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## A STUDY OF THE HYDROGEN ION CONCENTRATION GRADIENT ON ALGINIC ACID THIN LAYERS

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### SUMMARY

The various types of hydrogen ion concentration gradients formed along a chromatographic layer during elution with mineral acids, buffer solutions, and neutral salt solutions were thoroughly investigated. The results indicate that the formation of such gradients may be attributed to a process of absorption by the substrate and to ion-exchange.

Various analytical applications are envisaged.

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### INTRODUCTION

The chromatographic characteristics of alginic acid, its exchange mechanism and its applications in organic and inorganic chemistry to thin-layer chromatography have been described in previous papers<sup>1-4</sup>, where it was shown<sup>1</sup> that elution with strong mineral acids gives rise to a hydrogen ion concentration gradient along the layer, and that this is increasing when the initial concentration of the eluent becomes higher.

In the present work such behaviour was found not only with acidic eluents but also with buffers and solutions of neutral salts.

The possibility of making use of this formation of a hydrogen ion concentration gradient for analytical purposes has been examined.

### EXPERIMENTAL

#### *Preparation of the layers and solutions*

The solutions of metal salts were prepared as described previously<sup>1</sup>. 12 g of alginic acid in 50 ml of water was used for the preparation of 300  $\mu$  layers. For carboxymethylcellulose (CMC) layers, 4.0 g of the substrate in the form of the sodium salt in 40 ml of water and 4.5 g of the acid form in the same volume of water were employed.

"Chemetron" automatic equipment was used for layer application.

#### *Measurement of the hydrogen ion concentration gradient*

The following methods were employed: (1) Direct pH measurement on the sur-

face of the layer, using a special combined electrode (Polymetron). (2) Measurement of the pH of the suspensions obtained by dispersing equal portions of the layer (bands) in 5 ml portions of distilled water after elution. A glass electrode was used for the measurements, which were carried out after the suspensions had been agitated for 2 min.

The total acidity of the bands dispersed in 5 ml of water was determined to help in interpreting the data.

## RESULTS AND DISCUSSION

### Alginic acid layers

*Elution with neutral salt solutions.* The form of the pH gradient which arises along the layer is in this case opposite to that observed<sup>1</sup> during elution with strong mineral acids, as can be seen from the curves presented in Fig. 1. Table I shows a comparison

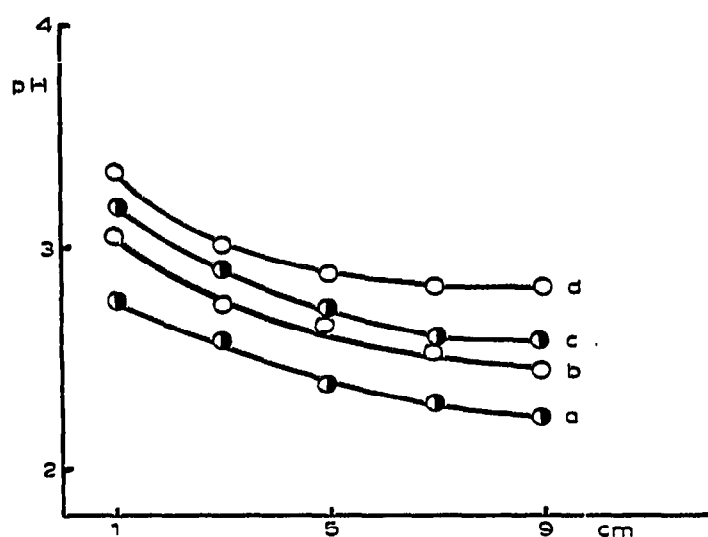


Fig. 1. pH values of alginic acid suspensions referred to the center of the strips. Eluents: (a) 1 *M* NaClO<sub>4</sub>; (b) 0.5 *M* NaClO<sub>4</sub>; (c) 0.3 *M* NaClO<sub>4</sub>; (d) 0.1 *M* NaClO<sub>4</sub>.

TABLE I

HYDROGEN ION CONCENTRATION GRADIENT ALONG THE LAYER FOR VARIOUS NaClO<sub>4</sub> CONCENTRATIONS IN THE ELUENT

pH<sub>1</sub> = pH measurement on the surface of the layer; 0.01 *N* NaOH (ml): acidimetric measurements of the total acidity.

