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ALGINIC ACID, A NEW THIN LAYER MATERIAL

PART I

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SUMMARY

Alginic acid is proposed as a new stationary phase for TLC. The effects of the distance moved by the solvent front, temperature, hydrogen ion concentration of the eluents and the thickness of the layers on the R_F values of some common elements have been extensively investigated. Characteristic R_F values of some common elements are reported. The chromatographic properties of alginic acid and carboxymethylcellulose were compared.

INTRODUCTION

Thin-layer chromatography had been extensively applied to many problems in organic chemistry¹ but it has only been used in inorganic chemistry in the last few years². Recently, selective stationary phases have been developed for numerous inorganic ions.

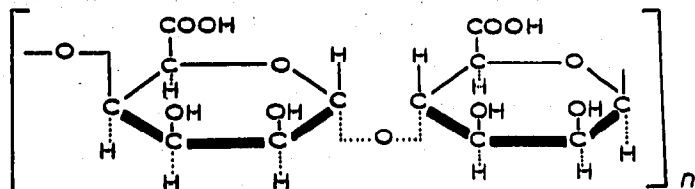
The substances employed as stationary phases can be collected in two distinct groups: (1) Substances employed as support and characterized by a great adsorption and low ion-exchange capacity such as silica gel, alumina and cellulose³. (2) Substances employed as support and characterized by a great ion-exchange capacity. The ion-exchangers more frequently employed are triisooctylamine (TIOA)⁴, di(2-ethylhexyl) phosphate (HDEHP)⁵ and synthetic resins⁶.

Alginic acid, proposed in this paper as a new stationary phase, is different from phases commonly employed because it is a support, an ion-exchanger and a complexing agent. Alginic acid together with sodium and calcium alginates have been extensively employed as ion-exchanger in column chromatographic separations of different elements^{7,8}. The peculiar characteristics of alginic acid depend upon its chemical and physical properties.

Properties of alginic acid

Recently, HIRST AND REES⁹ have shown that alginic acid is a linear polymer in which D-mannuronic and L-guluronic acids, linked together by 1-4 linkages, constitute

the monomeric units. No evidence of other forms of linkage or of branching was obtained. The most likely structure is



This type of structure indicates that different cations may be bound by the carboxyl and hydroxyl groups.

Alginic acid is insoluble in alcohol, ether and in the most common organic solvents and shows a great adsorption capacity. It is a weak acid which displaces CO_2 from carbonate solution and is very resistant to hydrolysis¹⁰.

The ion-exchanger characteristics of alginic acid are clearly seen from the I.R. spectra shown in Fig. 1 for alginic acid (1) and Co alginate (2). The strong band at 1735 cm^{-1} , assignable to the free carboxyl group of alginic acid, is not present in the Co alginate spectrum in which the strong band at 1600 cm^{-1} is assignable to the salified carboxyl group. No conclusive evidence is shown by I.R. spectra for the presence of coordination bonds of metal ions with hydroxyl groups. A very wide band, assignable to hydrogen bonds among hydroxyl groups, does not allow interpretation as a bonding action by two vicinal hydroxyl groups such as SCHWEIGER¹¹ found by other methods.

In Fig. 2 the R_M values *vs.* the log of the acid concentration in the eluent for some divalent and trivalent ions are reported. In both cases straight plots are observed provided that, for Ba(II) and Sr(II), the acid concentration in the eluent is between 0.05 and 0.5 *M*. Nevertheless the slope of the plots relative to the divalent ions is lower than that expected for an ion-exchange mechanism¹² but increases as the atomic number of the ion decreases.

