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SOLVENT-FRONT INDICATOR FOR PAPER CHROMATOGRAMS

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Fig. 1.

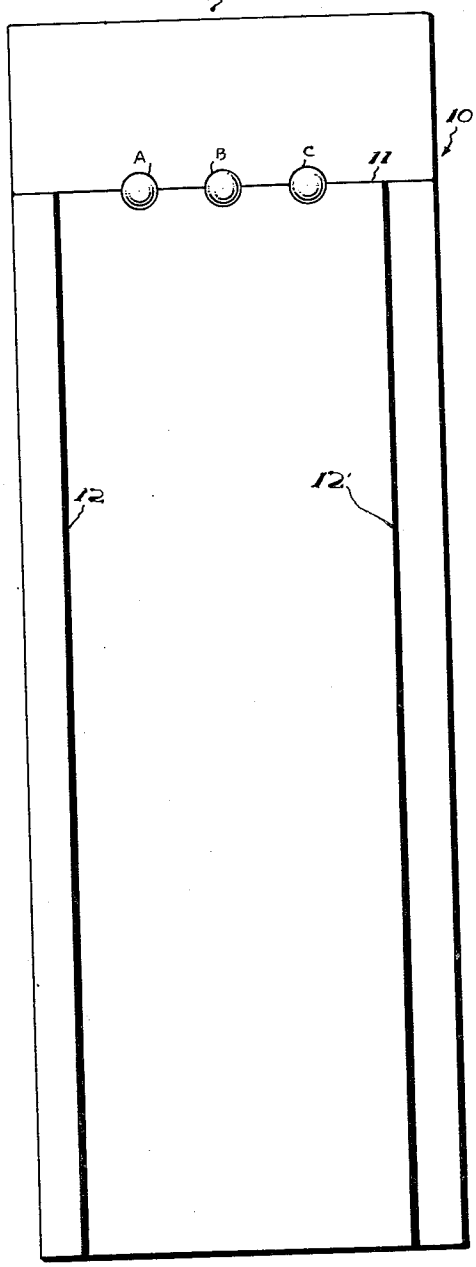
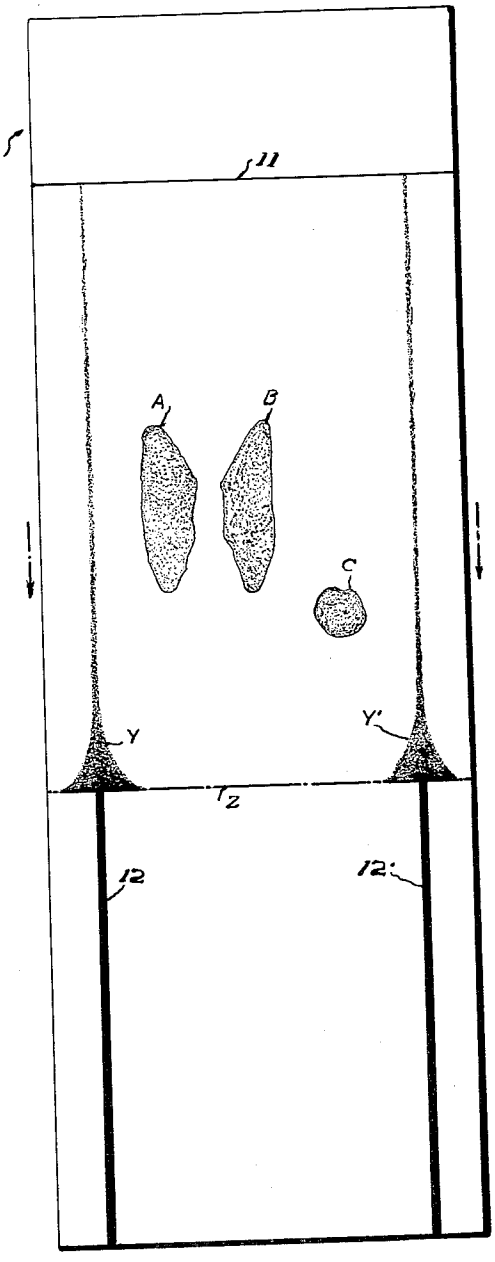


Fig. 2.



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SOLVENT-FRONT INDICATOR FOR PAPER CHROMATOGRAMS

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The invention described herein may be manufactured and used by or for the Government for governmental purposes, without the payment to me of any royalty thereon.

