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Note

Use of reversed-phase thin-layer chromatography for the identification of black inks from board felt markers and ball-point pens

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The general availability of a wide range of chemically bonded silica particles has given rise to a fast developing branch of chromatography, reversed-phase chromatography. The difficult problem of binding together these particles on a glass plate has recently been overcome by manufacturers and a technique for preparing home-made plates is available[1]. It is therefore now possible to perform thin-layer chromatographic (TLC) separations of very polar compounds easily and rapidly. The plates can be re-used after suitable washing[1].

The main advantages of this hydrophobic material are the virtual absence of interferences from vapours adsorbed from ambient air, the good reproducibility of the retention data, the absence of spot tailing in almost all instances, the ease of adjusting the R_F value in the practical range of 0.2–0.8 by adjusting the composition of simple mixtures of water and an organic solvent and the good stability in the pH range 2–9.

The main drawback is the low energy of the surface, which makes it poorly wet by water-rich solvents. Development is very slow with mixtures that contain more than 40% water[1,2].

In spite of its attractive features, reversed-phase TLC is still little used. We report here an analysis developed for a forensic laboratory and used by us as a demonstration of the principle and separation power of chromatography for chemistry students, namely the separation of the constituents of black inks. Whereas conventional china ink is made with carbon particles[3], many black inks, especially for board markers or ball-point pens, consist of mixtures of dyestuffs. TLC separation makes the identification of the ink used easy as the natures of the individual dyes can be established using a spectrophotodensitometer.

TLC separation of synthetic dyes has been performed on various systems such as Kieselguhr[4], cellulose[5] and silica coated with *p*-methylbenzenesulfonate[6]. Analyses of ball-point pen inks by paper chromatography have been reported[7,8] and a high-performance liquid chromatographic (HPLC) separation has been achieved by Colwell and Karger[9] on silica using 2% formamide in methanol as the mobile phase.

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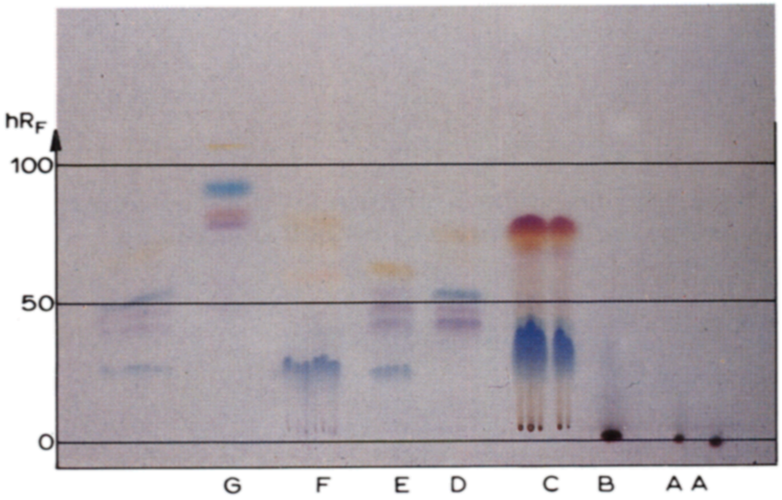


