

## HIGH-VOLTAGE PAPER ELECTROPHORESIS OF INORGANIC CATIONS

### CONDITIONS FOR CATHODIC MIGRATION AND MEASUREMENT OF MIGRATION RATES\*

D. GROSS

*Research Laboratory, Tate & Lyle Refineries Ltd.,  
Keston, Kent (Great Britain)*

(Received September 6th, 1962)

#### INTRODUCTION

It is well known that inorganic cations can be made to migrate to the anode by means of various complexing agents such as halogen acids, organic hydroxy-acids and poly-anions. The result is often a better separation or differentiation where a separation would be difficult or not at all possible in the cationic state. This subject was treated exhaustively in a recent up-to-date publication<sup>1</sup>.

In a previous article<sup>2</sup> some examples of separations of anodically migrating cations were given. In fact, almost only the alkali metals in a cationic state could be demonstrated to give such differential migration rates as to allow satisfactory resolution of their mixtures. A comprehensive bibliography of separations by paper electrophoresis was recently published<sup>3</sup>. The use of a complexing agent such as ethylenediaminetetraacetic acid (EDTA) in solutions of varying pH, the dependence of the movement of metallic ions on the degree of dissociation of the anionic complexes formed, on the concentration of ligand and pH conditions, were recently investigated and theoretically discussed<sup>4</sup>. The variable effect on the sign of migration of the concentration of the background electrolyte was also reported briefly but convincingly<sup>5</sup>. Other authors described the strong effect of pH on the migration behaviour of inorganic cations in the presence of organic complexing agents<sup>6</sup>.

It was for these reasons that it was decided to search for non-complexing electrolyte solutions less liable to be affected by slight variations in conditions and thus more suitable for routine experiments without the need for frequent rigorous standardization and checking.

#### EXPERIMENTAL

##### *Choice of background electrolyte*

Several guiding principles in the choice of electrolytes or buffer solutions have to be observed. Convenience of preparation, assurance of reproducible pH, steadiness of pH during the experiments, sufficient buffering power, low to medium degree of

\* The results of this paper were presented at Corso Estivo di Chimica, Rome, on September 21st, 1962.

dissociation in aqueous solutions, non-toxicity, non-inflammability, economic price of chemicals and low degree of corrosiveness. Several of the points have an enhanced importance in the case of high-voltage electrophoresis requiring large volumes of solution. A strong electrolyte such as HCl or HBr leads to high current density and correspondingly increased heat generation, making satisfactory control of experimental conditions at high voltages difficult.

It has always been the author's policy to find an electrolyte solution that could be made up conveniently and used for more than one group of compounds, irrespective of their organic or inorganic nature. For instance, a 0.05 *M* sodium tetraborate solution giving a pH of 9.2 can be used for the electrophoresis of sugars as well as of amino acids, even two-dimensionally<sup>7</sup>. Another electrolyte found very useful is an approx. 0.1 *M* ammonium carbonate solution of pH 8.9, suitable for alkali metals<sup>2</sup> and inorganic<sup>8</sup> as well as organic acids<sup>9</sup>. A 0.75 *M* solution of formic acid was previously found to be suitable for amino acids<sup>7</sup> and other organic acids. A particularly great advantage of the latter two solutions is that they are capable of being removed from the dried paper by moderate heating.

Some time ago an investigation was initiated to find possible applications in the inorganic field for the formic acid solution, with particular reference to the separation of  $\text{Ca}^{2+}$  from  $\text{Mg}^{2+}$ . It was soon discovered that both cations migrated towards the cathode at conveniently high and sufficiently different rates of migration to afford a clean separation. A systematic investigation of a great number of cations showed that nearly all behaved as cations under these conditions, and that they had, with few exceptions, quite interesting mobilities. Those that did not move were usually adsorbed or rather precipitated at the base line.

