Al(III) in mixtures of HCl and HF of various concentrations[145]. Moreover, eqn. (15) has been found valid[146] when a paper impregnated with zirconium phosphate (K^+ form) is used and the elution is carried out with highly concentrated solutions of KCl. In this case, the concentration of H^+ is naturally replaced by that of K^+ in eqn. (15).

Reading this extract one gains the impression that such "charge" determinations give meaningful results and have been generally applied. However, since then, severe limitations to this approach have become apparent. This emerged during the determination of the purity of a number of Co^{III} complexes, using the usual planar methods then at our disposal, namely paper partition chromatography, ion-exchange paper chromatography and paper electrophoresis. Doubts arose about the validity of such "charge" determinations when complexes such as $Co(NH_3)_6^{3+}$ behaved in some systems as if they had a charge of $+5$.

During the 1987 meeting on ion chromatography in Sils, the author noted that the authorities on ion exchange were unaware of these results, probably because they were obtained in terms of R_F and $R_M (= \log (1/R_F - 1)$ values or in terms of paper electrophoretic relative mobilities, and not in terms of K_d or capacity factors, as used in column chromatography; it was felt that a summary of the results, which were originally presented in various publications, would be of interest to chromatographers.

At this point the author would like to express his appreciation to Professor J. Inczedy for the help he gave in lengthy discussions and correspondence on this topic, which provided the impetus for this paper.

(i) Behaviour of Co^{III} complexes on sulphonic resin paper Amberlite SA-2

In 1969, Mazzei and Lederer[3] chromatographed $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$ and a number of divalent and trivalent metal ions and obtained the results shown in Fig. 2.
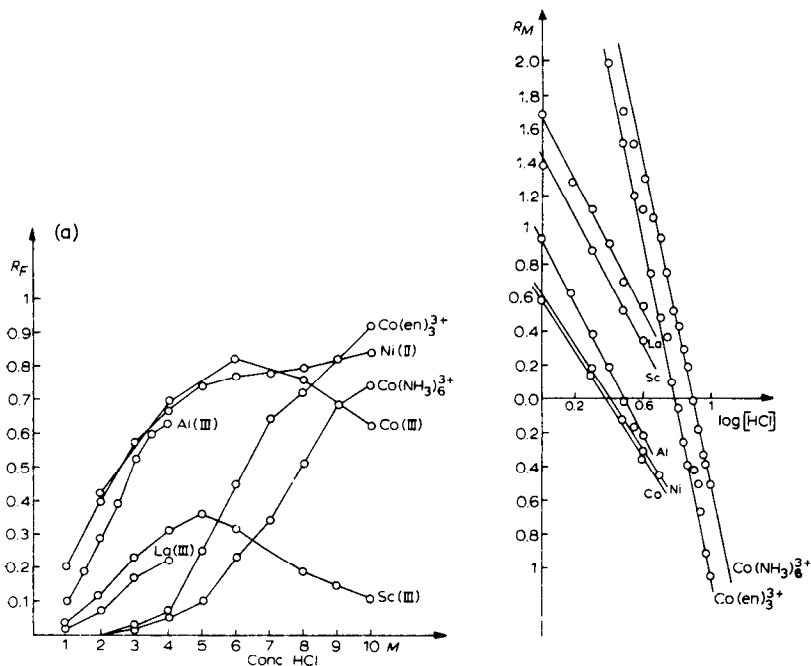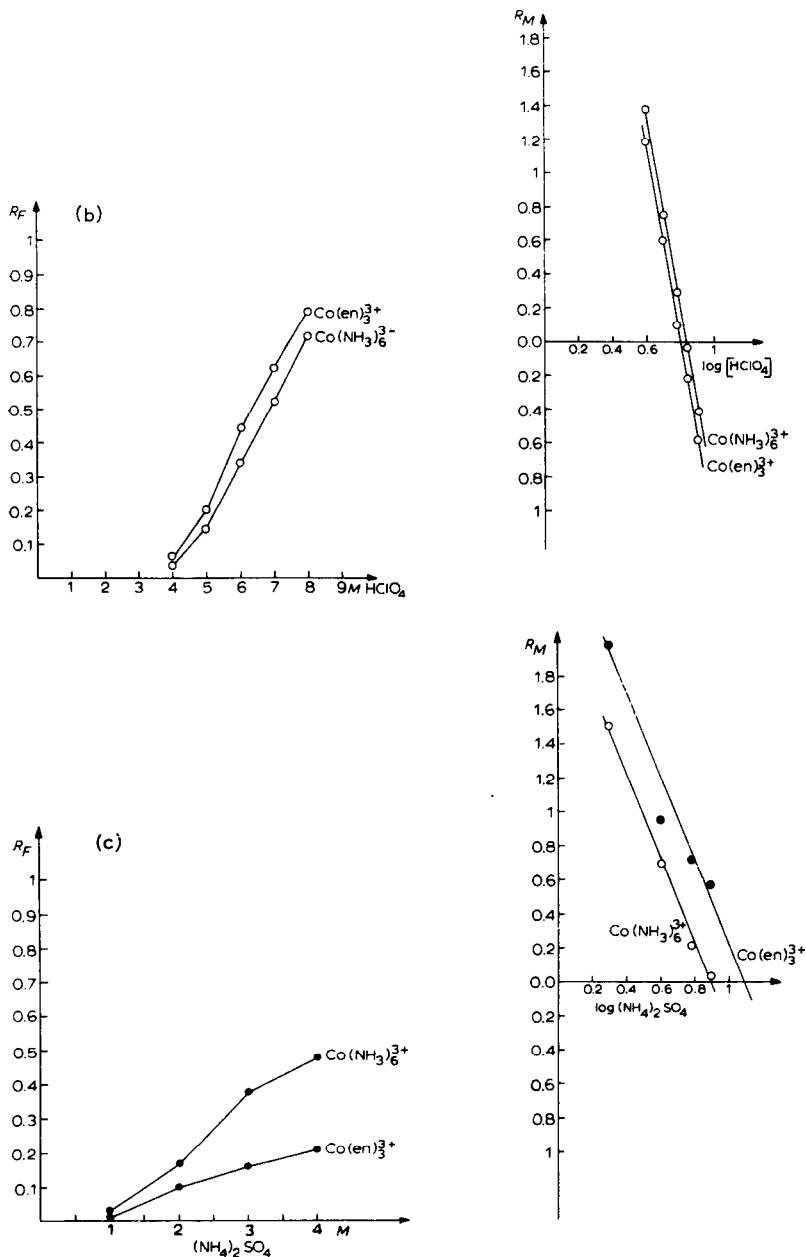