Distance <sup>a</sup> (cm)	1.0 <i>M</i> NaClO <sub>4</sub>		0.5 <i>M</i> NaClO <sub>4</sub>		0.1 <i>M</i> NaClO <sub>4</sub>	
	pH <sub>1</sub> <sup>b</sup>	NaOH <sup>c</sup> (ml)	pH <sub>1</sub>	NaOH (ml)	pH <sub>1</sub>	NaOH (ml)
0-2	2.3	14.25	2.4	14.35	2.4	14.40
2-4	1.9	15.45	2.2	15.85	2.3	16.30
4-6	1.7	16.05	2.0	16.45	2.2	16.80
6-8	1.4	17.75	1.7	16.95	2.1	17.00
8-10	1.2	18.00	1.5	17.90	2.1	17.00

<sup>a</sup> Distances of the front and rear limits of the band from the starting point.

<sup>b</sup> pH<sub>1</sub> values refer to the center of each band.

<sup>c</sup> Blank experiment: 16.30 ml of 0.01 *N* NaOH.

between the surface pH values of the layer and the total acidity of the individual bands.

It seems that the total acidity increases with increasing distance from the starting point, and consequently the surface pH decreases. Nevertheless, it is known that the total acidity is lower for some bands and higher for others compared with blank experiments (total acidity of analogous bands which have not been eluted), depending on the distance from the starting point and the concentration of salt in the eluent. For example, on elution with 0.1 *M* NaClO<sub>4</sub> only the first band has a total acidity less than the blank, while on elution with 0.5 *M* and 1 *M* solutions of NaClO<sub>4</sub>, bands two and three, respectively, are found to have a total acidity smaller than the blank. Such behaviour shows evidence of ion-exchange between bonded hydrogen ions and sodium ions together with the transfer of hydrogen ions towards the solvent front.

The formation of a pH gradient has a marked effect on the chromatographic behaviour of elements, as can be seen from Table II. Such a gradient plays the role of increasing the law of mass action effect and of decreasing the retention capacity along the layer.

TABLE II

*R<sub>F</sub>* VALUES OF METAL IONS ON ALGINIC ACID THIN LAYERS

Ion	NaCl (mole/l)			NaClO <sub>4</sub> (mole/l)	
	0.05	0.1	0.3	0.1	0.3
Tl(I)	0.13	0.15	(0-0.95)	0.16	0.31
Ag(I)	0.00	0.00	0.00	0.28	0.53
Co(II)	0.21	0.54	0.92	0.53	0.90
Ni(II)	0.20	0.54	0.92	0.52	0.90
Hg(II)	0.71	0.85	0.88	0.71	0.74
Mn(II)	0.33	0.60	0.95	0.59	0.95
Mg(II)	0.32	0.59	0.94	0.60	0.95
Cd(II)	0.27	0.61	0.94	0.39	0.83
Zn(II)	0.20	0.54	0.93	0.51	0.88
Ca(II)	0.06	0.21	n.d. <sup>a</sup>	0.22	n.d.
Sr(II)	0.04	0.05	0.07	0.05	0.07
Ba(II)	0.00	0.00	0.05	0.00	0.05
Cu(II)	0.02	0.08	0.25	0.07	0.20
Pb(II)	0.00	0.00	0.00	0.00	0.00
Bi(III)	0.00	0.00	(0-0.90)	0.00	0.00
In(III)	0.02	0.02	0.80	0.00	0.00
Th(IV)	0.00	0.00	0.00	0.00	0.00
Mo(VI)	0.00	0.00	0.00	0.00	0.00

<sup>a</sup> n.d. = not determined.

As could be expected, the cations found to be particularly affected were those having a small affinity for alginic acid and thus having higher *R<sub>F</sub>* values. Thus, it seems possible that some separations could be improved by making use of the fact that the formation of a positive hydrogen ion concentration gradient increases the difference between the *R<sub>F</sub>* values. In this way, with NaClO<sub>4</sub> and NaCl concentrations in the eluent equal to 0.05 *M*, Mg(II), Mn(II), Co(II), Ni(II) and Zn(II) can be clearly separated from Ca(II), Cu(II), Sr(II), Ba(II), Pb(II) and from tri- and tetravalent ions.

With the alkaline earth metals, which show different affinities for alginic acid<sup>3</sup>, we see that Ba(II) and Sr(II) do not respond to an increasing salt concentration of the eluent. On the other hand, Ca(II) and particularly Mg(II), which shows the lowest affinity for alginic acid, are markedly influenced.

In addition to the salt concentration in the eluent, the nature of the cation in the salt also has an important effect on the  $R_F$  values of the elements. Table III shows the  $R_F$  values for various elements with Li, Na,  $\text{NH}_4$ , and K nitrate solutions as eluents.