#### *Measurement of migration rates at pH 2.0*

The measurements were carried out using the previously described high-voltage apparatus<sup>10</sup> providing speed, precision and good reproducibility. Several  $\text{K}^+$  and  $\text{Zn}^{2+}$  spots distributed evenly on each sheet were used as reference markers and several sucrose spots as markers for electro-osmotic and hydrodynamic flow effects. Cellophane sleeves interposed between the sheet and the connecting paper wads reduced the electrolyte flow to a minimum, ensuring thus sufficiently steady hydrodynamic conditions in the sheet. All distances were measured from the front of the sucrose spot to the front of the cation spot. In view of the tendency of some of the cations to form irregular or unduly elongated spots, this was considered to be the most reasonable and dependable way of measurement. The deduction due to electro-osmosis was generally small and not more than 1.5 cm. The duration of the experiment at 100 V/cm was 20 min for most cations. The alkali metals were also run for 15 min. The correlation between the results at 15 and 20 min followed a linear relationship. The same applied to the potential-migration rate and the temperature-migration rate relationships, corresponding in the latter case to about +3% per 1° over the measured range of 6–15°. The mean temperature of the cooling water was taken as the temperature of the paper sheet, a fair assumption with the constancy of current during the experiment as an indication of efficient heat dissipation and steady conditions. The cooling system and electrical energy throughput were so adjusted that the system was under-run rather than taxed to the limit. Paper sheets of 12 in. × 22 1/2 in. (Whatman No. 3 MM), allowing the application without mutual interference of 8–10 streaks or

spots plus several marker spots were used throughout. Most applications were in the form of streaks, 2 cm wide. The reproducibility of results was generally good and amounted to well within 4% between individual sheets and better than that for replicates on the same sheet. The moisture uptake of the paper sheet at 1.5 lb./sq. in. was 140% ( $\pm 3\%$ ), compared with the weight of the dry paper.

The experimental conditions were consequently as follows: pH 2.0 (0.75 M formic acid solution), 100 V/cm, 6 mA/cm, 1.5 lb./sq. in., 20 min, 11° (mean temperature of cooling water), 140% moisture content.

Reagents for detecting heavy and alkaline earth metals were  $(\text{NH}_4)_2\text{S}$  or 8-hydroxyquinoline and  $\text{NH}_3$ -vapour, or better because of greater sensitivity 8-hydroxyquinoline dissolved in conc. ammonia solution<sup>11</sup>, for alkali metals bromophenol blue in ethanol, and for  $(\text{NH}_4)^+$  ninhydrin, which surprisingly was found to give a strong reaction with  $\text{K}^+$  and  $\text{Na}^+$  and a weaker one with  $\text{Li}^+$  and some of the alkaline earth metals. Tests indicated that this hitherto unreported reaction appeared to be based on some nitrogenous impurities in the paper, as test tube experiments failed to give this otherwise highly reproducible reaction.

The results at pH 2.0 are summarised in order of valency in Table I. All figures are corrected for electro-osmotic and hydrodynamic flow effects.

Although the migration rates of certain alkali metals at pH 8.9 had already been reported<sup>2</sup>, it was considered desirable to re-measure more accurately these and the rest of the cations as shown in Table I. The results confirmed more quantitatively the extremely small but real differences between  $\text{Rb}^+$ ,  $\text{Cs}^+$  and  $\text{K}^+$  and elucidated the fate of the alkaline earth metals in the system of ammonium carbonate solution. The latter was made possible by using several detecting reagents of which ninhydrin (see above) proved very useful. The experiments were run at 80 V/cm (4 kV), *i.e.* below the available capacity of 5 kV, in order to avoid any possible over-heating or temperature rise during the experiment. The results are summarised in Table II.

It is interesting to note that only the mono- and divalent cations possess appreciable mobilities under these conditions.

#### *Relative migration rates of some alkali metals*

It is useful to compare the results obtained for  $\text{Rb}^+$ ,  $\text{Cs}^+$  and  $\text{K}^+$  with those published earlier<sup>2</sup>. Migration rates, relative to  $\text{Rb}^+ = 1.000$ , are shown in Table III.

Considering that different equipment was used and the time interval between the two sets of measurements was about five years, the agreement appears to be quite satisfactory. The present apparatus affords better control of conditions and thus greater precision of measurements.