Fig. 2.

Fig. 2. (a) R_F vs. [HCl] and R_M vs. log [HCl] plots for Co^{II}, Ni^{II}, Al^{III}, Sc^{III}, La^{III}, [Co(NH$_3$)$_6$]$^{3+}$ and [Co(en)$_3$]$^{3+}$ on Amberlite SA-2 paper developed with hydrochloric acid. The slopes of the R_M vs. log [HCl] plots are Co^{II} = 1.7, Ni^{II} = 1.6, Al^{III} = 2.0, Sc^{III} = 1.92, La^{III} = 1.94, [Co(en)$_3$]$^{3+}$ = 4.6 and [Co(NH$_3$)$_6$]$^{3+}$ = 4.75. (b) R_F vs. [HClO$_4$] and R_M vs. log [HClO$_4$] plots for [Co(NH$_3$)$_6$]$^{3+}$ and [Co(en)$_3$]$^{3+}$ on Amberlite SA-2 paper developed with perchloric acid. The slopes of the R_M vs. log [HClO$_4$] plots are [Co(NH$_3$)$_6$]$^{3+}$ = 5.3 and [Co(en)$_3$]$^{3+}$ = 5.6. (c) R_F vs. [(NH$_4$)$_2$SO$_4$)] and R_M vs. log [(NH$_4$)$_2$SO$_4$] plots for [Co(NH$_3$)$_6$]$^{3+}$ and [Co(en)$_3$]$^{3+}$ on Amberlite SA-2 paper developed with aqueous ammonium sulphate. The slopes of the R_M vs. log [(NH$_4$)$_2$SO$_4$] plots are [Co(NH$_3$)$_6$]$^{3+}$ = 2.45 and [Co(en)$_3$]$^{3+}$ = 2.5. (From ref. 3).

Whereas the "slopes" for divalent metals such as Co^{II} and Ni^{II} were of the order of 1.7, those of trivalent metals such as Sc^{III} and La^{III} were between 1.9 and 2.0, and those of the Co^{III} complexes were 4.6–4.8 in hydrochloric acid and 5.3–5.6 in perchloric acid. In ammonium sulphate as eluent, the "charges" for the Co^{III} complexes were around 2.5.

(ii) "Charge" of Co^{III} complexes on a range of exchangers

We found the results of the above work to be surprising and felt that more data should be obtained for a wide range of cation exchangers. Table I shows the results obtained by Giannetta and Lederer[4], which clearly indicate that the "charge" so measured can vary from about 1 to 6, depending mainly on the resin and also on the eluent.

TABLE I

CHARGE EXHIBITED BY SOME COBALT COMPLEXES ON CATION-EXCHANGE PAPERS
(FROM REF. 4)

Cation-exchange paper	Eluent	"Apparent charge" or tangent of the R_M vs. log [LiCl] plot for	
		$Co(NH_3)_6^{3+}$	$Co(en)_3^{3+}$
Amberlite SA-2 paper	HCl	4.75	4.6
Amberlite SA-2 paper	LiCl	3.3	3.9
Cellulose phosphate paper, Whatman No. P20	LiCl	2.2	2.1
Cellullose citrate paper, Whatman No. CT30	LiCl	1.5	1.5
Carboxymethyl cellulose paper,Whatman No. CM50	LiCl	1.2	1.2
Zirconium phosphate paper	LiCl		2.4
Sulphonic acid cellulose paper (Macherey, Nagel and Co., Stark Sauer)	HCl	1.6	1.6

(iii) Effect of the "concentration" of the ion-exchange groups on the "charge"

This led us to suspect that the "charge" depends to a large extent on the number of exchange groups that can approach closely enough to a metal ion in order to exert an attraction or interaction. Cerrai et al.[5] showed that on a series of paper impregnated with di(2-ethylhexyl)orthophosphoric acid (HDEHP) liquid ion exchanger the "charge" changes with the concentration of the exchange groups, as shown in Fig. 3 and Table II, viz., from about 1.3 at low impregnation to about 3 at high impregnation.