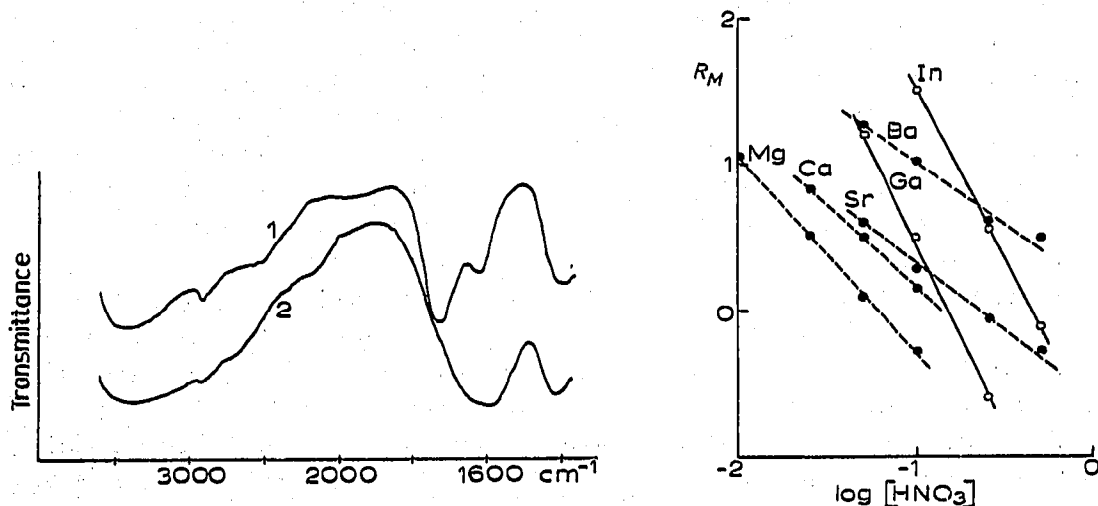


Fig. 1. I.R. spectra of (1) alginic acid and (2) Co alginate.

Fig. 2. R_M values *vs.* log HNO_3 concentration in the eluent.

On the basis of the reported results it is not possible to indicate the general operating mechanism of alginic acid. Nevertheless different behavior of the divalent compared to the trivalent ions examined is probable. With the latter an ion-exchange mechanism appears to predominate. Work is in progress on this problem.

EXPERIMENTAL

Solutions

For each element, 0.1 *M* solutions were prepared and titrated according to usual analytical procedures. All solutions were neutral or made weakly acid with HCl, HClO₄ or HNO₃. All ions were applied using solutions containing 1–15 μg/ml. For each element the amount employed (see Table IV) depended on the spot size and on the detecting reagent sensitivity.

Eluents

HCl, HClO₄, CH₃COOH, H₂O.

Detection reagents

- (1) 0.2 *N* NH₄HS solution.
- (2) 8-Hydroxyquinoline: 1% solution in ethanol followed by exposure to ammonia vapor.
- (3) Sodium rhodizonate: freshly prepared saturated aqueous solution.
- (4) Dithizone: 1% solution in chloroform.

Measurement of R_F values

In addition to the *R_F* values for the spot center those relative to the upper and lower edge of the spot are reported. Unless otherwise stated, *R_F* values were measured at room temperature (19–22°).

Preparation and properties of thin layers

Alginic acid is prepared by adding finely powdered sodium alginate to 2 *M* HCl. The Na alginate employed, SS/DJ, is commercially available from "Alginate Maton", 24 Rue Morillo, Paris 8 (France). The resulting product after acidification is separated, washed with distilled water to complete the removal of chloride ions, washed again with methanol and dried at room temperature. The particle size of the product depends upon the particle size of the sodium alginate employed. Actually, the thin layers were prepared from a powder which had been passed through a 150 mesh sieve before use. Alginic acid prepared in this way is substantially pure; by ashing a 0.5% by weight residue results, which contains only sodium and little iron, as shown by spectrographic analysis. To prepare four 7.5 × 15 cm thin-layer plates, 3.5 g of alginic acid are dispersed in 20 ml of water or, alternatively, in a water and methanol mixture 3:1 by volume. The amount specified is sufficient for coating one 20 × 20 cm plate. The slurry resulting from thorough stirring is immediately applied to the plates with the spreader blade set to give a 250 μ thick layer. Layers thicker than 250 μ, say 500 and 750 μ, may be obtained but have poor mechanical properties. The mechanical properties of the 250 μ layers vary considerably with the particle size of alginic acid. With a particle size between 100 and 120 mesh the layers are less

compact and slow down the movement of the eluent. This behavior is more evident with 500 and 750 μ layers. In such cases a tendency to cracking is also observed when alginic acid with smaller particle size is employed. Nevertheless, little variation in the R_F values is observed as data in Table I show. Before use, the thin layers were dried overnight at 18–22°.