#### *Mobilities*

Since it was established that under the given experimental conditions electrophoretic migration was a linear function of potential and time and independent of the size or design of the apparatus, and that all the significant physico-chemical factors such as temperature, moisture content of paper, conductance, and pH could be maintained constant, it seemed appropriate to calculate absolute mobilities from the migration rates for comparison with corresponding figures quoted in the literature. Table IV illustrates the agreement between mobilities derived from measurements in paper-stabilized

TABLE I  
MIGRATION RATES OF INORGANIC CATIONS AT pH 2.0  
(Conditions: 0.75 M formic acid soln.; 100 V/cm; 1.5 lb./sq.in.; 11°; 20 min)

Cation	Distance travelled towards cathode (cm)	$\frac{MK^*}{\text{rate}}$	Reagent	Colour in visible light	Colour in U.V. light	Remarks
Ag <sup>+</sup>	6.0	0.157	H.qu**	Brown	Dark (quenching)	
Hg <sup>+</sup>	0.0	0.0	H.qu.	Yellow	Dark	Precipitate
Cu <sup>+</sup>	17.6	0.461	H.qu.	Yellow	Dark	
Tl <sup>+</sup>	35.8	0.937	H.qu.	Brown	Dark	
Cs <sup>+</sup>	39.65	1.038	Bromophenol blue	Blue	—	Also ninhydrin blue colour
Li <sup>+</sup>	20.2	0.529	Bromophenol blue	Blue	—	Also ninhydrin blue colour
Na <sup>+</sup>	27.2	0.712	Bromophenol blue	Blue	—	Also ninhydrin blue colour
(NH <sub>4</sub> ) <sup>+</sup>	39.1	1.024	Bromophenol blue	Blue	—	Also ninhydrin blue colour
K <sup>+</sup>	38.2	1.000	Bromophenol blue	Blue	—	Also ninhydrin blue colour
Rb <sup>+</sup>	39.7	1.039	Bromophenol blue	Blue	—	Also ninhydrin blue colour
Ba <sup>2+</sup>	26.5	0.694	H.qu.	—	Greenish yellow	
Be <sup>2+</sup>	16.5	0.432	H.qu.	Pale yellow	Yellow	
Ca <sup>2+</sup>	27.9	0.730	H.qu.	—	Greenish yellow	
Cd <sup>2+</sup>	19.9	0.521	H.qu.	Yellow	Yellow	
Co <sup>2+</sup>	21.9	0.573	H.qu.	Orange	Dark	
Cu <sup>2+</sup>	16.9	0.442	H.qu.	Bright yellow	Dark	
Fe <sup>2+</sup>	21.3	0.555	H.qu.	Black	Dark	
Ni <sup>2+</sup>	24.3	0.558	H.qu.	Yellow	Dark	
Pb <sup>2+</sup>	21.75	0.569	H.qu.	Yellow	Dark	
Sr <sup>2+</sup>	0.0	0.0	H.qu.	Yellow	Bright yellow	Precipitate
Sr <sup>2+</sup>	24.9	0.652	H.qu.	—	Greenish yellow	
(UO <sub>2</sub> ) <sup>2+</sup>	8.95	0.234	H.qu.	Orange-brown	Dark	
Zn <sup>2+</sup>	21.8	0.571	H.qu.	Yellow	Bright yellow	