This invention relates to a solvent-front indicator for paper chromatograms, and more particularly to a method of indicating the progress of the solvent front of a paper chromatogram, so as to record the same by a permanent change in the appearance of one or more colored ink lines placed thereon.

Paper chromatography has assumed increased importance in recent years as a tool in analytical and microchemistry. Its principal uses lie in the resolution of mixtures in the recovery of small amounts of reaction products that cannot be recovered by conventional methods of crystallization, dialysis, etc., and in the identification of unknown reaction products and/or other small samples whose identity is postulated but not yet definitely known.

The technique of paper chromatography requires considerable skill and experience on the part of the operator. Various standard techniques of paper chromatography are known, all of which include the essential elements of a treated or untreated filter paper (or glass fiber) sheet on which drops of "spots" of one or more known and/or unknown samples are placed in a "starting line" or "base line," and are caused to migrate along the sheet by means of a solvent system consisting of one or more solvents. After the "spots" have migrated for a suitable distance from the starting line, they are fixed in place by removing the solvent, and made visible by means of a suitable chromogenic chemical, such as a spray of Ninhydrin (1,2,3-indantrione hydrate) or of an alcoholic solution of phosphomolybdic acid. The migration of the "spots" and solvent may be effected either in a vertical direction (descending or ascending) by suspension of the sheet, e.g., in a hooded tank, or horizontally by causing the solvent to migrate across the sheet while in a level position. In either case, the operator is beset with the uncertainty whether or not the solvent front has progressed far enough (or too far) for developing and fixing the "spots" on the paper chromatogram. Since the solvent is colorless, and the "spots" are likewise colorless in the majority of cases, prior to treatment with Ninhydrin or other chromogenic agent, it is very easy to make a mistake in timing and thereby spoil the entire procedure.

I have now found that this drawback can be overcome, and that progress of the solvent front on the paper chromatogram can be visually indicated and recorded, by drawing one or more "ink" lines perpendicular to the starting line and parallel to the edge of the paper chromatogram prior to applying the solvent. The "ink" must be soluble in the solvent system and must be not substantially absorbable by the paper or other material of the paper chromatogram sheet. In this manner, the operator merely need observe the distortion of the appearance of the ink line or lines as the solvent front proceeds, and is thus enabled to stop the migration of the solvent at the right time, by removing the sheet from the tank, and to fix the "spots" (e.g., with Ninhydrin), which completes the paper chromatogram.

By placing a pair of ink lines near either edge of the paper chromatogram, the operator is also enabled to observe the even progress of the solvent front, and to correct uneven migration of the latter, should it occur.

I have further found that lines drawn with ball point pen ink are entirely suitable for the practice of my invention. A typical suitable blue ball point pen ink is a mixture of 58% oleic acid, 42% Victoria Blue, and a trace of BA base; other suitable ball point pen inks are listed in Table I of an article by David E. Gould et al., "Inks for Ball Point Pens," on pages 37/38 of "The American Ink Maker," July 1951. Whether or not a particular ball point pen ink is compatible with (i.e., soluble in) a particular solvent contemplated for use in preparing a paper chromatogram, can be easily determined in advance by drawing an ink line and applying the solvent thereto; if it discolors, the ink is suitable. Thus, by means of this simple preliminary test, the operator need not even know the specific composition of the ball point pen ink. Another suitable ink compatible with most solvents used in paper chromatography is a water solution of a water soluble coal tar dye such as F&DC Blue #1; disodium salt of 4-(4-(N-ethyl-p-sulfobenzyl-amino)-phenyl)-(2-sulfonjumphenyl) - methylene)-(1-(N-ethyl-N-p-sulfobenzyl)-cyclo-hexadienimine).

The ink lines are suitable for use with acetone solvents, alcoholic solvents (e.g., butanol), liquid hydrocarbon solvents, aqueous solvent systems of sodium citrate and ammonium hydroxide, and many others.

Another requirement of a suitable ink (ball point pen ink, or other ink, such as a water solution of F&DC Blue #1) is that it must be substantially non-absorbable by the sheet material of the paper chromatogram; this, too, is easily ascertained by drawing an ink line on a sample sheet.

Having thus described the principles of my invention, I now proceed to describe its practice by means of a specific example illustrated in the appended drawings.

FIGURE 1 is an elevational view of a paper chromatogram in accordance with my invention, prior to application of the solvent, and

FIGURE 2 is an elevational view of the same, after application and subsequent removal of the solvent and development of the spots. It will be understood, of course, that this example is merely illustrative and is not intended to limit the scope of my invention.