Alginic acid gelatinizes even in weakly alkaline medium and the resulting gelatine retains solutes strongly and hinders the movement of the eluents. Consequently, only acidic and neutral solutions of elements and eluents can be employed.

TABLE I

 R_F VALUES ON ALGINIC ACID THIN LAYERS OF DIFFERENT THICKNESSESEluent: 0.1 M HClO₄.

Ions	Thin-layer thickness (μ)		
	250	500	750
Ag(I)	0.37 \pm 0.03	0.35 \pm 0.05	0.33 \pm 0.03
Tl(I)	0.20 \pm 0.04	0.18 \pm 0.04	0.16 \pm 0.03
Cu(II)	0.32 \pm 0.04	0.27 \pm 0.04	0.27 \pm 0.04
Bi(III)	0.00	0.00	0.00
Hg(II)	0.66 \pm 0.07	0.58 \pm 0.05	0.59 \pm 0.04
As(III)	0.47 \pm 0.04	0.48 \pm 0.05	0.45 \pm 0.04
Ni(II)	0.54 \pm 0.04	0.50 \pm 0.04	0.51 \pm 0.04
Co(II)	0.55 \pm 0.04	0.51 \pm 0.04	0.52 \pm 0.04

Movement of the solvent front

In a fixed time, the distance moved by the solvent front increases, as plots of Fig. 3 show, from the 750 μ (curve 1) to the 500 μ (curve 2) and finally to the 250 μ layer (curve 3). In any case the time dependence of the movement of the solvent front (v) is described by the simple relation (up to 30 min):

$$v^2 = a + bt$$

Ascending chromatography was employed.

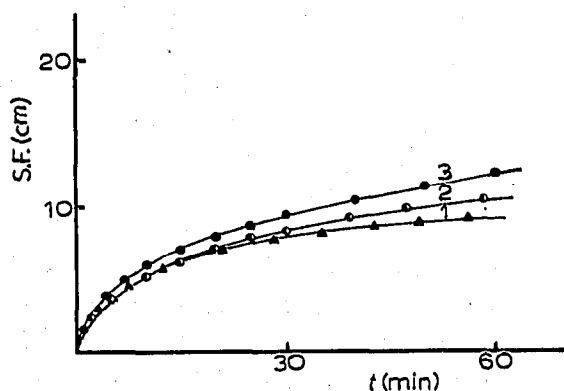


Fig. 3. Movement of the solvent front (S.F.) on alginic acid thin layers. (1) 750 μ ; (2) 500 μ ; (3) 250 μ .

Influence of the temperature

The temperature dependence of the movement of the solvent front at a fixed time, is shown in Fig. 4 for a 250μ thin layer. Indeed, according to the previous considerations, this layer shows the best properties. The plot exhibits a maximum at a temperature of 40° ; before this value the distance moved by the solvent front increases with increasing temperature, but beyond 40° falls rapidly as the temperature increases. Beyond 40° , the observed behavior is connected with the depolymerization of alginic acid which commences at this temperature and is more consistent at higher temperatures. In Table II the R_F values of some elements are reported for various temperatures. Up to 40° the average increase per degree, except for Pb(II), is about $4 \cdot 10^{-3} \text{ cm}/^\circ\text{C}$; for higher temperatures, owing to the depolymerization of alginic acid, anomalous values are observed.

TABLE II

EFFECT OF TEMPERATURE ON R_F VALUEAlginic acid thin layer, thickness 250μ . Eluent: $0.05 M$ HCl.

