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Note

Adsorption chromatography on cellulose

V. A simple chromatographic system for the identification of inks

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In a recent survey of the biomedical applications of chromatography[1], it was pointed out that modern instrumental chromatography is at present beyond the technical, scientific and financial possibilities of many potential users of chromatography, who would welcome simple arrangements for occasional use with their everyday problems. Although these comments were made mainly with reference to medical diagnosis, they are equally valid in other fields, where analytical chemistry plays or should play a role.

We report here some ideas concerning a field which we felt was a challenge in this direction, namely the identification of writing inks on documents in relation to police scientific investigations. The main problem here is that of performing in police stations, which have neither the usual laboratory facilities nor a constant need for such, work usually carried out by graduates of the Institut de Police Scientifique (Lausanne), with a good training in chemical analysis.

A thesis by Tappolet (2) dealt with the high-performance thin-layer chromatographic (HPTLC) analysis of writing inks. It was shown that most commercial inks produce 3–5 zones under good resolution conditions. Hence there seems to be no need to resort to high-efficiency systems for chromatographic recognition.

Horváth[3] stated that "It was particularly irritating that, without getting assurances from the weather bureau that the humidity was in a certain range, it did not make sense to start TLC work that day since the results were greatly affected by the moisture content of the silica". Preliminary work with silica thin layers along the lines of the work of Tappolet[2] indicated the validity of Horváth's comments, so instead of abandoning thin layers, like Horváth, we merely abandoned silica gel and replaced it with paper strips.

EXPERIMENTAL

In developing the system described here, we considered also the availability of the various components used and the environmental acceptability of the eluents, etc., as one can hardly suggest to a police officer that he dispose of poisons or acids down the drain.

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The apparatus consisted of the following items, for which we also indicate the local price, converted to US$. The apparatus can be fitted into a small suitcase.

(1) A simple balance, as can be purchased in shops selling hunting equipment, where it is sold for compounding home-made cartridges. Price US$ 30. It is shown in Fig. 1 and has an accuracy of about 10 mg. Thus in weighing out 1-g amounts the accuracy is 1% or even better.

(2) Chromatography jars were made from large glass coffee jars, to which cork stoppers were fitted with office clips attached to form J-shaped hooks for the paper strips. The price of the corks was about $ 1.

(3) We found that the usual Whatman No. 1 or 3MM papers yielded fairly good chromatograms with aqueous solvents and either of these papers can be used. One large sheet of Whatman No. 1 paper (price *ca.* US$ 0.50) yields enough paper for about 200 individual chromatograms, thus providing another reason for preferring paper strips to silica gel thin layers.

(4) Development: in view of environmental considerations, we used only aqueous eluents. Cellulose eluted with aqueous salt solutions was shown recently[4,5] to be essentially a reversed-phase adsorption system, in which the cellulose functions as a polar stationary phase and adsorption is easily and predictably controlled by the concentration and the type of the salt in the eluent. We obtained good results with solutions of ammonium sulphate, which has the attraction that it is very soluble, but for most separations 1% sodium chloride solution also yields good chromatograms. In electrolyte solutions of reasonable strength the few ions present in tap water cause no measurable changes, so when distilled water is unavailable tap water will suffice. Complete development to the upper border of the paper takes 20–25 min.

(5) Documentation may be effected by merely keeping the developed chromatograms. However, as some dyestuffs in the inks may decompose on storage, we preferred to make a photographic record. Excellent photographic equipment for the recording of thin-layer chromatograms is available, but in view of our aim to keep costs as low as possible we adopted a Polaroid Image System E camera and a "Pola-

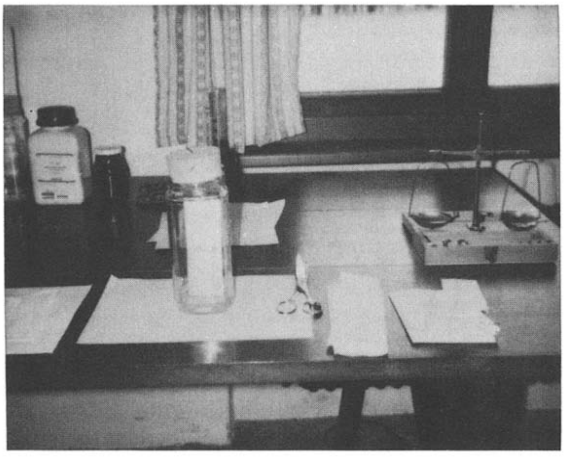

Fig. 1. The entire "laboratory", excluding the camera with which the picture was taken. The equipment can easily be set up on any desk or table.

roid close up Stand", which was principally designed for the copying of photographs on a 1:1 scale. The entire photographic equipment costs about US$ 200 and is therefore the main expense item of the system. The chromatograms of inks yielded poor, pale copies with this system and we found that a Hama Polarisations-Folie, Stärke 0.75 mm, had to be placed in front of the camera lens in order to obtain photographs of the same intensity as the original chromatogram. However, they show a slight brownish tinge in comparison with the original. This can be corrected by placing a colour standard, made with various suitably coloured felt pens, next to the chromatogram, as shown in Fig. 2.