More particularly, numeral **10** denotes a rectangular sheet of Whatman No. 1 chromatographic filter paper. A pencil line **11** is drawn about 1"–1½" from the "top" of the sheet to mark a conventional "starting line" or "base line." In accordance with the present invention there are drawn two ball point pen ink lines **12**, **12'** perpendicular to the starting line at a distance of about ⅛"–¼" from either long edge of the sheet.

"Spots" A, B, and C, are placed on the "starting line" **11**, laterally of ink lines **12**, **12'**, as shown in FIGURE 1; "spot" A being a sample of known chemical composition in solution and "spots" B, and C being unknown samples in solution. A solvent (e.g., 75 ml. acetone in 100 ml. water) is now applied to the top of the sheet and caused to migrate in the direction of the arrows appearing to the left and right of FIGURE 2. The progress of the solvent front along the sheet by noting the progressive change in appearance of ink lines **12** and **12'** (as at Y, Y'), and when it has progressed far enough, say to the position Z (as shown in FIGURE 2), the sheet is removed, the solvent is evaporated, and the spots, which were carried along by the solvent for the distances marked on FIGURE 2, are made visible by application of a chromogenic agent (e.g., Ninhydrin spray). The flared appearance of the ink, at Y, Y', on lines **12**, **12'**, is due to the

carriage of cumulative amounts of ink by the solvent in the course of the migration of the solvent front.

In this example, spots A and B have traveled the same distance, and assumed substantially identical configuration, which indicates to the analyst that previously unknown sample B probably corresponds to the chemical composition of known sample A (this can be ascertained with greater assurance by reproducing the result with a different solvent). On the other hand, spot C has traveled farther than either samples A and B, and it can be concluded therefrom with certainty that sample C is of a chemical composition different from known sample A.

It will thus be seen, that by the practice of my invention, the operator is enabled to pick the right time for terminating the migration of the solvent along the sheet of the paper chromatogram, and to avoid failure that otherwise might be caused by letting the solvent front and one or more of the "spots," carried by the solvent, run off the paper.

It is also evident that the operator can determine at any time that the solvent front proceeds in a direction perpendicular to the starting line and remains substantially parallel therewith. The permanently changed appearance of the ink line (as at Y, Y') furnishes a permanent record of the observance of proper procedures in the preparation of the paper chromatogram.

The term "paper" as used in the specification, is deemed to include other sheet material suitable for chromatography, as, for instance, glass fiber sheets. The same applies to the terminology of the claims.

Although I have illustrated and described the preferred form of my invention, it will be obvious that various changes may be made therein, wtihout departing from the spirit of the invention or from the scope of the subjoined claims.

I claim:

1. In a method of paper chromatography, wherein a chemical substance is placed on the starting line of a paper chromatogram, and a solvent is applied to said starting line and caused to migrate along siad paper chromatogram in a direction perpendicular to said starting line, whereby chemical substance is left on said paper chromatogram in a new location intermediate said starting line and the line of farthest progress of the front of said solvent: the improvement which comprises the steps of forming an ink line perpendicular to said starting line and parallel to an edge of said paper chromatogram, the ink of said ink line containing coloring matter soluble in said solvent and not substantially absorbable by the paper of said paper chromatogram, said ink line being laterally spaced from said deposit of chemical substance on said starting line; and terminating the migration of said solvent at a distance of the solvent front from said starting line sufficient to permit the deposit of chemical substance on said paper chromatogram in said new location, said distance of said solvent front being indicated by a permanent change in the appearance of said ink line resulting from contact of said solvent with said ink line.

2. The method according to claim 1, wherein said ink is ball point pen ink.

3. The method according to claim 1, wherein said ink is a water solution of a water-soluble coal tar dye.

4. In a method of paper chromatography, wherein a chemical substance is placed on the starting line of a paper chromatogram, and a solvent is applied to said starting line and caused to migrate along said paper chromatogram in a direction perpendicular to said starting line, whereby chemical substance is left on said paper chromatogram in a new location intermediate said starting line and the line of farthest progress of the front of said solvent; the improvement which comprises the steps of forming a pair of ball point pen ink lines perpendicular to said starting line and parallel to and proximate to a pair of edges of said paper chromatogram, said ink lines being laterally spaced from said deposit of chemical substance on said starting line; and terminating the migration of said solvent at a distance of the solvent front from said starting line sufficient to permit the deposit of chemical substance on said paper chromatogram in said new location, said distance of said solvent front being indicated by a permanent change in the appearance of said ink lines resulting from contact of said solvent with said ink lines.

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