SECTIONS

CAPILLARY ANALYSIS OF CERTIFIED FOOD DYES.*

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The application of capillarity to chemical analysis began in 1861 with the work of Schonbein (1) in differentiating natural products by use of filter strips, but it was not until 1909 that Goppelsroeder (2) applied the method extensively using a number of media besides paper. He named the procedure capillary analysis. Since then Kunz-Krause (3) introduced the method into Pharmacy, Platz (4) applied it to Homeopathy, and Lasseur and Marchal (5) to Bacteriology. Boutaric (6) and Freundlich (7) have made extensive investigations of the mechanism of capillary chemistry. Mulliken (8), as well as Schonbein and Goppelsroeder have attempted to apply the method to dyes but have admitted the lack of reliability of their procedures.

The present investigation was begun with the purpose of developing a shorter method of detecting and separating the certified food dyes. The more extended use of the certified dyes which will be made necessary by the recently enacted Food, Drug and Cosmetic Act, makes their rapid detection highly desirable.

An examination of present chemical methods of analysis of these dyes (9, 10) makes readily apparent the laborious technique involved in some of these, and the indefiniteness of conclusions obtained in others. The method involving successive dye extractions with selective immiscible solvents (11) at different p_H values is very lengthy and does not give complete separations. The method of spot testing on dyed animal and vegetable fibers (10) involving color reactions with different reagents lacks reliability. When mixtures of dyes are present, obscured and false color reactions make positive identification an almost hopeless task. It was felt that the method of capillary analysis offered possibilities of providing a more rapid and positive method of analysis of these dyestuffs.

To carry out the capillary analysis under controlled conditions, a special cabinet was built. This is shown, with front cover removed, in Fig. 1. The compact case, 41 x 11 x 32 cm. has been constructed so as to provide an hermetically sealed compartment when front and back glass plates have been bolted on. The metal frame is soldered at all joints. Rubber gaskets, 1 cm. thick, provide a seal between the frame of the case and the glass plates. An inlet and an outlet tube are connected in the interior to air diffusing chambers running lengthwise across top and bottom. A cross bar bears spring clips from which the capillarizing strips may be suspended. Set screws, specially sealed to prevent leakage of air, can be operated from outside the case after it is sealed, to raise or lower the strips into the 30-cc. beakers containing the dye solutions. The beakers are held in place by metal rings soldered to the bottom of the case. Paraffin covers, with slits 11 x 1 mm. were designed to prevent excessive evaporation of water from the dye solutions, and facilitate the control of humidity within the case.

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Previous investigators, Heinrici (12) and Rojahn (13) have recognized the need of humidity control, but were unable by their methods to obtain sufficiently constant conditions. Too high a humidity causes diffuse capillary images. Too low a humidity gives capillary rises too short in height to permit adequate differentiation. A relative humidity of 60% (±5) was found to be optimum.