(6) Ultraviolet fluorescence detection: a battery-operated small UV lamp, the "Ultra-violet lamp for Stamps" from Leuchtturm/Lighthouse is available from stamp-dealers at a price of US$ 25 and performs very well in semi-darkness, *e.g.*, inside an open cupboard. Only one of the dyes of the twelve blue and black inks examined is fluorescent in the ultraviolet region; felt pen No.12 (see below) has a pink fluorescent spot, which corresponds to the second slowest spot ($R_F \approx 0.45$). In the case of the set of coloured felt pens (a total of 30 pens) only one fluorescent spot was found.

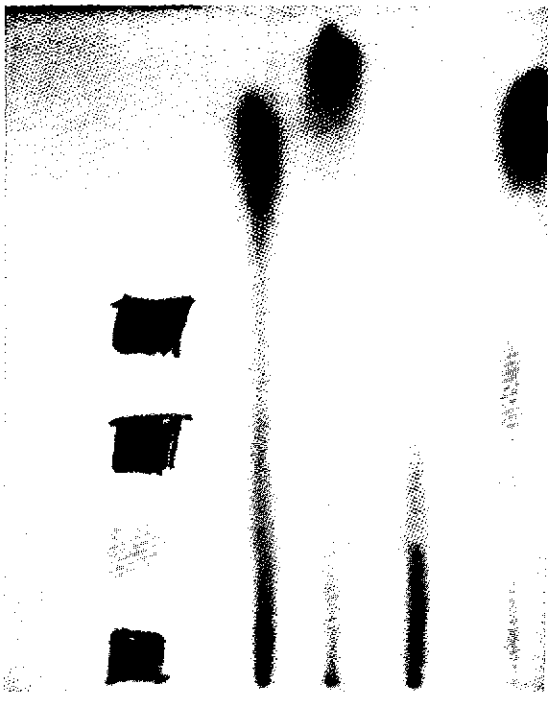

Fig. 2. Polaroid photograph of a chromatogram of four felt pen inks run side by side on a strip of Whatman No.1 paper with 1% sodium chloride as eluent. Colour samples are placed next to the chromatogram, to permit correction for the change in colour due to the photographic process.

RESULTS

The ink of one of the felt pens used for preliminary work was developed with a range of concentrations of ammonium sulphate from 0.005 to 1.3 *M*. The results are shown in Table I. A black ink pen yields a chromatogram with a fast blue spot, then a yellow spot and two red spots in the lower half of the chromatogram (the chromatogram on the right in Fig. 2). It can be seen that increasing the salt concentration lowers the R_F values of all four constituents, as expected. The blue and yellow spots separated well with all eluents, except the very dilute one.

A box of coloured felt pens, as sold for use in schools, was examined (Migros 36 Faserschreiber 7202.335, Switzerland). These pens gave the chromatograms shown schematically in Fig. 3. With many of the colours, the manufacturer employed different ratios of the same yellow and red colours and of the fast-moving blue colour. Some purple constituents yield elongated trails. Only one brick-red dye exhibited UV fluorescence (chromatogram 29). Most chromatograms showed one or two separated spots and the remainder showed three or four spots. Only five pens yielded chromatograms with "comets".

We decided to estimate the number of theoretical plates developed in such a chromatogram. Taking the fast-moving blue spot, we calculated about 1000 theoretical plates. This could certainly be improved by either developing for a longer distance or by using cellulose thin layers instead of paper. Longer development would have made copying more difficult and thin layers would have made the technique more complicated and expensive. We believe neither would have increased the amount of information obtained from such a chromatogram.

We then examined twelve blue and black writing pens: blue, (1) Pental Sign Pen, (2) Pilot Oasis, (3) Pentel "Super Ball" Japan. (4) Pentel Ultra fine S590 Japan (Blue), (5) Markana 33; black, (6) Clici Swiss Made Caran d'Ache 836009, (7) Pentel Sign Pens Japan, (8) Pentel Ultra fine S590 Japan (Black), (9) Pilot Fineliner, (10) Papermate Precise Roller 0.5 mm, (11) Compo, (12) Feutre Bic Porous Pen.

Pens 1 and 3 gave a strongly adsorbed spot at the origin, each with a short forward comet (one purple, the other blackish). Pen 5 gave a single spot at R_F 0. Pens 2, 6, 7 and 8 gave a fast-moving blue spot, and also two or three other constituents, each different from the others. Pen 4 gave the fast-moving blue spot and a slight short forward comet from the origin. Pens 9–12 have already been shown in Fig. 2. Although there are elongated comets in five of the twelve chromatograms, all pens can

TABLE I

R_F VALUES OF THE FOUR SEPARATED DYES FROM A FEUTRE BIC POROUS PEN (BLACK) IN SOLUTIONS OF AMMONIUM SULPHATE AS ELUENTS ON WHATMAN No. 3MM PAPER