If the "charge" measured in ion-exchange equilibria corresponds to the charge on the ion present in a solution properties, then the other properties of such solutions should be such as to agree with the ion-exchange results. This has been discussed extensively in relation to the charge on proteins and will be discussed at the end of this paper. Here we shall consider two properties which give evidence for the actual "charge" in solution.
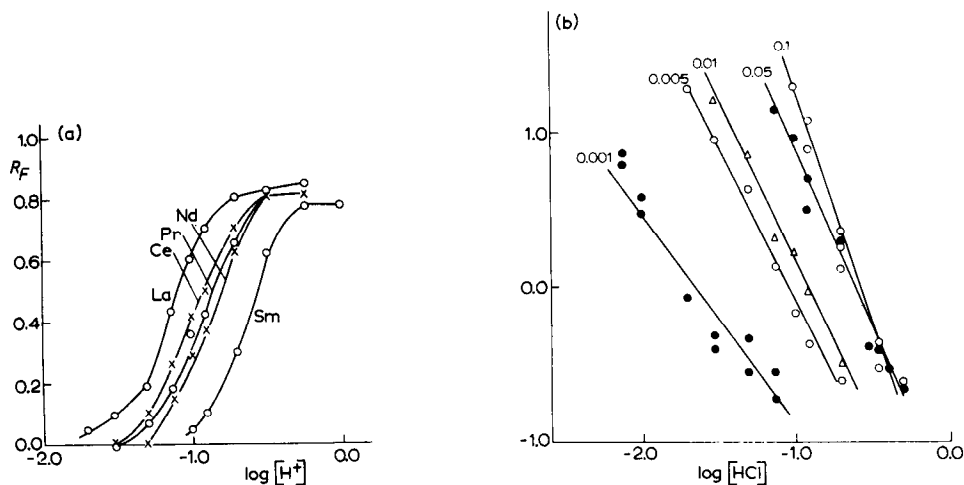
Fig. 3. (a) R_F *versus* $\log [H^+]$ plots for papers impregnated with 0.005 M HDEHP. (b) R_M *versus* $\log [H^+]$ plots for papers impregnated with various concentrations of HDEHP. (From ref. 5).

(iv) Activity coefficients

Activity coefficients obtained from electrochemical measurements are available in the literature for various concentrations of pure salts, but data for, *e.g.*, La^{3+} in 1 M hydrochloric acid are usually not available and would be difficult to measure with any accuracy.

If we examine Table III for 0.1 M solutions[6], we find that monovalent cations as in $AgNO_3$ or CsCl have activity coefficients near unity, *viz.*, 0.734 and 0.756, respectively. With a divalent cation such as in $CaCl_2$ or $CoCl_2$, the activity is only 0.518 and 0.523, respectively, in other words reduced by about 50%. Trivalent cation salts, such as $AlCl_3$, have an activity of 0.337.

To give a kinetic picture, the Al^{3+} in 0.1 M $AlCl_3$ attracts the Cl^- so closely that to all intents the particle preponderant in such a solution is best pictured as an ion pair $[Al^{3+} + 2 Cl^-]^+$.

TABLE II

SLOPES OF R_M *VERSUS* LOG [HCl] PLOTS FOR PAPER IMPREGNATED WITH VARIOUS AMOUNTS OF HDEHP (FROM REF. 5)

Metal ion	HDEHP concentration in impregnating solution (M)				
	0.1	0.05	0.01	0.005	0.001
La^{III}	-3.02 ± 0.47	-2.29 ± 0.23	-2.12 ± 0.46	-1.99 ± 0.26	-1.34 ± 0.18
Ce^{III}	-2.82 ± 0.58	-2.68 ± 0.21	-2.40 ± 0.35	-2.17 ± 0.51	-1.40 ± 0.18
Pr^{III}	-2.98 ± 0.37	-2.47 ± 0.36	-2.20 ± 0.56	-2.10 ± 0.43	-2.06 ± 0.30
Nd^{III}	-3.08 ± 0.61	-2.40 ± 0.41	-2.28 ± 0.73	-2.00 ± 0.41	-2.27 ± 0.52
Sm^{III}	-3.11 ± 0.69	-2.69 ± 0.35	-2.66 ± 0.53	-2.12 ± 0.62	-1.71 ± 0.48

TABLE III

MEAN ACTIVITY COEFFICIENTS (FROM REF. 6)

M	$AgNO_3$	$AlCl_3$	$BaCl_2$	$Ba(ClO_4)_2$	$Ba(NO_3)_2$	$CaBr_2$	$CaCl_2$	$Ca(ClO_4)_2$	$Ca(NO_3)_2$
0.1	0.734	0.337	0.508	0.524	0.431	0.532	0.518	0.557	0.488
0.2	0.657	0.305	0.450	0.481	0.345	0.491	0.472	0.532	0.429
0.3	0.606	0.302	0.425	0.464	0.295	0.481	0.455	0.532	0.397
0.4	0.567	0.313	0.411	0.459	0.262	0.482	0.448	0.544	0.378
0.5	0.536	0.331	0.403	0.462		0.490	0.448	0.564	0.365
0.6	0.509	0.356	0.397	0.469		0.504	0.453	0.589	0.356
0.7	0.485	0.388	0.397	0.477		0.521	0.460	0.618	0.349
0.8	0.464	0.429	0.397	0.487		0.542	0.470	0.654	0.344
0.9	0.446	0.479	0.397	0.500		0.567	0.484	0.695	0.340
1.0	0.429	0.539	0.401	0.513		0.596	0.500	0.743	0.338
1.2	0.399	0.701	0.411	0.545		0.664	0.539	0.853	0.337
1.4	0.374	0.936	0.424	0.581		0.746	0.587	0.992	0.337
1.6	0.352	1.284	0.439	0.622		0.846	0.644	1.161	0.339
1.8	0.333	1.819	0.455	0.674		0.968	0.712	1.372	0.342
2.0	0.316			0.718		1.119	0.792	1.634	0.347
2.5	0.280			0.868		1.654	1.063	2.62	0.362
3.0	0.252			1.047		2.53	1.483	4.21	0.382
3.5	0.229			1.287		3.88	2.08	6.76	0.407
4.0	0.210			1.545		6.27	2.93	10.77	0.438
4.5	0.194			1.826		10.64	4.17	17.02	0.472
5.0	0.181			2.13		18.43	5.89	26.7	0.510
5.5	0.169					31.7	8.18	41.7	0.551
6.0	0.159					55.7	11.11	63.7	0.596

(v) Paper electrophoretic mobilities

Here we can measure the mobility of an ion in an excess of background electrolyte. For example, Table IV shows mobilities of metal ions in various concentrations of perchloric acid[5]. The conditions here are similar to those in ion-exchange equilibria. The values shown are relative and illustrate that all divalent and polyvalent metal ions travel with similar speeds and that this speed is about half of that of a monovalent cation (in this instance we used Tl^+; other monovalent ions were not run in these experiments because of the difficulty in finding suitable detection reagents).