Ions	5°	20°	26°	35°	52°	70°
Pb(II)	0.00	0.00	0.00	0.02 ± 0.02	0.04 ± 0.04	0.15 ± 0.04
Tl(I)	0.14 ± 0.04	0.20 ± 0.05	0.23 ± 0.05	0.27 ± 0.06	0.46 ± 0.06	0.95 ± 0.05
Cu(II)	0.16 ± 0.03	0.21 ± 0.04	0.23 ± 0.04	0.26 ± 0.05	0.41 ± 0.05	0.85 ± 0.05
Hg(II)	0.75 ± 0.05	0.82 ± 0.06	0.90 ± 0.06	0.94 ± 0.06	0.95 ± 0.05	0.95 ± 0.05
As(III)	0.52 ± 0.04	0.58 ± 0.04	0.63 ± 0.04	0.68 ± 0.05	0.90 ± 0.04	0.96 ± 0.04
Ba(II)	0.02 ± 0.02	0.05 ± 0.04	0.09 ± 0.04	0.12 ± 0.04	0.25 ± 0.05	0.66 ± 0.05

Influence of the acid concentration

The concentration and nature of the mineral acids employed does not appreciably influence the distance moved by the solvent front. On varying the hydrogen ion concentration in the range 10^{-7} – $4 M$, no notable variation in the movement of the different mineral acids, for a fixed time, is observed. The movement of the solvent front does not depend upon the acid concentration and this fact clearly shows the considerable resistance of alginic acid to hydrolysis.

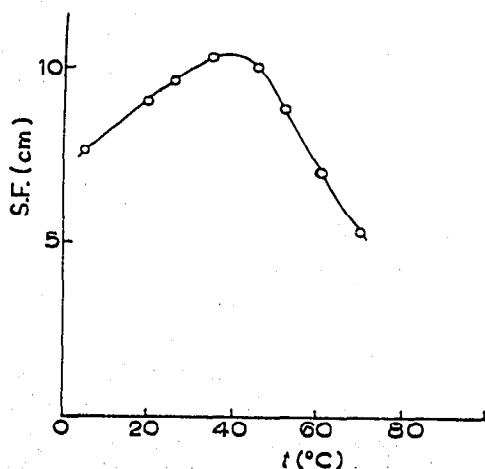


Fig. 4. Temperature dependence of movement of the solvent front (S.F.).

Comparison between alginic acid and carboxymethylcellulose

The chromatographic properties of alginic acid have been compared with those of carboxymethylcellulose and its sodium salt because among the substances employed in TLC, carboxymethylcellulose is the one that is most structurally similar to alginic acid. Thin layers of the same thickness are compared and in Table III R_F values of several ions on alginic acid and carboxymethylcellulose thin layers are reported; HCl at different concentrations was the eluent. In the case of the sodium salt of carboxymethylcellulose, the R_F values are different from those reported in Table III but the ions are all retained in an identical manner and remain at the origin, excepting Tl(I), with 0.01 *M* HCl as eluent.

TABLE III

 R_F VALUES ON ALGINIC (AA) AND CARBOXYMETHYLCELLULOSE (CM) THIN LAYERS

Ions	0.01 <i>M</i> HCl		0.1 <i>M</i> HCl		1 <i>M</i> HCl	
	AA	CM	AA	CM	AA	CM
Pb(II)	0.00	0.23 ± 0.04	0.03 ± 0.03	0.92 ± 0.05	0.37 ± 0.03	0.96 ± 0.04
Tl(I)	0.08 ± 0.03	0.70 ± 0.09	(—)	0.80 ± 0.08	0.00	0.00
Bi(III)	0.00	0.00	0.03 ± 0.03	0.50 ± 0.09	0.94 ± 0.06	0.97 ± 0.03
Cu(II)	0.02 ± 0.02	0.46 ± 0.09	0.34 ± 0.04	0.93 ± 0.03	0.95 ± 0.05	0.97 ± 0.03
Fe(III)	0.04 ± 0.04	(—) ^a	0.12 ± 0.09	0.94 ± 0.03	0.93 ± 0.07	0.97 ± 0.03
Ni(II)	0.05 ± 0.04	0.50 ± 0.04	0.60 ± 0.05	0.96 ± 0.03	0.95 ± 0.05	0.97 ± 0.03
Ba(II)	0.00	0.49 ± 0.06	0.09 ± 0.04	0.92 ± 0.05	0.30 ± 0.04	0.96 ± 0.04

^a (—) = diffuse.

Because the formation of an acid front, different from that of the solvent, has been observed in paper chromatography^{12,13}, the composition of the solvent front along the thin layers has been examined in order to correlate it with the previous results.