Concentration of $(NH_4)_2SO_4$ (M)	*R_F value*			
	Fast blue spot	*Yellow spot*	*Purple spot*	*Red spot*
0.005	0.91	0.80	0.38	0.05
0.05	0.93	0.62	0.39	0.04
0.53	0.66	0.41	0.22	0.02
1.3	0.62	0.37	0.19	0.015

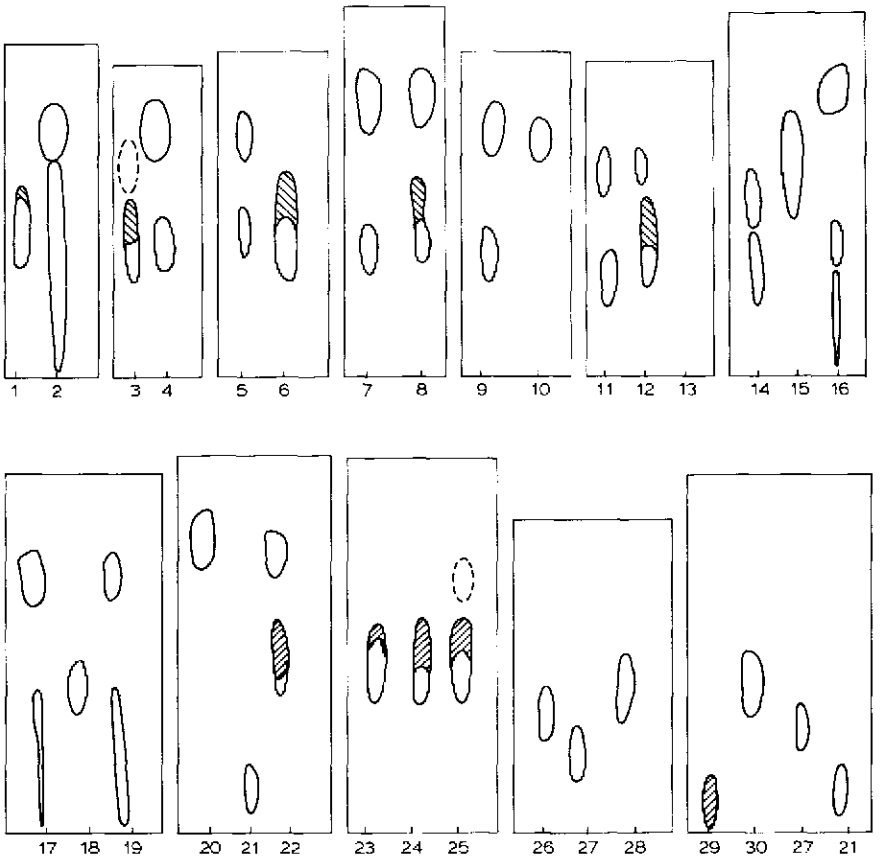

Fig. 3. Schematic representation of 30 chromatograms of coloured felt pens. From left to right: (1) shows a strong yellow spot with an adjacent faster weak red spot (hatched); (2) has a predominant blue fast moving spot and a violet comet from the origin; (3) a strong yellow and a strong red adjacent spot (hatched) and ahead of it a weak blue spot; (4) a strong yellow spot and a fast blue spot; (5) a red spot and a fast blue spot; (6) a yellow spot preceded by a strong red spot (hatched); (7) a violet comet from the origin, a yellow spot and the fast blue spot; (8) the yellow spot preceded by about an equally intense red spot (hatched) and the fast blue spot; (9) a weak yellow spot and a fairly strong blue spot; (10) a single fast blue spot; (11) the same as 9, except with a strong yellow and a weak blue spot; (12) a yellow spot preceded by a very strong red spot (hatched) and a weak blue spot; (13) a violet comet; (14) two red spots, the slower being more purple; (15) a single red spot; (16) a violet comet, the yellow spot and the fast blue spot; (17) almost identical with 16; (18) a single yellow spot; (19) a violet comet and the fast blue spot; (20) a single blue spot; (21) a single dark red spot; (22) a weak yellow spot with an adjacent strong red spot (hatched), preceded by the fast blue spot; (23–25) these are all different ratios of the yellow and the adjacent red (hatched) spot, 25, also has a faint fast blue spot; (26) a single red spot; (27) a single violet spot; (28) a single red spot; (29) a single red spot (UV fluorescent); (30) a single yellow spot.

be readily distinguished from each other by comparing the colour patterns and spot number. Only pen 12 gave one fluorescent spot (the second from the start).

We feel that the system proposed could prove of value in the examination of documents. We have not, so far, dealt with the extraction of the ink from the docu-

ment, or with the problem of the decomposition of inks with time. This will be dealt with by one of us (M.S.) in due course.

The amount of ink placed on the chromatogram in these preliminary studies was a short line of 2–3 mm directly from the pen. Thus a four-letter word would yield enough material for six to eight chromatograms.

We have recently surveyed the literature on paper chromatography with aqueous solvents (to be published) and feel that our system could have applications in various fields, such as the study of anthocyanins and other water-soluble pigments in plants in field work or the detection of some amino acids in urine in cases of aminoaciduria.

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