TABLE III

 $R_F$  VALUES OF METAL IONS ON ALGINIC ACID THIN LAYERS

Ion	Eluent			
	0.1 M $\text{LiNO}_3$	0.1 M $\text{NaNO}_3$	0.1 M $\text{NH}_4\text{NO}_3$	0.1 M $\text{KNO}_3$
Tl(I)	0.12	0.16	0.17	0.23
Co(II)	0.26	0.53	0.53	0.54
Cd(II)	0.12	0.39	0.41	0.47
Zn(II)	0.15	0.51	0.56	0.56
Mg(II)	0.56	0.60	0.65	0.68

There is a remarkable difference between the  $R_F$  values obtained on elution with lithium nitrate and with the other nitrates in agreement with the negative  $\Delta F^\circ$  of  $\text{Li}^+$  relative to  $\text{H}^+$  in ion-exchange equilibria<sup>5</sup>.

*Use of buffer solutions as eluents.* Fig. 2 shows the changes in the hydrogen ion concentration gradient along the layer on elution with buffer solutions of  $\text{pH} < 3$  prepared from sodium acetate and nitric acid. All the curves except (b) show the formation of a pH gradient. While in the case of curve (a) the pH variation is in

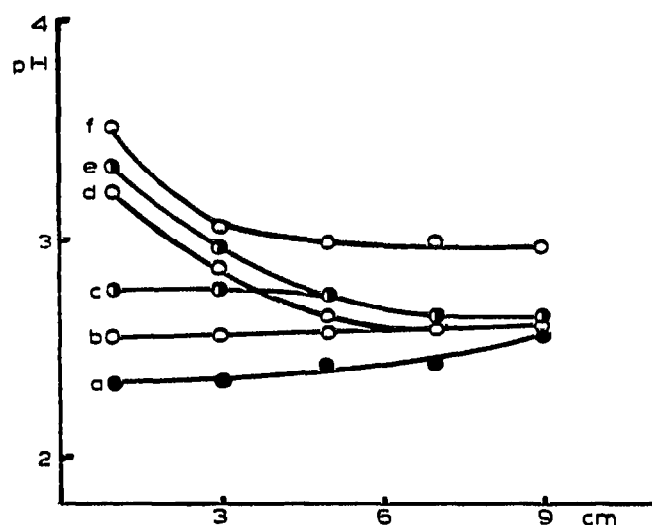


Fig. 2. pH values of alginic acid suspensions referred to the center of the strips. Eluents:  $\text{CH}_3\text{COONa} + \text{HNO}_3$  solutions. Curves (a, b and c): sodium acetate concentration 0.2 mole/l and  $\text{pH} = 1.00$ ; 1.22 and 1.40. Curves (d, e and f): sodium acetate concentration 0.4, 0.2 and 0.1 mole/l and  $\text{pH} = 2.70$ .

agreement with the behaviour observed on elution with strong mineral acids<sup>1</sup>, and may be attributed to an absorption process; curves (c, d, e and f) show evidence of ion-exchange between bonded hydrogen ions and sodium ions. Such a process leads to an increase in the hydrogen ion concentration along the layer, the effect being greater for higher concentrations of sodium ions in the eluent. This is clearly shown by curves (d, e and f). The effect of the ion-exchange process exactly balances the effect of the process of absorption by the substrate, as shown by curve (b).

The behaviour shown in Fig. 2 may also be observed with a solution prepared from sodium acetate and a suitable excess of a strong mineral acid; phenomena analogous to those found when solutions of KCl and HCl are used as eluents may also be seen.

The possible analytical applications are interesting. Figs. 3 and 4 show the variation of the chromatographic characteristics, as a function of the eluent pH, for some representative cations. The differences between the  $R_F$  values of the cations, which differ little in their affinities for alginic acid, are increased.