In the case of the two series of data which (although they cannot be directly compared) both show that in eluent solutions from 0.1 to 1.0 M the existence of an M^{2+} or M^{3+} is most unlikely owing to the strong interactions between the metal ions and their counter anions. Hence the high "charges" exhibited in ion-exchange equilibria were perplexing. Some light was thrown on this problem by the paper electrophorestic behaviour of metal ions in 0.5 M sulphate solution at pH 1.

A series of metals were examined by Chakrabortty[7], as shown in Table V. Here monovalent metals, such as Ag^+ and Tl^+, move fastest, followed by divalent metal

$CdCl_2$	$Cd(NO_3)_2$	$CdSO_4$	$CoCl_2$	$Co(NO_3)_2$	$CrCl_3$	$Cr(NO_3)_3$	$CsCl$	$CuCl_2$
0.2280	0.516	0.150	0.523	0.521	0.331	0.319	0.756	0.510
0.1638	0.467	0.103	0.479	0.474	0.298	0.285	0.694	0.457
0.1329	0.445	0.0822	0.463	0.455	0.294	0.279	0.656	0.431
0.1139	0.433	0.0699	0.459	0.448	0.300	0.281	0.628	0.419
0.1006	0.428	0.0615	0.462	0.448	0.314	0.291	0.606	0.413
0.0905	0.426	0.0553	0.470	0.451	0.335	0.304	0.589	0.411
0.0827	0.426	0.0505	0.479	0.458	0.362	0.322	0.575	0.411
0.0765	0.428	0.0468	0.492	0.468	0.397	0.344	0.563	0.412
0.0713	0.431	0.0438	0.511	0.480	0.436	0.371	0.553	0.415
0.0669	0.436	0.0415	0.531	0.493	0.481	0.401	0.544	0.419
0.0599	0.449	0.0379	0.578	0.526	0.584	0.474	0.529	0.427
0.0546	0.463	0.0355	0.634	0.566		0.565	0.518	0.436
0.0504	0.481	0.0338	0.699	0.613			0.509	0.446
0.0469	0.498	0.0327	0.773	0.668			0.501	0.457
0.0441	0.518	0.0321	0.860	0.730			0.496	0.468
0.0389	0.573	0.0317	1.120	0.926			0.485	0.496
0.0352		0.0329	1.458	1.189			0.479	0.522
0.0325		0.0356	1.832	1.535			0.475	0.549
0.0306			2.22	1.984			0.474	0.575
0.0291				2.57			0.474	0.599
0.0279				3.33			0.475	0.623
0.0270							0.477	0.650
0.0263							0.480	0.676

ions. The trivalent metal ions move very little, either positively (*e.g.*, Al^{III} and Y^{III}) or negatively (*e.g.*, La^{III} and Sc^{III}).

Two important points are that: (i) it can be shown that in 0.5 M sodium sulphate at pH 1 the preponderant species is HSO_4^-, which is almost the same anion as the functional groups in a sulphonic resin; (ii) it is known that most of these anionic moving trivalent metal ions did not form sulphate complexes, because in the crystalline double salts (alums) the cation is present as a hexahydrate, *viz.*, the movement is governed by ion-pair formation between an $[M^{III}(H_2O)_6]^{3+}$ ion and a number of anions, probably HSO_4^- or SO_4^{2-} ions.

We feel that the comparison of the ion exchange equilibrium data and the electrophoretic data suggest that retention of trivalent metal ions probably involves ion-pair formation between the hydrated metal ion and the sulphonic groups, which is the same as seen in electrophoresis, *i.e.*, ion-pair formation with hydrogensulphate anions. This would explain why apparent charges of 5–6 are possible with $Co(NH_3)^{3+}$ and $Co(en)_3^{3+}$.

The apparent charge of 3 on metal ions such as Al^{III} seems to be due to a coincidence of several factors and further is observed only in some resins.

TABLE IV

DISTANCE MOVED BY METAL IONS IN HClO$_4$ BY HIGH-VOLTAGE ELECTROPHORESIS
(FROM REF. 5)

Paper: Whatman No. 1. Experiments conducted at 3–4°C for 2 h at 300 V. Camag apparatus.