Mn <sup>2+</sup>	21.9	0.573	H.qu.	Orange	Dark
Ni <sup>2+</sup>	21.3	0.558	H.qu.	Yellow	Dark
Pb <sup>2+</sup>	21.75	0.569	H.qu.	Yellow	Dark
Sn <sup>2+</sup>	0.0	0.0	H.qu.	Yellow	Bright yellow Precipitate
Sr <sup>2+</sup>	24.9	0.652	H.qu.	—	Greenish yellow
(UO <sub>2</sub> ) <sup>2+</sup>	8.95	0.234	H.qu.	Orange-brown	Dark
Zn <sup>2+</sup>	21.8	0.571	H.qu.	Yellow	Bright yellow
Al <sup>3+</sup>	17.9	0.468	H.qu.	Brownish yellow	Bright yellow
Bi <sup>3+</sup>	3.9	0.102	H.qu.	Yellow	Dark
Ce <sup>3+</sup>	21.02	0.550	H.qu.	Reddish brown	Dark
Fe <sup>3+</sup>	8.45	0.221	H.qu.	Black	Dark
La <sup>3+</sup>	22.5	0.589	H.qu.	Brownish yellow	Yellow
Sb <sup>3+</sup>	0.0	0.0	H.qu.	Brownish yellow	Dark Precipitate
Ti <sup>3+</sup>	10.4 (8.4)	0.272 (0.219)	H.qu.	Brownish yellow	Dark Brown band develops only after several hours
Ce <sup>4+</sup>	6.77	0.177	H.qu.	Grey	Dark Contained also band of Ce <sup>3+</sup>
Th <sup>4+</sup>	10.2	0.267	H.qu.	Brownish yellow	Brown
Ti <sup>4+</sup>	8.4	0.219	H.qu.	Yellow	Dark
Zr <sup>4+</sup>	0.5	0.013	H.qu.	Yellow	Dark Trailing

\*  $M_K$  = Migration rate of cation

= Migration rate of potassium

\*\* H.qu. = 8-hydroxyquinoline.

TABLE II  
MIGRATION RATES OF INORGANIC CATIONS AT pH 8.9.  
(Conditions: approx. 0.1 M ammonium carbonate soln.; 80 V/cm; 1.5 lb./sq. in.; 11°; 20 min)

Cation	Distance travelled towards cathode (cm)	$M_K^*$ (relative migration rate)	Reagent	Colour in visible light	Colour in U.V. light	Remarks
Ag <sup>+</sup>	4.7	0.148	H.qu. **	Brown	Dark (quenching)	
Hg <sup>+</sup>	15.6	0.492	H.qu.	Yellow	Dark (quenching)	
Cu <sup>+</sup>	1.6	0.050	H.qu.	Yellow	Dark (quenching)	
Tl <sup>+</sup>	25.1	0.792	H.qu.	Brown	Dark (quenching)	
Cs <sup>+</sup>	32.5	1.025	Bromothymol blue	Blue	—	
Li <sup>+</sup>	16.8	0.529	Bromothymol blue	Blue	—	
Na <sup>+</sup>	23.1	0.729	Bromothymol blue	Blue	—	
K <sup>+</sup>	31.7	1.000	Bromothymol blue	Blue	—	
Rb <sup>+</sup>	33.1	1.044	Bromothymol blue	Blue	—	
Ba <sup>2+</sup>	15.8	0.498	H.qu.	—	Greenish yellow	Some precipitate and trailing to base line
Be <sup>2+</sup>	0.0	0.0	H.qu.	Pale yellow	Yellow	Ppt. and trail. to anode
Ca <sup>2+</sup>	13.1	0.413	H.qu.	—	Greenish yellow	Ppt. and trail. to base line
Cd <sup>2+</sup>	0.0	0.0	H.qu.	Yellow	Yellow	Ppt.
Co <sup>2+</sup>	4.3	0.130	H.qu.	Orange	Dark (quenching)	Ppt.
Cu <sup>2+</sup>	0.0	0.0	H.qu.	Yellow	Dark (quenching)	Ppt.
Fe <sup>2+</sup>	0.0	0.0	H.qu.	Black	Dark (quenching)	Ppt.
Mg <sup>2+</sup>	15.0	0.473	H.qu.	Pale yellow	Whitish yellow	