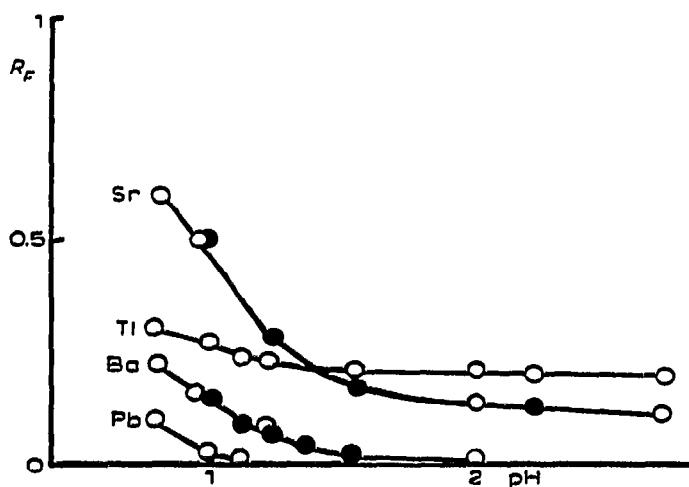


Fig. 3. The relationship of  $R_F$  values to pH for some elements. Eluents:  $\text{CH}_3\text{COONa} + \text{HCl}$  ( $\bullet-\bullet$ ) and  $\text{CH}_3\text{COONa} + \text{HNO}_3$  ( $\circ-\circ$ ) solutions with equal molarities of sodium acetate (0.2 mole/l).

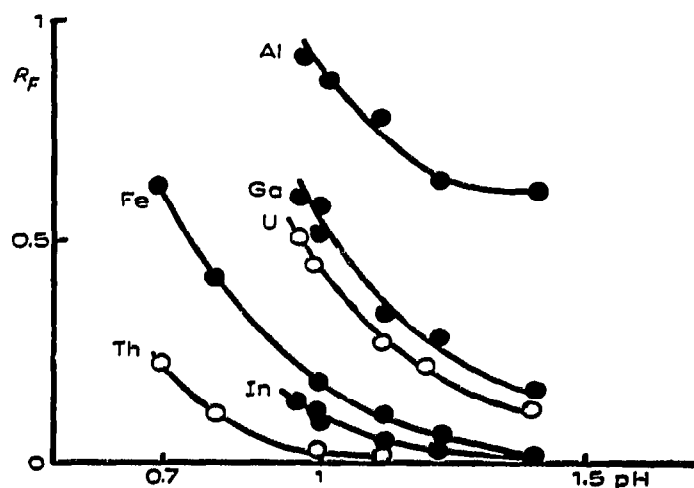


Fig. 4. The relationship of  $R_F$  values to pH for some elements. Eluents:  $\text{CH}_3\text{COONa} + \text{HNO}_3$  solutions with equal molarities of sodium acetate (0.2 mole/l).

At pH values for which a positive hydrogen concentration gradient is observed (curves c, d, e and f in Fig. 2), such an increase is due to the hydrogen ions produced by the ion-exchange between the Na<sup>+</sup> ions and the carboxyl groups of alginic acid.

This effect, although predominant, is not the only one; the law of the mass action effect and the complexing action of the acetate ions, the concentration of which increases as the pH of the eluent increases, also participate.

The separation of Al(III), Ga(III) and In(III) is of considerable interest. These elements give compact bands which may possibly be useful for quantitative determination.

Such separations cannot be achieved by elution with solutions of strong mineral acids having the same pH as the buffer solutions<sup>2</sup>. If KCl + HCl buffer is used as the eluent instead of HCl, at the same pH, a better differentiation is observed between the  $R_F$  values of the metal cations. The separation of Ba(II), Sr(II), Ca(II), and Mg(II) can be considered as an example. These ions have already been separated<sup>2</sup> using HCl (pH  $\leq$  1.4) as the eluent. A marked improvement is observed when a mixture of HCl and KCl at the same pH is used, even with a low concentration of the KCl (0.05 mole/l).

TABLE IV

$R_F$  OF METAL IONS ON ALGINIC ACID THIN LAYERS

Eluents: KCl + HCl solutions with equal molarities of KCl (0.05 mole/l).<sup>1</sup>

Ion	pH of the eluent			
	2.00	1.43	1.10	0.91
Co(II)	0.28	0.57	0.80	0.95
Ni(II)	0.27	0.56	0.80	0.95
Cu(II)	0.08	0.26	0.52	0.68
Pb(II)	0.00	0.00	0.04	0.09
Ba(II)	0.00	0.03	0.12	0.20
Sr(II)	0.06	0.21	0.41	0.52
Ca(II)	0.24	0.45	n.d. <sup>b</sup>	n.d.
Mg(II)	0.36	0.62	0.90	0.96
Zn(II)	0.26	0.52	0.75	0.95
Cd(II)	0.36	0.65	0.94	0.96
Fe(III)	e.s. <sup>a</sup>	e.s.	0.26	0.64
Bi(III)	0.00	0.00	0.00	0.13
Al(III)	0.12	0.39	e.s.	e.s.
Ga(III)	0.03	0.18	0.47	e.s.
In(III)	0.02	0.16	0.66	0.95
Th(IV)	0.00	0.00	0.00	0.07
Mo(VI)	0.00	0.00	0.00	0.04

<sup>a</sup> e.s. = elongated spot.