Metal ion	Distance in mm corrected for electroosmotic flow (with H$_2$O$_2$)		
	0.1 M HClO$_4$	0.5 M HClO$_4$	1.0 M HClO$_4$
Tl^I	93	76	61
UO$_2$$^{2+}$	45	35	35
Bi^{III}	46	49	50
Cd^{II}	60	56	52
Pb^{II}	74	57	57
Cu^{II}	61	47	50
Fe^{III}	52	45	47
Co^{II}	61	56	51
Ni^{II}	62	56	51
Mn^{II}	70	57	50
Zn^{II}	62	57	51
Al^{III}	69	56	49
Y^{III}	67	59	52
La^{III}	75	65	58
Zr^{IV}	28 comet	37 comet	38 comet
Th^{IV}	49	55	55

TABLE V

DISTANCES MOVED BY METAL IONS IN 0.5 M SODIUM SULPHATE SOLUTION ADJUSTED
TO pH 1 WITH SULPHURIC ACID IN 1 h AT 360 V (FROM REF. 7)

Metal ion	Distance moved (mm)	Metal ion	Distance moved (mm)
Ag^I	39	In^{III}	−14
Tl^I	50	Ga^{III}	−6
Fe^{III}	21	Bi^{III}	−12
Co^{II}	22	Co(NH$_3$)$_6$$^{3+}$	12
Ni^{II}	21	Sc^{III}	−14
Zn^{II}	23	Y^{III}	9
Cd^{II}	19	La^{III}	−6
Cu^{II}	23	Th^{IV}	−30
Mn^{II}	13	Zr^{IV}	−19
Mg^{II}	26	Ti^{IV}	−24
Be^{II}	7	V^{IV}	−11
Pb^{II}	0 (precipitated)	Ge^{IV}	−4
Hg^{II}	0	CrO$_4$$^{2-}$	−70
Fe^{III}	−9	MoO$_4$$^{2-}$	−30
Al^{III}	9	UO$_2$$^{2+}$	−44
Cr^{III}	4		

This leaves some interesting questions open. Fig. 3 shows log [HCl] against R_M relationships over the ranges 1–12 M hydrochloric acid and 4–9 M perchloric acid. The activities of hydrochloric acid and perchloric acid are certainly not constant in this range. Linearity over the whole range is thus unexpected and so far unexplained.

"CORRECTION" OF THE SLOPE BY THE USE OF ACTIVITY COEFFICIENTS

It has been stated on various occasions that the "charge" determined by plotting log K_d (or R_M) against log [eluent concentration] gives incorrect values. It was suggested that the "theoretical" slopes of 3 and 2 for tri- and divalent cations can be obtained by multiplying the eluent concentration by the activity coefficient of the eluent.

The first such proposal was refereed some 30 years ago for this journal by Professor G. Alberti and I am grateful for his criticism, in which he pointed out that in an equation such as (see page 3):

$$K = G \cdot \frac{[M^{x+}]_r}{[M^{x+}]} \cdot \frac{[H^+]^x}{[H^+]_r^x}$$

we must know not only the activity coefficients of the eluent, but also that of the sulphonic groups of the resin and that of the metal ion in solution and in the resin. These cannot be determined readily but probably cancel each other. However, for an "accurate determination" the activity coefficients of all four terms in the equation would have to be introduced.

SOME CONSIDERATIONS OF INORGANIC EXCHANGERS

A typical inorganic ion exchanger fully investigated for its properties is zirconium oxide. For anion exchange it was postulated that there are free zirconium cations on the surface which attract anions from solution. The law of mass action plot for the CrO_4^{2-} anion yields exactly a slope of 2, and this was taken as evidence that the adsorption was ion exchange, i.e., of an electrostatic nature[8].

However, in paper electrophoretic experiments with chromate anions, chromate migrates in a Zr^{IV} or Th^{IV} solution as a cation, viz., in solution an ion pair with a residual positive charge is formed. This strongly suggests that the equilibrium between chromate and Zr cations on the surface of zirconium oxide involves the same kind of association[9].

The adsorption of Co^{III} complexes on a range of inorganic exchangers was examined by Lederer and Battilotti[10,11]. The general behaviour of Co^{III} complexes in electrophoresis should also be mentioned here. The electrophoretic sequence changes radically according to the type of ion pairs (or outer-sphere complexes) formed with the electrolyte. In acetate or chloride, for example, the Co^{III} complexes with three "charges" travel at about the same speed. In sulphate or other anions prone to hydrogen bonding with ammine groups, the complexes with ammine groups are strongly retarded, whereas those with heterocyclic nitrogen such as dipyridyl complexes have high speed. In electrolytes with hydrophobic anions such as ClO_4^- or

TABLE VI

RELATIVE ELECTROPHORETIC MOVEMENT OF COBALT(III) COMPLEXES IN VARIOUS
ELECTROLYTES (AFTER REF. 13)

en = Ethylenediamine; dip = dipyridyl; ophen = o-phenanthroline.

Electrolyte	$[Co(en)_3]^{3+}$	$[Co(dip)_3]^{3+}$	$[Co(ophen)_3]^{3+}$
1 M CH$_3$COONa	49	50	42
1 M LiCl	33	31	27
1 M NaClO$_4$	72	14	8
1 M Cl$_3$CCOONa	38	0	−8
0.5 M Na$_2$SO$_4$	−2	22	21
1 M NaH$_2$PO$_4$	23	45	42

Cl$_3$COO$^-$ it is the large complexes such as the dipyridyl complexes that move very little
while the ammine complexes move at high speed (see Table VI, taken from refs. 12
and 13).