	5-9	0.186	H.qu.	Orange	Dark	Some ppt. and trail.
Mn <sup>2+</sup>	5.9	0.186	H.qu.	Yellow	Dark	
Ni <sup>2+</sup>	5.9	0.186	H.qu.	Yellow	Dark	Ppt.
Pb <sup>2+</sup>	0.0	0.0	H.qu.	Yellow	Bright yellow	Ppt.
Sn <sup>2+</sup>	0.0	0.0	H.qu.	Yellow	Bright yellow	Trail. to base line
Sr <sup>2+</sup>	15.2	0.479	H.qu.	—	Greenish yellow	Anodic trail.
(UO <sub>2</sub> ) <sup>2+</sup>	0.0	0.0	H.qu.	Brown	Dark	Ppt.
Zn <sup>2+</sup>	0.0	0.0	H.qu.	Yellow	Bright yellow	
Al <sup>3+</sup>	0.0	0.0	H.qu.	Yellow	Bright yellow	Ppt. and anodic trail.
Bi <sup>3+</sup>	0.0	0.0	H.qu.	Yellow	Dark	Ppt.
Ce <sup>3+</sup>	0.0	0.0	H.qu.	Brown	Dark	Ppt.
Fe <sup>3+</sup>	0.0	0.0	H.qu.	Black	Dark	Ppt.
La <sup>3+</sup>	0.0	0.0	H.qu.	Yellow	Dark	Ppt.
Sb <sup>3+</sup>	0.0	0.0	H.qu.	Yellow	Dark	Ppt.
Ti <sup>3+</sup>	0.0	0.0	H.qu.	Yellow	Dark	Ppt.
Ce <sup>4+</sup>	0.0	0.0	H.qu.	Grey	Dark	Ppt.
Th <sup>4+</sup>	0.0	0.0	H.qu.	Brown	Brown	Ppt. and anodic trail.
Ti <sup>4+</sup>	0.0	0.0	H.qu.	Yellow	Dark	Ppt.
Zr <sup>4+</sup>	0.0	0.0	H.qu.	Yellow	Dark	Ppt.

\*  $M_K = \frac{\text{Migration rate of cation}}{\text{Migration rate of potassium}}$   
 \*\* H.qu. = 8-hydroxyquinoline.

TABLE III

COMPARISON OF MEASUREMENTS OF SOME RELATIVE MIGRATION RATES

Cation	$M_{Rb}$		Remarks
	Previous results	Present results	
K <sup>+</sup>	0.962	0.958	Apparatus of similar design but different dimensions and precision
Rb <sup>+</sup>	1.000	1.000	
Cs <sup>+</sup>	0.988	0.982	

systems under varying conditions and the discrepancies between these figures and those derived from conductivities at infinite dilution.

Although the agreement between previous and present results was fairly good and the discrepancy, due to the tortuosity factor, between mobilities as measured in paper-stabilized systems and in free electrophoresis was of the expected order of magnitude, some figures seemed to be outside the limit of error and consistently higher. It was then discovered that there was a measurable effect due to the machine direction or arrangement of the cellulose fibres in the paper. The effect of this on the flow of solvent in paper chromatography is well known, but it was not suspected to affect the mechanism of paper electrophoresis.

#### *An additional tortuosity effect*

It was found that cutting the paper sheet in the machine direction produced under the given conditions consistently higher results when compared with those obtained by cutting the paper across the machine direction. The differences were always significant though varying with the batch of paper, between 4 and 10%. There was also a noticeable difference in the current flowing through the paper, the sheet cut along the machine direction requiring more current. The results in Table V demonstrate the magnitude of the effect found when measuring some inorganic and organic ions of varying mobilities in certain batches of paper after having established the machine direction.

The existence of this migration-retarding effect was only discovered because of the high reproducibility of the mobility measurements. Any discrepancy greater than 3% was suspect and electrical, timing and cooling conditions were checked

TABLE IV

COMPARISON OF IONIC MOBILITIES  
(in  $\text{cm}^2 \text{V}^{-1} \text{sec}^{-1} \cdot 10^6$ )

Ion	Mobilities (in paper) at 11°				Mobilities <sup>12</sup> (free electrophoresis) at 18° (from conductivities)
	Previous results <sup>8,9</sup>		Present results		
	pH 2.0	pH 8.9	pH 2.0	pH 8.9	
K <sup>+</sup>	—	32.32	31.83	33.02	64.6
Rb <sup>+</sup>	—	33.56	33.08	34.48	67.5
Cs <sup>+</sup>	—	33.16	33.04	33.84	68.0
Na <sup>+</sup>	—	—	22.66	24.06	43.4
Li <sup>+</sup>	—	—	16.81	17.5	33.4
Cl <sup>-</sup>	32.65	31.6	—	—	65.5