<sup>b</sup> n.d. = not determined.

### Carboxymethylcellulose (CMC) layers

Buffer solutions are widely used as eluents in chromatography; in particular, their use in ion-exchange chromatography has the additional purpose of preventing the formation of a pH gradient along the layer<sup>6</sup>. We thought it would be useful to extend our study to include carboxymethylcellulose layers, which have the advantage over alginic acid that they can be used in buffer solutions at pH > 3.

Use of acetic acid buffer as the eluent. Fig. 5 shows the pH changes along the layers, obtained by using buffer solutions of sodium acetate and acetic acid, with equimolar concentrations, as eluents on carboxymethylcellulose layers; curves (a, b, c) are in the acid form and curves (d, e, f) are in the form of the sodium salt. The variation of pH along the layer proved to be greater when the acid form was used.

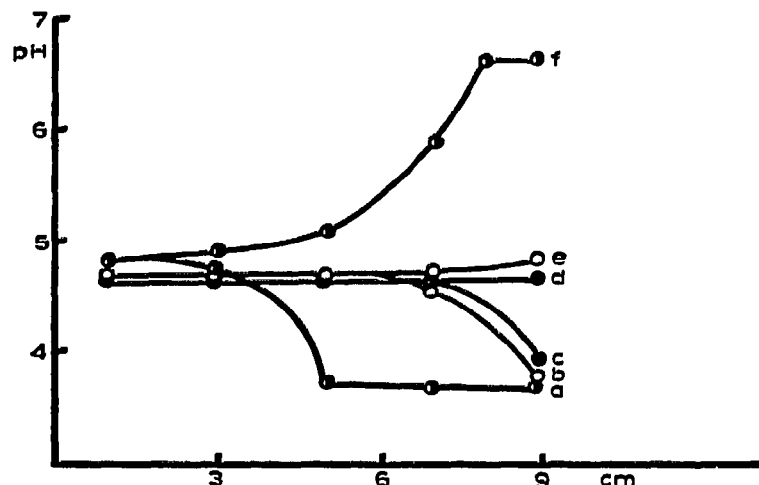


Fig. 5. pH values, referred to the center of the strips, of carboxymethylcellulose (curves (d, e and f)) and its sodium salt (curves (a, b and c)), suspensions. Eluents: buffers with equal molarities of sodium acetate and acetic acid. (a and f) 0.1 M; (b and e) 0.5 M; (c and d) 1 M.

Parallel acidimetric measurements and surface pH measurements were carried out. Table V shows the data obtained by measuring the pH on the surface and in suspension, together with values of total acidity expressed as 0.01 M NaOH. Comparison of data obtained by different methods indicates that the pH of the suspension increases as the total acidity of the band is diminished, while the surface pH decreases,

TABLE V

HYDROGEN ION CONCENTRATION GRADIENT ALONG THE LAYER OF SODIUM CARBOXYMETHYLCELLULOSE FOR BUFFER SOLUTIONS EQUIMOLAR WITH RESPECT TO SODIUM ACETATE AND ACETIC ACID IN THE ELUENT

pH<sub>1</sub> = pH measurement on the surface of the layer. pH<sub>2</sub> = measurement of the pH of suspensions. 0.01 N NaOH (ml): acidimetric measurements of the total acidity.

Distance <sup>a</sup> (cm)	Buffer solution								
	1 M			0.5 M			0.1 M		
	pH <sub>1</sub> <sup>b</sup>	pH <sub>2</sub>	NaOH <sup>c</sup> (ml)	pH <sub>1</sub>	pH <sub>2</sub>	NaOH (ml)	pH <sub>1</sub>	pH <sub>2</sub>	NaOH (ml)
0-2	4.6	4.60	15.9	4.6	4.66	8.65	4.8	4.85	2.90
2-4	4.4	4.60	15.8	4.6	4.68	8.65	4.8	4.92	2.70
4-6	4.3	4.63	13.5	4.6	4.69	7.20	4.9	5.08	2.00
6-8	4.3	4.64	12.5	4.5	4.72	7.10	5.3	5.90	0.60
8-10	4.2	4.66	11.0	4.5	4.82	4.95	5.6	6.62	0.50

<sup>a</sup> Distances of the front and rear limits of the band from the starting point.