On zirconium(IV) oxide, as well as on titanium(IV) oxide, zirconium phosphate,
alumina and thorium(IV) oxide, the hydrogen bonding ammine-type complexes are
strongly retained, similar in sequence to those on a sulphonic ion exchanger, whereas
on silica, the opposite sequence is observed, as shown in Fig. 4. The R_M vs. log [LiCl]
plots are linear, showing that the adsorption follows a law of mass action equation
with a slope of ca. 1. On inorganic ion exchangers there seems also to be strong
evidence for ion pairing with the exchange groups. Depending on the exchanger,
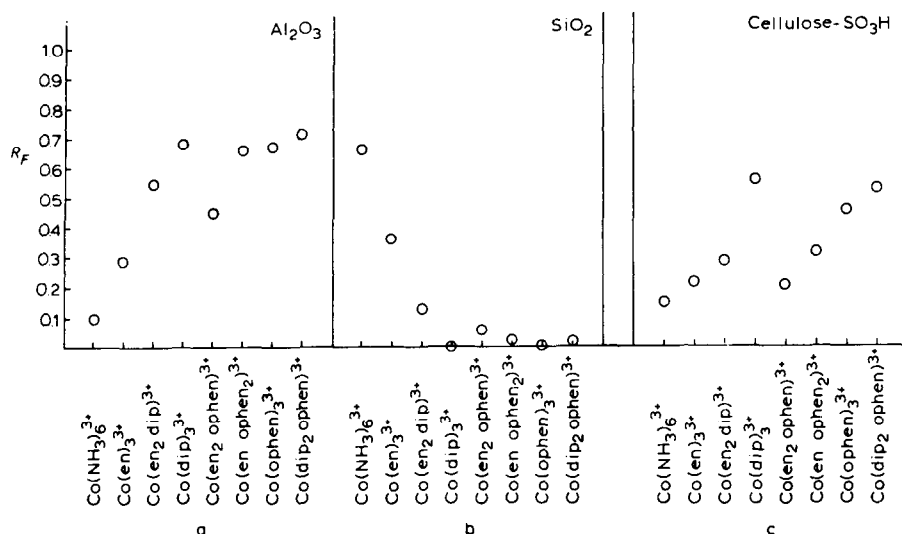different mechanism may operate.

Fig. 4. Graphical representation of the R_F values of cobalt(III) complexes: (a) on MN Polygram Alox N thin
layers developed with 0.1 M lithium chloride solution; (b) on MN Polygram Sil G thin layers developed with
0.1 M lithium chloride solution; (c) on MN ion-exchange paper with strongly acidic (sulphonic) groups
developed with 0.5 M LiCl. (From ref. 10).

ION-EXCHANGE CHROMATOGRAPHY OF PROTEINS

The application of law of mass action-type equations has received the attention of numerous workers. We quote here from a thorough study by Kopaciewicz *et al.*[14]:

> Since there are both acidic and basic residues within the same protein molecule, its net charge is pH-dependent. Under acidic conditions, basic amino acids are ionized, while carboxyl ionization is suppressed, and the protein obtains a net positive charge. Conversely, under basic conditions carboxyl groups are ionized, while the amino groups are neutral, and the protein accumulates a negative charge. Depending on the ratio of acidic to basic amino acids, at some intermediate pH, the net charge will be zero and a protein is said to be at its isoelectric point (p*I*). The exact pH of the isoelectric point is determined by both the type of amino acids and molecular structure. The amphoteric nature of a protein is best examined by a pH titration curve. Since a protein can exhibit net positive, neutral or net negative charge, depending on its p*I* and solution pH, the "net charge" concept has been used to predict retention behavior on ion-exchange columns. The general features of this concept are that proteins: (1) will not be retained on ion-exchange columns at their p*I* because they have no net charge; (2) will be retained above their p*I* on anion-exchange columns because they have a net negative charge; (3) will be retained below their p*I* on cation-exchange columns because they have a net positive charge; and (4) will show a correlation between net charge, as demonstrated by the titration curve and retention on ion-exchange columns. This hypothetical relationship between net charge and chromatographic retention is illustrated in Fig. A. A titration curve depicts net charge (*Z*), and a retention map (retention time *versus* pH) represents pH-dependent chromatographic behavior.
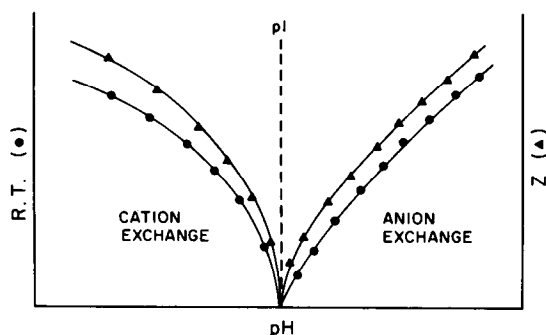

Fig. A. The hypothetical relationship between protein net charge and chromatographic retention. Net charge, *Z*, is depicted by a titration curve, and pH-dependent chromatographic behavior is represented by a retention map (retention time *vs.* pH).

Straight-line log *K'* *versus* log [NaCl] graphs were obtained, *e.g.*, Fig. 5. The effect of the cation and the anion of the eluent was also examined (see Table VII). The conclusions in ref. 14:

CONCLUSIONS

> The retention of proteins on high-performance ion-exchange columns cannot be satisfactorily explained by the "net charge" concept alone. Approximately three-fourths of the proteins studied deviated from this concept in that they were retained at their respective p*I*. Thus, the importance of protein p*I* has apparently been

overemphasized. Non-systematic deviations from this concept are not the result of non-ionic interactions but, rather, are due to the nature of the protein molecule itself. Intramolecular charge asymmetry promotes differences in electrical potential on the surface of the protein. These regions of localized charge are present even when the net charge of the molecule is zero (at the pI) and are postulated to orient or "steer" the protein with respect to oppositely charged surfaces, such as ion-exchange support. An estimation of stationary-phase surface double-layer thickness indicates that it is small relative to protein dimensions; thus, this "steering effect" may be important during the adsorption desorption process.

In addition to the charge characteristics of the protein itself, the nature of the support surface and the mobile-phase displacing ion are also important. Retention on both strong anion- and cation-exchange columns was shown to be affected by the choice of displacing salt. The extent of this effect varied among columns. In some cases, protein retention was altered as much as 100%. Individual proteins may respond differently to specific salts. This phenomenon may be linked to the thermodynamic activity of the displacing ion itself.