TABLE V  
TORTUOSITY EFFECT  
(Due to machine direction of paper)

Ion	pH 2.0 Conditions: 100 V/cm, 13°, 15 min						pH 8.9 Conditions: 80 V/cm, 13°, 20 min			
	Paper batch A		Paper batch B		Current mA/cm		Paper batch C		Current mA/cm	
	across M.D. (cm)	along M.D. (cm)	across M.D. (cm)	along M.D. (cm)	across M.D.	along M.D.	across M.D. (cm)	along M.D. (cm)	across M.D.	along M.D.
(NH <sub>4</sub> ) <sup>+</sup>	31.2	35.5	31.8	33.8	6.0	6.7			8	10
K <sup>+</sup>	30.6	34.7	31.3	33.3			31.25	32.0		
Na <sup>+</sup>	21.7	24.55	22.4	24.0			22.4	23.1		
Li <sup>+</sup>	16.4	18.4	16.7	18.3			16.7	17.3		
Lysine	13.4	15.2	14.0	15.2						
Alanine	8.75	9.75	9.0	9.75						
Glutamic acid	5.95	6.65	6.1	6.65						

immediately. As long as batches of paper were cut by sheer routine or chance in the same way, no discrepancies were bound to appear. But once the routine was reversed or a batch was particularly bad in this respect, the differences called for an investigation.

Whether this effect is simply due to an extended tortuous path caused by a different arrangement of the cellulose fibres with respect to the direction of migration, is at present difficult to decide. Although the trend of the current consumption would support the assumption that the electrical resistance is correspondingly affected, the actual differences appear to be rather too high for a strict correlation.

#### DISCUSSION

High-voltage paper electrophoresis should be particularly useful for separation of inorganic ions because of their relatively high rates of migration and the avoidance of disturbing diffusion effects during the extremely short runs at high-potential gradients. It should be possible to make use of very small differences in mobilities, provided suitable background electrolytes can be found to give maximal differentiation and thus optimal resolution.

A systematic investigation of background electrolytes, involving the measurement of migration rates of a great number of inorganic cations under the rigorously controlled conditions of a suitable precision high-voltage apparatus<sup>10</sup>, established the usefulness of an electrolyte solution consisting of a 0.75 *M* formic acid solution of pH 2.0. Under these conditions most cations migrate to the cathode at varying rates, offering many interesting separation possibilities. Migration rates in this electrolyte and in an approx. 0.1 *M* ammonium carbonate solution of pH 8.9 were measured. Mobilities in paper-stabilized systems for some ions were calculated and compared with theoretical mobilities. An unknown additional tortuosity effect was observed and measured.

It would appear that the potential applicability of the technique of paper electrophoresis to problems of inorganic chemistry is far from exhausted. Higher standards of precision of measurement and better control of experimental conditions

could lead to more examples of exploitation of even very small differences in mobilities. The advantage of the formic acid solution is that the time for some separations has been reduced to one-third, *i.e.* a few minutes.

#### ACKNOWLEDGEMENTS

Thanks are due to Mr. R. W. BUTTERS for his valuable technical assistance and to the directors of Tate & Lyle Refineries Ltd. for the permission to publish this communication.

#### SUMMARY

Conditions were investigated under which most inorganic cations are non-complexed and migrate at varying rates towards the cathode. A background electrolyte consisting of a 0.75 *M* formic acid solution of pH 2.0 was found particularly suitable for this purpose. Another electrolyte solution, *viz.* an approx. 0.1 *M* ammonium carbonate solution of pH 8.9 was again thoroughly investigated for the migration behaviour of many cations. The measured migration rates were tabulated and mobilities calculated and compared. A new tortuosity effect was observed. All measurements were made, using a previously described high-voltage apparatus<sup>10</sup> of ample precision ensuring good reproducibility of results.

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