Carboxymethylcellulose: a binder for thin-layer chromatography of lipids and indoles*

We have found carboxylmethylcellulose (CMC) to be an effective binder with silicic acid for thin-layer chromatography (TLC) of fatty acid derivatives and indoles.

Cellulose and its derivatives have been used as adsorbents in TLC but to our knowledge they have not been recommended as binders for silicic acid. For instance, RANDERATH[1-3] studied nucleic acid derivatives on pure cellulose powder with and without plaster of Paris as a binder. TEICHERT *et al.*[4] separated alkaloids using pure cellulose impregnated with formamide and WOLLENWEBER[5,6] has used cellulose powder with plaster of Paris for chromatography of synthetic food coloring matter and amino acids. Brinkmann Instruments, Inc. distributes carboxymethylcellulose and other cellulose derivatives with and without a $CaSO_4$ binder.

The converse utility of cellulose derivatives as binders themselves appears to have escaped notice.

Materials and methods

The adsorbent is prepared as follows: silicic acid (Mallinckrodt 100 mesh No. 2847) is slurried with 3 *N* HCl for approximately 10 min, rinsed with distilled water until the slurry reaches pH 4.5 then rinsed with acetone and dried overnight at 105°. The silicic acid is sieved and that grade passing through a No. 325 mesh (44 μ) screen is used for the adsorbent. 28.5 g washed silicic acid is mixed with 1.5 g carboxymethylcellulose (No. 70 Premium, Low Viscosity grade) in 60 ml warm distilled water. This will make eight 200 × 200 mm plates with the apparatus for application purchased from C. Desaga, Heidelberg, Germany. The plates are air dried overnight then activated before use at 100° for 30 min.

Silica Gel G** was prepared by adding 30 g of dry gel to 80 ml distilled water.

All thin-layer chromatograms were run in tanks at a uniform temperature of 26°.

Results

Fig. 1 illustrates the chromatographic qualities of Silica Gel G and silicic acid–CMC. Both develop adequate separations of the mono-, di- and triglycerides, methyl esters and alcohols. However, even with larger quantities, the hydrocarbon n-octadecane is not observed on plates prepared with Silica Gel G and it takes twice as long to get similar results with the Silica Gel G.

The neutral and acidic indoles ran similarly on both adsorbents in the modified aqueous system, isopropanol–ammonia–water (100:10:5)[7]. Fig. 2 indicates the differences when they are run in 2-butanone–hexane (18:82)[8]. It is interesting to note here, that indole-3-acetic, indole-3-pyruvic, indole-3-butyric and indole-3-propionic acid begin to separate on the CMC plate but remain at the origin on the Silica Gel G plate.

When the acidic indoles are run under the same conditions in 2-butanone–hexane (25:75) they move from the origin with different R_F values yet still remain at the origin with Silica Gel G.

Silica Gel G was not an adequate adsorbent when using KCl (20 % w/v in water)[9]

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** Brinkmann Instruments Inc., Great Neck, L.I., N.Y.

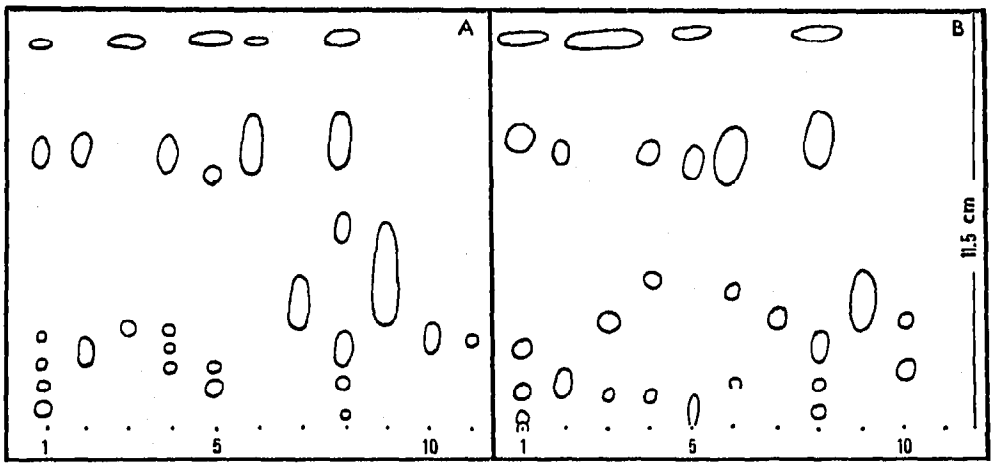
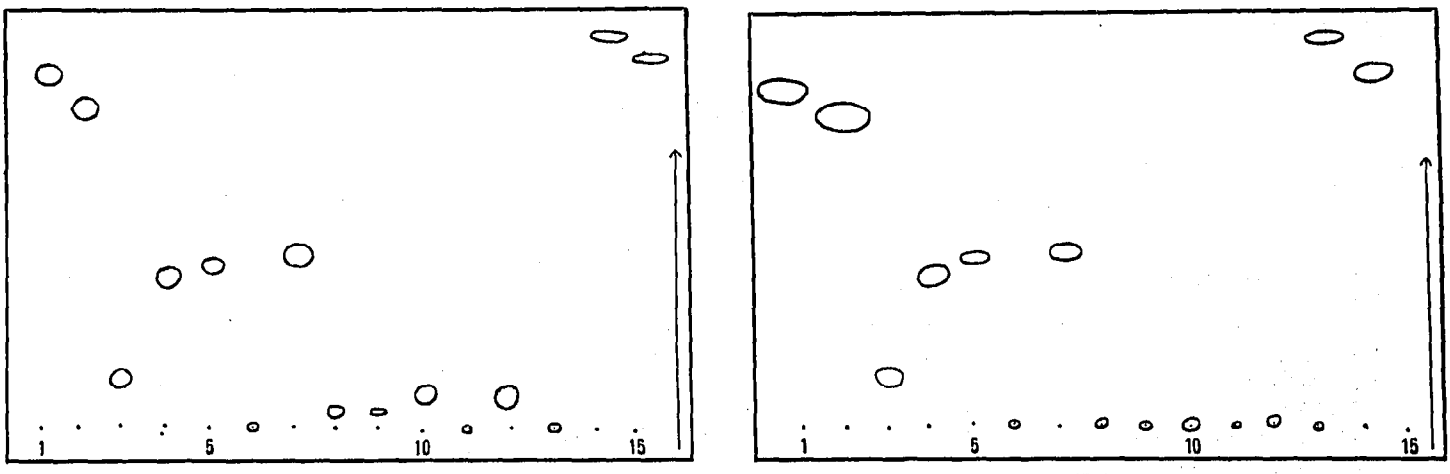