Preliminary tests with universal pH indicator solutions sprayed, after development, on to the carboxymethylcellulose (sodium salt) thin layers, led us to identify two different fronts, the nearer one being due to the hydrogen ions. The R_F value of the acid front is identical with R_F values of the ions examined, except for a few cases, and substantiates the results obtained with the sodium salt of carboxymethylcellulose. With alginic acid and carboxymethylcellulose these tests are not conclusive owing to the intrinsic acidity of the thin layers.

However an apparent measure of pH was made as follows:

After development, the thin layers were divided in strips 2 cm by height; the material of each strip was dispersed in 3 ml of distilled water and the pH of such dispersions measured with a glass electrode. The results for alginic acid are reported in Fig. 5. The consistency of such data is clearly shown by $\Delta\text{pH} = 1$ being observed between two adjacent curves in accordance with a change of ten times in the acid concentration of the eluent. From these data it is clearly shown that on alginic acid thin layers the hydrogen ion concentration of the solvent front changes only slightly compared with sodium carboxymethylcellulose in which two distinct fronts have been observed by pH measurement.

The behavior of carboxymethylcellulose is similar to that of alginic acid al-

though the changes of hydrogen ion concentration of the solvent front are greater with carboxymethylcellulose, particularly with an 0.01 *M* acid concentration in the eluent. Furthermore curve (d) of Fig. 5 shows that, with H₂O as eluent, the thin layer pH is near to that observed with an 0.01 *M* acid concentration in the eluent. The sodium carboxymethylcellulose employed was batch No. 132 commercially available from Carl Schleicher und Schüll.

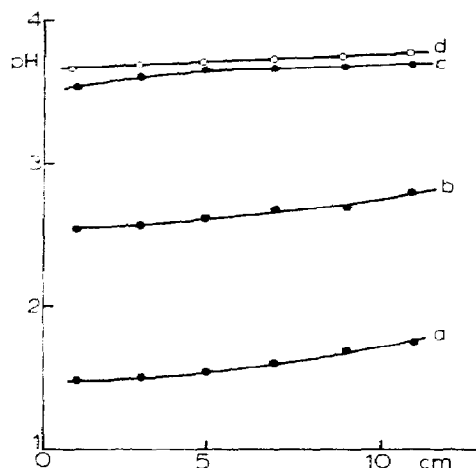


Fig. 5. pH values relative to the center of the strips (see text) for HNO₃ concentration in the eluent: (a) 1 *M*; (b) 0.1 *M*; (c) 0.01 *M*; curve (d) is relative to water as eluent.

Potentialities of alginic acid thin layers

The *R_F* values of some metal ions are reported in Tables IV and V. They have been obtained employing water and acetic, perchloric and hydrochloric acids of varying concentrations as developing solvents. A typical chromatogram is shown in

TABLE IV

R_F VALUES OF COMMON ELEMENTS ON ALGINIC ACID THIN LAYERS

Ions	Amount (μg)	Eluents		Detection reagents ^a
		H ₂ O	0.5 <i>M</i> CH ₃ COOH	
Pb(II)	5	0.00	0.00	1
Tl(I)	10	0.00	0.00	1
Hg(II)	5	0.03 : 0.03	0.06 : 0.06	4
Cu(II)	5	0.00	0.00	1
Bi(III)	3	0.00	0.00	1
As(III)	15	0.53 : 0.03	0.54 : 0.05	1
Fe(III)	1.5	0.03 : 0.03	0.04 : 0.04	2
Al(III)	0.5	0.02 : 0.02	0.04 : 0.04	2
Ni(II)	1.5	0.00	0.00	1
Zn(II)	2.3	0.00	0.00	2
Ba(II)	10	0.00	0.00	3
Mg(II)	1	0.03 : 0.03	0.05 : 0.05	2

^aThe numbers refer to the detecting reagents as specified in the text.