A non-mechanistic model for the retention of proteins on HPIEC columns was developed. Calculations of Z (the number of charges associated with the adsorption/desorption process) for β-lactoglobulin indicates that the number of charged sites involved in binding may be greater or less than the net charge of the protein. This would explain the lack of correlation between retention and titration curves for β-LAC. There appears to be a positive correlation between Z and protein retention.

show that, as with trivalent ions, the net charge as measured by titration or in electrophoresis has no relevance for the Z measured by ion-exchange equilibria. Also, the anion and cation of the eluent can change the retention, evidently by ion-pair formation.

TABLE VII

INFLUENCE OF VARIOUS IONS ON THE RETENTION OF CYTOCHROME c AND LYSOZYME ON A STRONG CATION-EXCHANGE (SCX) COLUMN

Ion	Relative retention[**] on SCX[***] column		Retention ratio[§], LYS/CYT c	Ion	Relative retention[***] on SCX[***] column		Retention ratio[§] LYS/ CYT c
	CYT c	LYS			CYT c	LYS	
Anion[*] (sodium salt):				Cation[*] (chloride salt):			
Fluoride	0.79	0.88	1.38	Lithium	1.00	1.00	1.22
Chloride	0.68	0.64	1.16	Sodium	0.58	0.55	1.16
Bromide	0.58	0.51	1.10	Potassium	0.57	0.55	1.19
Phosphate	0.54	0.57	1.29	Ammonium	0.59	0.58	1.19
Sulphate	0.90	0.85	1.16	Magnesium	0.56	0.54	1.18
Acetate	0.74	0.74	1.22				
Tartrate	0.98	0.99	1.25				
Citrate	1.00	1.00	1.23				

[*] Chromatography was performed at pH 6. The ionic strength of buffer B was 0.5.
[**] Unity refers to the longest retention time obtained with a 20-min gradient.
[***] SCX refers to the Pharmacia Mono S column.
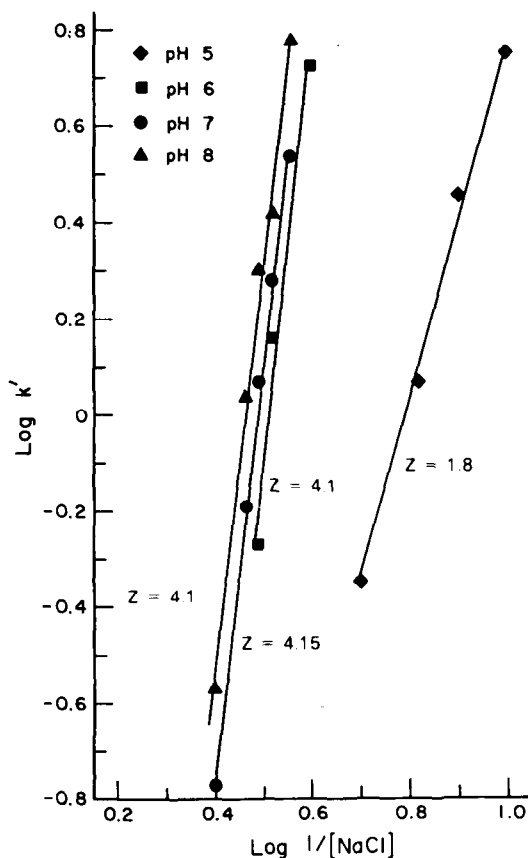[§] Ratios were calculated from the actual retention times.

Fig. 5. Estimation of the number of charge interactions (Z) occurring between β-lactoglobulin and the surface of a strong anion-exchange column. β-Lactoglobulin was chromatographed isocratically at several pH values on a 25 × 0.41 cm I.D. silica-based SAX column. (From ref. 14).

CONCLUSIONS

Werner, at the turn of the century, observed that complexes of the type $Co(NH_3)_6^{3+}$ interacted strongly with anions in aqueous solution to the extent that the spectra of, e.g., chlorides and sulphates differed considerably. In the consideration of ion-exchange equilibria, the importance of such ion pairing or outer-sphere complexing has often been underestimated, and the possibility of ion pairing with the functional groups of the exchanger has not been sufficiently appreciated. The determination of the "charge" of a metal ion by the use of ion-exchange equilibria is strongly influenced by such ion pairing, especially when the metal ion has a charge of more than +2. Similar limitations have also been reported with proteins.

APPENDIX

Here I reproduce a letter to me from Professor Inczedy (with her permission) in which he discusses the problem of the "charge" on a metal ion.

"Concerning the problem of the ion exchange of cobalt–hexamine and similar complex cations, I have read your papers carefully and I have read also my papers which were published many years ago. After long consideration I would like to answer your questions as follows:

(1) If we use strongly acidic ion exchangers of the traditional type (styrene–DUB copolymers, sulphonated), the log (distribution ratio) vs. log [eluent ion (hydrogen ion) concentration] in the acidity range 0.1–1 M follows the ideal equation. Hence the slopes for Li, Na, K, Rb, Cs and NH_4 ions in hydrochloric and perchloric acid and for Tl and Ag ions in perchloric and nitric acid are very close to -1.

Similarly, the slopes for divalent ions (Cu, Zn, VO, Ca, Sr, Ba, Ni, Co, Mg, Mn, Fe, TiO) are very close to -2 in hydrochloric acid, and for Pb in nitric acid solutions. See [1] [2] [3].