Fig. 1. Ascending chromatography of commercial grades of fatty acid derivatives. A. Silicic acid–CMC. B. Silica Gel G. (1) Methyl palmitate; (2) Palmitic diglycerides; (3) Methyl palmitoleate; (4) Tripalmitin; (5) Methyl stearate; (6) Tristearin; (7) Stearyl alcohol; (8) Triolein; (9) Oleyl alcohol; (10) Cholesterol; (11) *n*-Octadecane; Solvent: pet. ether–ethyl ether–acetic acid (98:10:1). Time: A, 30 min; B, 60 min. Chromogenic reagent: 2′,7′-Dichlorofluorescein.

as a solvent because the layer blistered. Fig. 3 indicates the performance of silicic acid–CMC in this solvent which took 15 min to run as opposed to 45 min with Silica Gel G. The results of this separation indicate that this may be a useful solvent system for two-dimensional thin-layer chromatography of indoles.

Discussion

Carboxymethylcellulose, 70 Premium Low Viscosity, was chosen for its ready solubility in water, physiological inertness and binding effectiveness. A range of other grades are available[10] and advantage might be taken of their properties for use with other adsorbents and separations.



(A) (B)

Fig. 2. Ascending chromatography on silicic acid–CMC. A. Silicic acid–CMC. B. Silica Gel G. (1) Skatole; (2) Indole; (3) Tryptophol; (4) Indole-3-acetonitrile; (5) Indole-3-carbinol; (6) Indole-3-acetamide; (7) Ethyl indole-3-acetate; (8) Indole-3-acetic acid; (9) Indole-3-pyruvic acid; (10) Indole-3-butyric acid; (11) Indole-3-lactic acid; (12) Indole-3-propionic acid; (13) Indole glycolic acid; (14) 1,2-Dimethylindole; (15) 2,3-Dimethylindole. Solvent: 2-butanone–hexane (18:82). Time: A, 30 min; B, 45 min. Chromogenic reagent: Ehrlich's reagent.

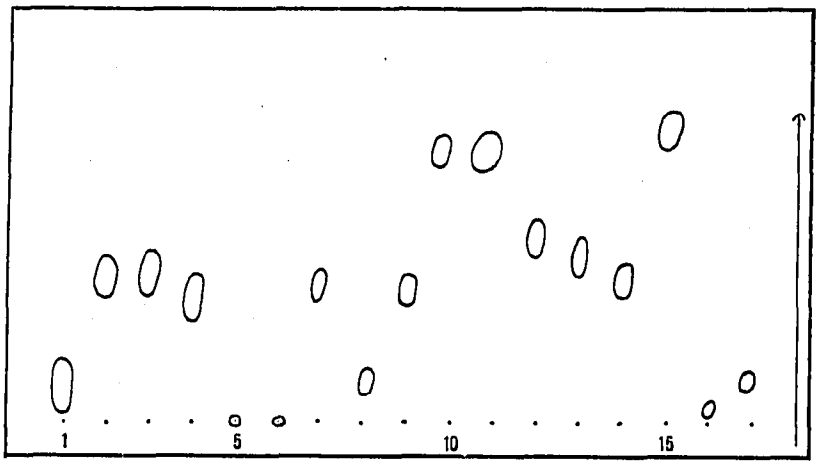


Fig. 3. Ascending chromatography of indoles on silicic acid–CMC. (1) Skatole; (2) Indole; (3) Tryptophol; (4) Indole-3-acetonitrile; (5) 2,3-Dimethylindole; (6) 1,2-Dimethylindole; (7) Indole-3-acetamide; (8) Ethyl indole-3-acetate; (9) Indole-3-acetic acid; (10) Indole-3-pyruvic acid; (11) Tryptamine-HCl; (12) Serotonin (5-Hydroxytryptamine); (13) 5-Methoxytryptamine; (14) 5-Methyltryptamine; (15) 5-Hydroxytryptophan; (16) N,N-Diethyltryptamine; (17) N,N-Dimethyltryptamine. Solvent: KCl 20% w/v in water. Time: 15 min. Chromogenic reagent: Ehrlich's reagent.

We have found that in all cases the running time for silicic acid–CMC is about half that for Silica Gel G. The resolution with some solvent systems is better with this adsorbent than with Silica Gel G. The binding properties are such that the very smooth, thin layer strongly adheres to the plate, is not the least bit powdery, and may be written on with pencil without breaking the layer.

It is not possible to use concentrated H_2SO_4 as a chromogenic reagent for fatty acid analysis with CMC, but the following reagents do give distinctive color reactions for the lipids: 2,7 dichlorofluorescein[11], α-cyclodextrin[11], Rhodamine 6G[12] and iodine vapour[11]. Ehrlich's reagent[7] is effective for the indoles.

Acknowledgements

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