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A FEW EXPERIMENTS WITH THIN-LAYER CHROMATOGRAPHY ON CHARCOAL

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SUMMARY

Plates for thin-layer chromatography on charcoal were prepared using polyethylene as binder. The problem of detection was overcome in several ways and the adsorptive powers of silica gel and charcoal layers were compared using two sets of substances: dyes and amino acids. The charcoal employed was a stronger adsorbent than silica gel. The R_F data of the substances mentioned, in several solvents, are represented as chromatographic profiles.

INTRODUCTION

Although charcoal has been used for a very long time as a powerful adsorbent in organic chemistry, its use for chromatographic purposes is very scarce. To our knowledge only very few papers have been published dealing with TLC on charcoal[1,2].

The main difficulty in using charcoal for TLC lies in its colour, *i.e.* blackness, which prevents one from detecting spots on it in the usual manner. Even fluorescent substances remain invisible on charcoal under UV light. We tried to overcome this difficulty in several ways. The first consisted of pouring a suspension of powdered cellulose or silica gel in methanol over the charcoal thin-layer plate. During the drying of the suspension a part of the material from the spots diffused by capillary action into the upper white layer, and the spots, if coloured or fluorescent, became visible. Detection reagents could also be applied. The second method consisted of applying a piece of triple gauze (the size of the plate) wetted in methanol over the surface of the black layer. Immediately afterwards a strip of filter paper was applied over the gauze. The dry paper absorbed a part of the methanol from the charcoal layer, wetted with methanol from the triple gauze. In such a manner a part of the material from the spot passed into the paper, and the spots could be made visible. The third method consisted of applying and pressing a strip of filter paper evenly over the surface of the still wet chromatogram immediately after interruption of chromatography. As a fourth alternative, we also tried to transfer the spots from the charcoal layer onto a strip of paper or a strip-like layer of silica gel by transversal capillary ascent, *i.e.* we immersed the longitudinal edge of the plate in methanol and allowed it to diffuse into the white material (carrying with it the spots). However, this method is applicable only for chromatograms with only one point of application.

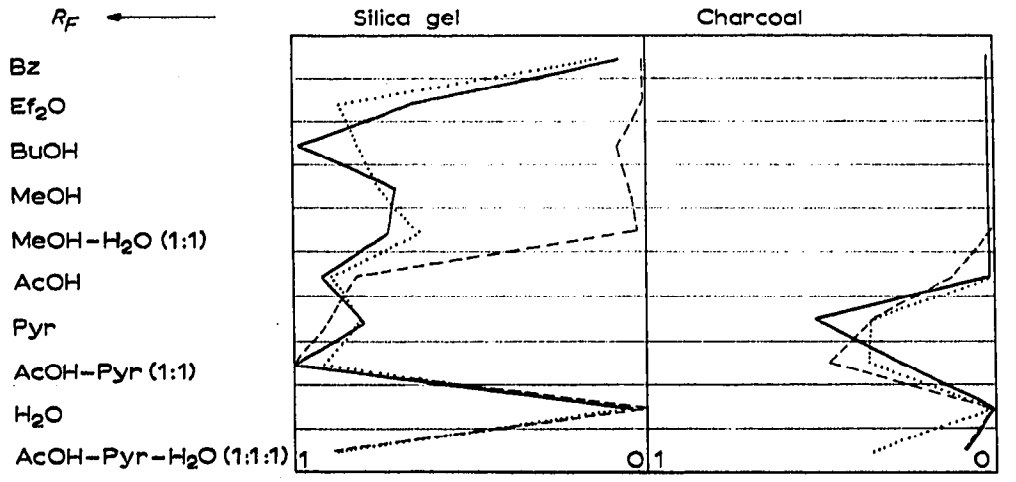

Fig. 1. Chromatographic profiles (spectra) of fluorescein (———), eosine (— — —), and 2,6-dichlorophenylindophenol (· · · · · ·) in ten solvents on silica gel and charcoal containing polyethylene as binder. Bz = benzene, Pyr = pyridine; the other abbreviations are those used in *Chemical Abstracts*.

It is evident that all these difficulties with detection can be easily overcome by working with radioactive substances. In that case, the plates can be simply scanned using some commercial apparatus.