For trivalent ions the slope is between 2 and 3. For example, for La^{3+} and Y^{3+} in hydrochloric acid it is close to three [1], but the slope for iron(III) and aluminium is between 2 and 3. For titanium the slope is close to 2, corresponding to the TiO^{2+} [or $Ti(OH)_2^{2+}$] ion [3].

[1] F. W. Strelow, *Anal. Cnem.*, 32 (1961) 1185.
[2] F. W. Strelow, R. Rethemeyer and C. J. C. Bothma, *Anal. Chem.*, 37 (1965) 106.
[3] F. W. Strelow and H. Sondorp, *Talanta*, 19 (1972) 1113.

The mentioned phenomena are valid, of course, only for traditional ion-exchange resins of high capacity and of the strongly acidic type. In those systems the ion-exchanger phase can be assumed to be a strong electrolyte system. The deviations from the ideal slope for the trivalent ions can be attributed to partial hydroxo or chloride complex formation and also to steric problems discovered by you.

Salmon published a paper [4], 30 years ago in which the partial charge of the complex is determined by saturation of the ion exchanger with the solution of the complex. From the capacity of the resin and from the adsorbed amount of the metal ion the partial charge is calculated.

It is a very interesting statement in your paper that by lowering the capacity of the ion-exchanger phase the slope number "decreases". Thus the ion-exchange sites are not accessible by the polyvalent ions owing to steric problems. This effect is very similar to that which we found earlier, *viz.*, that the divalent tetracyanozincate ions are bound only monovalently to the anion exchanger, and the ion exchange sites are shared between cyanide and $Zn(CN)_4^{2-}$ ions [5].

[4] J. E. Salmon, *Rev. Pure Appl. Chem. (Australia)*, 6 (1956) 24.
[5] J. Inczédy and Frankow, *Period. Polytech.*, 11 (1967) 53.

(2) The nickel–, cobalt–, copper–, cadmium–and zinc–ammine complexes are bound very strongly to sulphonic acid-type resin. This phenomenon is based on the fact that the ammine complex cations, surrounded with NH_3 ligands, are not hydratable in comparison with aquo complex ions. Especially those cations are the most strongly adsorbed for which the coordination sphere is completely saturated, *e.g.*, $Ni(NH_3)_6^{2+}$ or $Cd(NH_3)_6^{2+}$. The volume of the resin saturated with the mentioned ammine complex cations is smaller than that of the hydrated cations (*i.e.*, aquo complexes). The interaction between the resin sites and the ammine complex ion is rather strong. One cannot speak any more about a strong electrolyte system. The ions and the fixed ionic groups are very close to each other, and the electrostatic force is dominating. The behaviour of ethylenediamine complexes is similar, but different for those complex cations in which the coordinated neutral ligand is much larger (dipyridyl or *o*-phenanthroline, etc.).

An ion-exchanger experiment with trivalent cobalt hexammine complex and with a conventional ion-exchange resin is almost impossible, because the adsorption of the complex ions and the water release are very high, and the resin cannot be regenerated completely.

(3) The "unusual high slopes" of the $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$ ions obtained by you may be attributed to the formation of associated species. The concentration of the acid (hydrochloric or perchloric acid) used in the experiments is also high, 4–10 M, where the water activity is low, and the formation of the associated species and ion pairs is highly favoured. The medium does not correspond to the usual strong electrolyte systems, where well hydrated simple ions in fairly dilute aqueous solutions are present. In your

system in the strongly acidic solutions the hydrophobic complex cations may form binuclear species with attached chloride or perchlorate ions, which are expelled from the aqueous phase to the hydrophobic phase, but negatively charged resin particles are embedded in the paper.

(4) The electrophoretic mobility of the simple cations depends on the volume of the hydrated ion and on the total charge. Since the volume of the hydrated ions is not perfectly known, and the number of the water molecules in the hydrate sphere change according to the temperature, it is very difficult to predict the mobility values of the cations. Li^+ has a high charge density, being a very small ion, and its hydrate sphere is large. Therefore, its mobility is very low. Since the mass increases in the order $Li < Na < K < Rb < Cs$, the hydrate sphere decreases and the mobility increases from Li^+ to Cs^+. However, the mass of NH_4^+ is small (less than that for Na^+) and its mobility is similar to that of K^+ because NH_4^+ has a lower hydration tendency than Na^+. Divalent metal ions have a double charge, but their hydration ability is therefore also higher. Ca^{2+} is more mobile than Mg^{2+}, having a larger mass, but has a lower hydration ability. This is true also for Pb^{2+}, which has an even greater mass but a very small hydrate sphere and a high mobility. Cu^{2+} and Zn^{2+} are of medium mass (63.5 and 65), but their mobilities are low, similar to that of Mg^{2+}. Hence it is more difficult to predict the behaviour of the trivalent ions. In certain cases, by increasing the ion mass, the mobility also increases, e.g., $Al^{3+} < Cr^{3+} < Fe^{3+}$. I am afraid one cannot draw unambigous conclusions from the data mentioned.

Conclusions; (1) you are completely right, that in those systems where the ion-exchange phase is "dilute", i.e., is of low capacity, the polyvalent ions cannot access corresponding ion-exchange sites, and therefore the value of the ionic charge seem to be lower than the real value. The data in Table III in the paper by Cerrai, Ghersini, Lederer and Mazzei [J. Chromatogr., 44 (1969) 161] are very convincing.

(2) In the experiments reported in the paper by Mazzei and Lederer [J. Chromatogr., 40 (1969) 197], the ion-exchanger system is very different from the conventional hydrated systems. In the strongly acidic heterogeneous mixed solid phase used, unusual interactions and also kinetic hindrance must be assumed, which are very probably responsible for the very high slopes obtained for the log R_M vs. log (acid concentration) diagrams."

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