TABLE V

 R_F VALUES OF COMMON ELEMENTS ON ALGINIC ACID THIN LAYERS

Ions ^a	HCl (moles/l)			HClO ₄ (moles/l)		
	0.01	0.05	0.5	0.01	0.05	0.5
Pb(II)	0.00	0.00	0.22 ± 0.02	0.00	0.00	0.09 ± 0.03
Tl(II)	0.08 ± 0.03	0.20 ± 0.06	(—)	0.06 ± 0.03	0.12 ± 0.03	0.27 ± 0.03
Hg(II)	0.78 ± 0.06	0.83 ± 0.08	0.96 ± 0.04	0.66 ± 0.06	0.63 ± 0.06	0.78 ± 0.06
Cu(II)	0.02 ± 0.02	0.19 ± 0.04	0.95 ± 0.05	0.00	0.16 ± 0.04	0.94 ± 0.06
Bi(III)	0.00	0.03 ± 0.03	0.53 ± 0.05	0.00	0.00	0.00
As(III)	0.54 ± 0.03	0.56 ± 0.04	0.56 ± 0.04	0.47 ± 0.03	0.50 ± 0.03	0.50 ± 0.03
Fe(III)	0.04 ± 0.04	0.05 ± 0.05	0.91 ± 0.09	0.05 ± 0.05	0.08 ± 0.08	(—)
Al(III)	0.04 ± 0.04	(—) ^b	0.92 ± 0.08	0.05 ± 0.05	(—)	0.93 ± 0.07
Ni(II)	0.05 ± 0.04	0.33 ± 0.03	0.94 ± 0.06	0.03 ± 0.03	0.30 ± 0.04	0.93 ± 0.07
Zn(II)	0.03 ± 0.03	0.30 ± 0.06	0.93 ± 0.07	0.03 ± 0.03	0.30 ± 0.05	0.94 ± 0.06
Ba(II)	0.00	0.05 ± 0.03	0.26 ± 0.05	0.00	0.05 ± 0.03	0.25 ± 0.04
Mg(II)	0.09 ± 0.09	0.43 ± 0.05	0.96 ± 0.04	0.09 ± 0.09	0.42 ± 0.05	0.96 ± 0.04

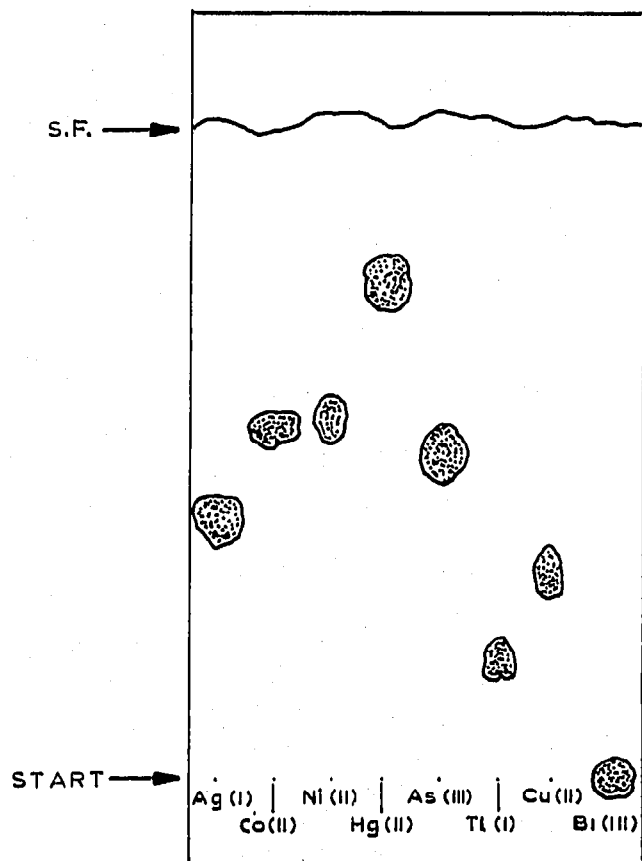
^a Detecting reagents and ion amounts are the same as in Table IV.^b (—) = diffuse.Fig. 6. Typical chromatogram on alginic acid thin layer. Eluent, HClO₄, 0.1 M; development time approx. 45 min.

Fig. 6. The reported data show the possibilities of alginic acid for TLC separations of elements.

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