After having overcome this first difficulty, *i.e.* detection, we were interested to see how large the difference in adsorptivity was between charcoal and silica gel. Firstly, we chose two sets of substances: three dyes as representatives of aromatic, rather lipophilic substances, and three amino acids as representatives of very hydrophilic or aliphatic compounds. We chromatographed these substances in a number of simple solvents chosen just for the sake of this preliminary investigation. The measured R_F values were plotted in diagrams commonly called "chromatographic spectra", or more accurately "chromatographic profiles". In Figs. 1 and 2, the diagrams

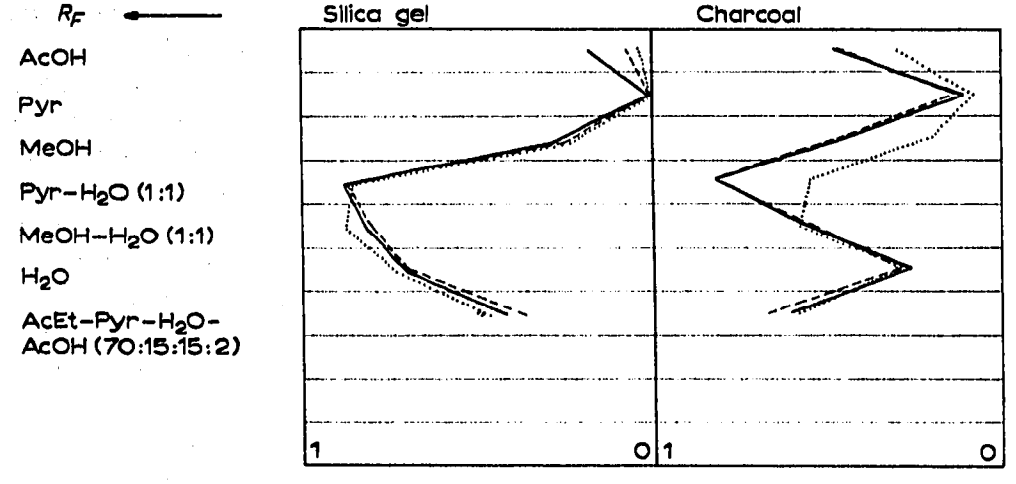

Fig. 2. Chromatographic profiles (spectra) of leucine (———), isoleucine (— — —), and tyrosine (· · · · · ·) in seven solvents on silica gel and charcoal containing polyethylene as binder. AcEt = ethyl acetate; for other abbreviations, see the legend to Fig. 1.

clearly show that charcoal is a more powerful adsorbent than silica gel and that the "profiles" on both adsorbents differ sufficiently to justify a further study of TLC on charcoal layers or layers containing charcoal.

EXPERIMENTAL

Materials

Silica gel (according to Pitra), 5–40 μ particle diameter; Charcoal (Karborafin—akt. uhlí), purchased from Chema, Horní Počernice; Polyethylene (unpolymerized), 3–50 μ particle size, from Plast-Labor S.A., Switzerland. The solvents were of pure or analytical grade.

Procedure

The adsorbent (20 g) and the binder (polyethylene, 2 g) were suspended in 100–150 ml of methylene chloride, shaken thoroughly for 1–2 min and poured onto a thin glass plate (5 × 20 cm). The plate with the suspension was moved (rocked) in a horizontal position to make the layer even and then allowed to dry for 20 min. The dry plate was put into an oven and heated for 10 min at 140°. During the heating the polyethylene polymerised and became insoluble in organic solvents, while at the same time fixing the adsorbent particles to the glass plate and to each other. Application of the substances and chromatographic development were carried out in the usual manner. For the detection see above.

REFERENCES

1 T. F. BRODASKY, *Anal. Chem.*, 35 (1963) 343.
2 R. PERRON AND C. MADELMONT, *Bull. Soc. Chim. France*, (1967) 3443.

DISCUSSION

HAIS: A great deal of work has been done on charcoal column chromatography by CLAESON and the Swedish school in general. The curved isotherm of untreated charcoal, which has been attributed to adsorption sites widely differing in affinity, seems to have been the main obstacle (part of the solutes was irreversibly bound). This has been overcome, to a large extent, by saturating the strongest adsorption sites, *e.g.* by stearic acid. I wonder whether such phenomena would also affect TLC or whether the adsorption isotherm is linear, at least, in its initial portion corresponding to the low concentrations of solutes usually applied on TLC layers. I would rather think that the isotherm on untreated charcoal is curved just at its beginning.