Fig. 1

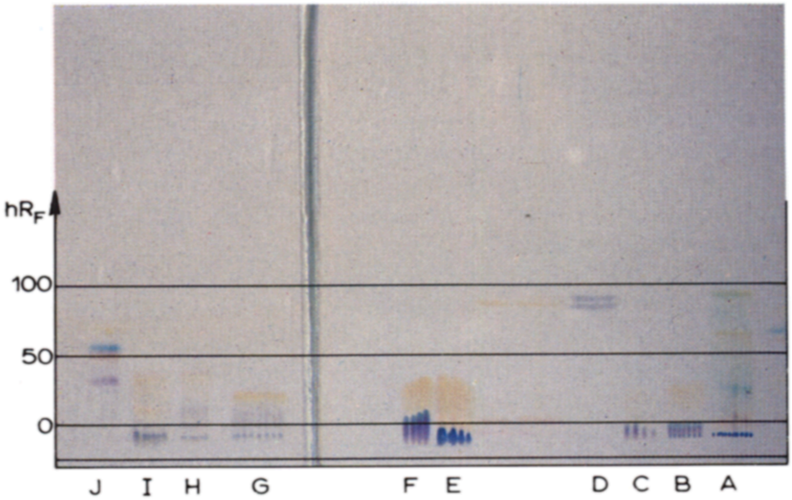


Fig. 2

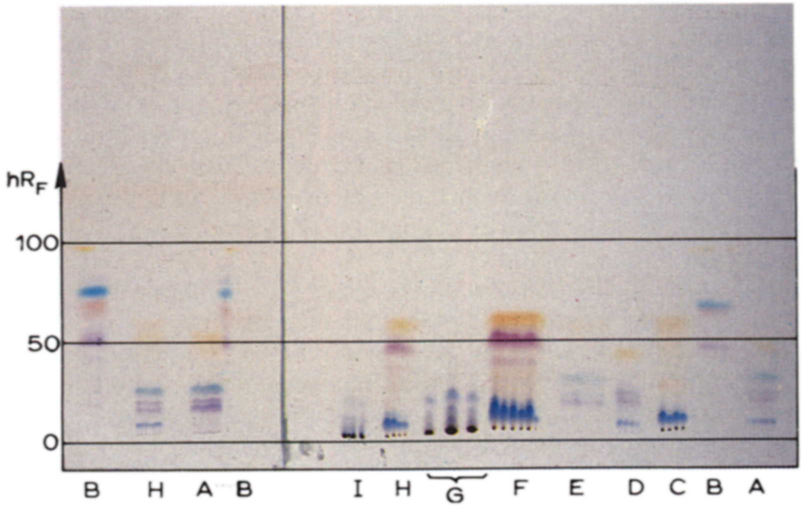


Fig. 3

To our knowledge there has been no report of the separation of these dye mixtures on TLC plates coated with alkyl-grafted silica, although dye mixtures have been separated successfully by reversed-phase TLC on paraffin-impregnated cellulose, silica gel and cellulose[10].

EXPERIMENTAL

TLC plates prepared with silica particles chemically bonded to ethyl, *n*-octyl or *n*-octadecyl groups were obtained from Merck (Darmstadt, G.F.R.) under the trade names RP-2, RP-8 and RP-18, respectively. The average particle size was 7 μm (ref. 2). Developments were carried out in a Camag (Muttenz, Switzerland) N-chamber. Because of the relatively low volatility of the solvents used, there was no need for a sandwich system.

The solvents were of spectrograde quality and were used without further purification. The developing solvent was prepared by careful mixing of 3.5 cm^3 of a phosphate buffer solution and 6.5 cm^3 of ethanol. The buffer solution (pH 2.8) was prepared from 0.1 *M* sodium dihydrogen orthophosphate solution, the pH being adjusted by adding orthophosphoric acid.

Samples were deposited 1 cm from the edge of the plate and the plate was immersed 0.5 cm in the developing solvent. Development was carried out to a distance of 4 cm, which usually took about 1 h.

Densitograms were obtained using a TRD 2 spectrophotodensitometer (Vernon, Paris, France) equipped with a tungsten lamp. No filter was used.

RESULTS AND DISCUSSION

An aqueous solution of the sample cannot be used, as it would not wet the sorbent layer. Two application techniques were used. With small-tipped markers and pens, simple pressure of the felt tip or pen on the layer yielded a convenient spot of diameter 0.2–0.5 mm. With large-size board markers or pens with cartridges, one drop of ink was mixed with a few drops of methanol and the sample was applied on the layer using a glass capillary.

We found that the use of plates with a concentration zone is inadequate. As the solvent migrates through the inactive zone, pushing forward the sample to the chromatographic bed, some separation takes place and strong tailing of some compounds occurs. Not all sample components reach the small gap at the same time and the separation is poor, with broad and tailing spots. To our knowledge, this is the first reported failure of the concentration zone system.

Fig. 1. Photograph of a developed TLC plate (Merck RP-18). Conditions as described in text. Samples: A, china ink; B, Waterman black; C, Veleda (felt); D, Somarco (felt); E. Somarco (ball point); F, Bic (ball point); G, Reynolds (felt).

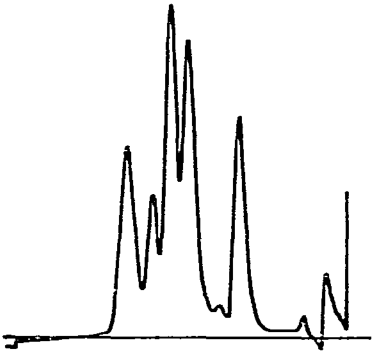
Fig. 2. As Fig. 1 for A, Pentel (felt); B, unknown ink from an Omniscribe recorder; C, Immont blue; D. ink from a Vernon recorder; E, Bic (blue ball point); F, Waterman cartridges (for fountain pen); G, Somarco (ball point); H, Stylist (felt); I, unknown; J, Reynolds (felt).

Fig. 3. As Fig. 1 for black felt pens: A, Somarco; B, Reynolds; C, Staedtler; D, Somarco; E, Bic; F. Plan Master; G, Onyx; H, Stabilo; I, black ink from Waterman.