<sup>b</sup> pH<sub>1</sub> values refer to the center of each band.

<sup>c</sup> Blank experiment: 0.3 ml of 0.01 N NaOH.

at least for high buffer concentrations. This fact suggests that the phenomena occurring on the surface and in the interior of the same layer may be different. The presence of a film of liquid on the layer has a critical effect on the surface pH measurements. To obtain reproducible results, it is therefore necessary to make the measurements immediately after elution.

Comparison of the acidimetric data with those of the suspension pH finally shows the greater variation of the total acidity compared with the corresponding values of the pH, especially for high buffer concentrations. In particular, the variation of the total acidity for a buffer concentration of 1 *M* is not accompanied by any appreciable variations in pH.

The phenomenon which we have demonstrated by acidimetric and pH studies is confirmed by the chromatographic behaviour of ions reported in Table VI. It will be seen that the  $R_F$  values obtained by elution with 0.1 *M* buffer are particularly low compared to those obtained with more concentrated buffers. This behaviour agrees perfectly with the greater variation of pH observed in the case of the less concentrated buffer solution. Table VI also shows the results obtained by LEDERER<sup>6</sup> on carboxymethylcellulose ion-exchange papers. From the agreement of the data, apart from the general reduction in  $R_F$  values in work on thin layers, it seems reasonable to conclude that the phenomenon found by us is also observed on ion-exchange papers. Such behaviour may be attributed mainly to an ion-exchange process.

TABLE VI

$R_F$  VALUES OF METAL IONS ON SODIUM CARBOXYMETHYLCELLULOSE (CMC) THIN LAYERS AND WHATMAN CM-50 PAPER<sup>5</sup>

Eluents: 1 = 1 *N* sodium acetate-1 *N* acetic acid; 2 = 0.5 *N* sodium acetate-0.5 *N* acetic acid; 3 = 0.1 *N* sodium acetate-0.1 *N* acetic acid.

Ion	Eluent 1		Eluent 2		Eluent 3	
	CM-50	CMC	CM-50	CMC	CM-50	CMC
Ag(I)	0.5	0.49	0.36	0.36	0.17	0.14
Tl(I)	0.48	0.57	0.46	0.46	0.25	0.21
Hg(II)	0.78	0.81	0.56	0.76	0.09	c.s. <sup>a</sup>
Cd(II)	0.72	0.61	0.44	0.36	0.08	0.03
Pb(II)	0.53	0.37	0.27	0.13	0.04	0.00
Cu(II)	0.63	0.55	0.34	0.28	0.04	0.00
Co(II)	0.70	0.62	0.52	0.38	0.10	0.04
Ni(II)	0.68	0.61	0.52	0.38	0.10	0.04
Zn(II)	0.72	0.62	0.45	0.35	0.09	0.04
Mn(II)	0.75	0.58	0.52	0.38	0.13	0.08
Be(II)	0.85	0.79	0.71	0.55	0.12	0.06
Mg(II)	0.84	0.78	0.62	0.53	0.17	0.09
Ca(II)	0.65	0.57	0.45	0.35	0.08	0.04
Sr(II)	0.62	0.55	0.48	0.38	0.11	0.06
Ba(II)	0.52	0.40	0.35	0.26	0.05	0.02
Bi(III)	0.88	0.84	0.6	0.50	0.05	0.02
Al(III)	0.78	0.65	0.36	0.18	0.04	0.00
Ga(III)	—	0.51	—	0.09	—	0.00
In(III)	—	0.91	—	0.55	—	0.00

<sup>a</sup> e.s. = elongated spot.



## CONCLUSION

In the course of this work consideration was given to the various types of hydrogen ion concentration gradients which are formed on alginic acid and carboxymethylcellulose layers during elution with strong acids, buffers, and neutral salt solutions. In all the cases it was found that the formation of a gradient of hydrogen ions along the layer is due to: (1) An ion-exchange process; (2) A process of absorption of acid by the substrate

The absorption process is not limited to acids, but also occurs with salts, as shown by preliminary conductivity experiments on layers of sodium carboxymethylcellulose eluted with  $\text{NaClO}_4$  solutions of different concentrations. These showed the presence of a salt concentration gradient along the layer.

The various types of pH gradients, coupled with the law of mass action effect, may also be usefully employed for analytical purposes, in so far as they tend to increase the differences between the affinities of various elements for the substrate, and thus often make possible improved separation of the elements themselves. As an example we cite the sharp separation of Al(III), Ga(III) and In(III).

## ACKNOWLEDGEMENT

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