TURINA: The chromatographic process on a thin layer of charcoal is different from the process in charcoal columns because the ratio of liquids and solids in TLC is smaller than in column chromatography. For this reason, the classical isotherms of adsorption are not valid in the case of TLC with charcoal as the adsorbent, and we must find a new model for the chromatographic process on it.

HAIS: Charcoal is an interesting adsorbent. I wonder whether you tried non-polar solvents (aliphatic hydrocarbons) for non-aromatic substances of intermediate polarity. One would expect, in such a case, that polar functional groups would lower

the R_F value. In aqueous solvents, charcoal would act as a non-polar adsorbent and a reverse sequence would be expected. I remember such a change of sequence in the case of pregnene derivatives which we tried to resolve on charcoal columns. The separation was not very sharp.

Only two papers dealing with TLC on charcoal were cross-referenced in our bibliography 1960–1965, so I can confirm PROCHÁZKA's statement that this has not been a popular adsorbent.

PROCHÁZKA: Yes, we tried also non-polar solvents such as hexane or benzene, but not yet with non-aromatic substances of intermediate polarity. Only with the three dyes mentioned. I did not mention it, but we intend to use radioactive compounds and thus overcome the difficulties with the detection.

JÄNCHEN: I would like to add a few hints concerning previous work on TLC using charcoal which might have escaped attention:

BRODASKI at the Upjohn Company in Kalamazoo separated antibiotics on strongly bound charcoal layers. Detection was performed by bioautography (1962). ALEXANDER, coworker of HESSE, reported (in a printers' journal, I believe) on TLC on dark layers, *e.g.* Fe_2O_3 or charcoal. Detection was made by spraying the layer with silica gel containing an elution solvent. KIRCHNER reported at the ACS meeting in 1964 on TLC on side by side layers of charcoal and silica gel on the same plate. Development was done two-dimensionally, on charcoal in the first dimension, then development into the silica area.

DEUTSCHER: Wenn ein Tropfen einer Lösung von Fluorescein auf einer Glasplatte eintrocknet, so kann der Farbstoff durch UV-Licht nicht zur Fluorescenz angeregt werden. Durch Besprühen mit Glycerin geht der Farbstoff in Lösung ohne seine Form auf der Unterlage zu verändern, und fluoresziert im UV-Licht. Diese Möglichkeit der Sichtbarmachung gelingt auch auf den manchmal etwas dunklen, z.B. durch anodische Oxidation in Oxalsäure–Chromsäure hergestellten Aluminium-Oberflächen. Für die Sichtbarmachung fluoreszierender Substanzen auf Platin-Schwarz ist diese Methode nicht geeignet. Ist diese Methode auch für Kohleschichten anwendbar?

PROCHÁZKA: Ich danke für den Hinweis. Das Anfeuchten mit Glycerin haben wir nicht probiert, aber wir werden es tun. Ich habe aber das Gefühl, es wird nicht gelingen.

IRVINE: I was very interested in the paper by PROCHÁZKA, since our group has done some (unpublished) work with charcoal TLC and chromatography on charcoal-loaded paper. We too have been unable to detect compounds on charcoal by fluorescence, but have had some success locating the compounds by transferring them (at right angles) onto a paper strip and then applying conventional UV or chromogenic spray techniques.

In view of suggestions in the literature that charcoals differ in their content of polar and non-polar sites, and further to Dr. HAIS' comments, I would like to ask whether you have had the opportunity to compare different kinds of charcoal in your TLC system?

PROCHÁZKA: We too tried to transfer the solutes transversally from the charcoal chromatogram to some white material (paper, silica gel layer). This method was successful in principle but not very convenient.

As regards various sorts of charcoals, we have no results as yet. One of the aims

of our work, however, is to elaborate a suitable method for the study of adsorptive properties of charcoals from various sources.

DEYL: Why did you use predominantly polar solvents and only pure solvents or simple mixtures? Why didn't you try the usual mixtures for the separation of amino acids where you would probably obtain better separation?

PROCHÁZKA: As amino acids are very hydrophilic, I had to start the investigation with more polar solvents. I did not investigate the common mixtures suitable for a good separation of amino acids because I was not interested at this point in the separation of amino acids but in the study of the properties of charcoal layers. Therefore I also used pure solvents or simple mixtures.

DEYL: Did you try chromatography of proteins, nucleic acids or other macromolecules?

PROCHÁZKA: As regards the chromatography of higher-molecular-weight compounds, I did not study them because I did not yet have enough time to start their investigation. But we intend to study different classes of substances, different modes of detection and different types of charcoals as well.