As the black inks studied are unknown mixtures of dyestuffs, the best chromatographic system is the one which gives the largest number of spots. The best results were obtained with RP-18 plates. The pH of the solvent has a critical effect on the number of spots and their resolution. A strongly acidic buffer was found to be necessary. Alcohols gave better results than acetonitrile; ethanol was preferred to methanol because of its lower volatility.

Figs. 1–3 show some of the results. Obviously black china ink exhibits one spot only, with infinite retention. The black inks from different manufacturers exhibit a number of different coloured components, with a wide range of different dyes and concentrations. Some of these dyes are strongly retained, others weakly.

Densitometry of the developed plates also provided valuable information, and was much more accurate than visual examination of the plate. Figs. 4 and 5 show such densitograms for two different ink samples. The relative percentages of each dye permit the derivation of a "fingerprint" for the various inks studied. The identification and authentication of small ink samples was possible. Positive authentication could be achieved, if needed, by running a UV and visible spectrum of each dye component of the mixture. The amount of information supplied by the TLC densitogram is close to 100 bits with the system used, which is already large compared with the number of brands of black ink available[11].



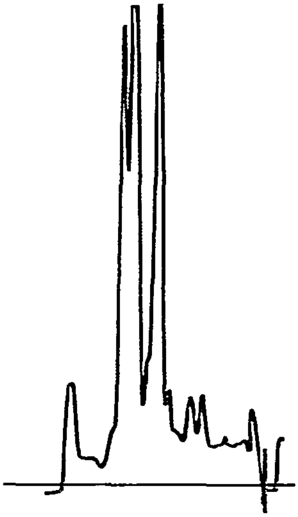


Fig. 4. Photodensitogram of sample E in Fig. 1 (Somarco ball point).

Fig. 5. Photodensitogram of sample B in Fig. 3 (Reynolds black felt).

It is easy to run such separations for a class of up to about 20 students. Each student can put a spot with a pen on a TLC plate and a clear chromatogram is obtained in 15 min, exhibiting arrays of brightly coloured spots which illustrate dramatically the resolving power of chromatography, its fields of application, its principle and its etymology. The sharpness of resolution also permits some further work in the laboratory on comparison between the performances of various plates, the influence of development length, etc. TLC is much quicker and simpler than HPLC as a preliminary introduction or for limited laboratory work and it is much cheaper;

many runs can be made simultaneously, clean-up is not necessary and plates can be re-used a number of times.

A comparison of Figs. 1 and 4 and Figs. 3 and 5 offers some insight into what actually takes place in LC columns. Figs. 4 and 5 show excellent chromatograms, with almost symmetrical peaks and no tailing, and would be considered acceptable by all chromatographers. Nevertheless, as shown by the photographs in Figs. 1 and 3, there is definitively some tailing, although very slight. The dye density behind the spots is very small and the effect is markedly enhanced by the non-linear response of the eye, a phenomenon too often overlooked[12]. This shows that a very small amount of dye, probably of any other polar compound chromatographed, remains sorbed for a long period on alkyl-bonded silica phases. We have not investigated this phenomenon in detail but have shown that the amount of dye sorbed is too small to be measured; it does not affect the chromatographic properties of the phase. It is possible that this sorption takes place mainly on the external surface of the particles, where the organic layer bonded to the silica surface is prone to be worn by friction between particles. This would explain our observations, but this assumption has not been tested.

REFERENCES

1 A. M. Siouffi, T. Wawrzynowicz, F. Bressolle and G. Guiochon, *J. Chromatogr.*, 186 (1979) 563.
2 A. M. Siouffi, G. Korosi and G. Guiochon, *J. Chromatogr. Sci.*, 18 (1980) 324.
3 *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 11, Interscience, New York, 2nd ed., 1966, p. 611.
4 W. Reiners, *Z. Anal. Chem.*, 216 (1966) 272.
5 P. Wollenweber, *J. Chromatogr.*, 7 (1962) 557.
6 L. van Dessel and P. van Regenmortel, *Z. Anal. Chem.*, 247 (1969) 280.
7 M. Swarup and S. S. Tripathi, *Int. Crim. Police Rev.*, 278 (1974) 126.
8 M. Afshar, *Bull. Med. Leg. Toxicol.*, 21 (1978) 485.
9 F. L. Colwell and B. L. Karger, *J. Ass. Offic. Anal. Chem.*, 60 (1977) 613.
10 J. Gasparič and J. Churaček, *Paper and Thin-layer Chromatography*, Wiley, New York, 1978, pp. 309–311.
11 H. Kaiser, *Anal. Chem.*, 42, No. 2 (1970) 24A.
12 E. Stahl, *Pharmazie*, 2 (1956) 633.