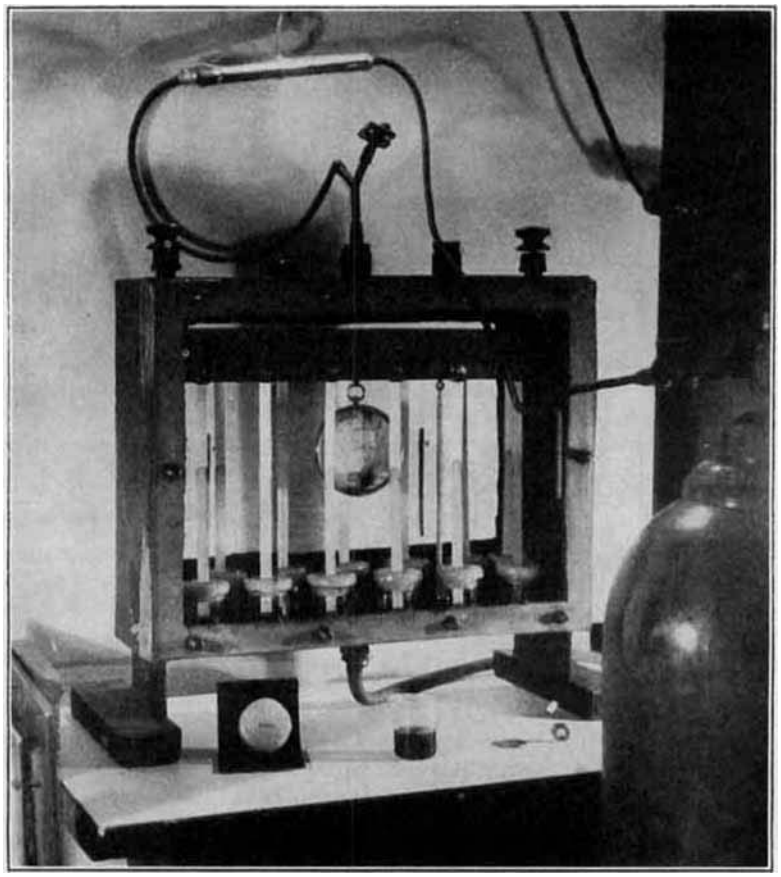

Fig. 1.

Since the rapid evaporation of water from the capillary strips would cause a 100% saturation of the air in a few minutes within the small compartment, provision for rapid removal of this moisture must be made. The use of drying agents within the case is of no value for rapidly reducing the humidity. The only satisfactory method is to pass a stream of air through the case, by vacuum or pressure pump, at a velocity sufficient to bring about the desired humidity.

The air which enters the case must first have its relative humidity reduced almost to zero if it is to be an effective drying agent. An attempt was made to adapt the method of Sweetman (14) who suggested controlling the humidity of air by passing it first through supersaturated salt solutions. A number of solutions listed in *International Critical Tables* (15) were tried. However, since large volumes of air are involved, the original moisture content of the air soon dilutes the salt solutions and upsets the equilibrium. It was found that a flow of approximately one liter of dry air per minute had to be passed through the case to maintain a fairly constant optimum humidity. Solid drying agents, capable of being regenerated, can be used, but a practical solution of the problem is to use compressed air, available commercially in tanks of 200 cu. ft. and having an extremely low moisture content. Regulating the flow of this air permits satisfactory humidity control within the case.

A wet-dry bulb hygrometer cannot be used since it gives off considerable water vapor, which would have a marked effect in the small space of the cabinet. A calibrated horse-hair hygrometer proved satisfactory. Grant (16) has shown this instrument to be sufficiently accurate.

Temperature fluctuations of a few degrees did not affect the reliability of the results. Room temperature was maintained at 24° C. ± 2. The air passing from the outlet tube was forced through 20 mm. of mercury to maintain a constant pressure.

The filter paper from which the capillary strips are made is Schleicher & Schüll No. 604. This was selected, in preference to No. 597 or 598, used by other investigators, because of its soft texture which permits rapid and uniform capillary penetration of solutions. The paper is cut into strips 1 cm. wide and 22 cms. long. This length was found sufficient since maximum height to which the dyes rose within the period of operation was 16 cms. The depth to which the capillary strips are immersed was found to have no effect upon capillary rise. For purposes of uniformity in measuring capillary rise, all strips were immersed 1 cm. below the surface of the liquids.

For the purposes of this investigation, the thirteen permitted water soluble coal tar food dyes[1] were selected. These dyes, described in Bulletin 1390 and Supplement 1 of the Department of Agriculture are termed certified when they comply with the standards set by the Food and Drug Administration. Government certified dyes were used throughout this work. These dyes are salts of acid dyestuffs. Their colored ions are negatively charged and their behavior as a group might be expected to be analogous. However the differences in their chemical composition is in most instances sufficient to encourage the idea that they would be differently affected by other substances in solution. It was therefore decided to adapt the work of investigators in other fields (17), (18), (19) of the influence of certain electrolytes and other substances, upon the capillary rise of these dyes.

In carrying out the capillary analysis, the following technique is employed. The dye is dissolved in water and the appropriate concentration of reagent added. The optimum concentration of dye is of the order of 0.1 millimolar. If a colored food or drug product is being examined, the dye must first be removed from the product by the standard method of double dyeing on wool (9). It is then stripped with dilute ammonia solution, the liquid evaporated to dryness, and the residue made up to a concentration of about 0.1 millimolar. About five to ten cc. of the dye solution is then placed in the beaker in the apparatus, and a capillary strip is suspended from the cross bar and passed through the paraffin slit so that it hangs slightly above the surface of the solution. In the apparatus described, thirteen determinations can be started and run simultaneously. As soon as all of the strips are in place over the solutions, the glass plates are clamped on and a current of air allowed to pass through for a few minutes until the relative humidity falls to 55%. The cross bar is then lowered so that all the strips are simultaneously immersed in their respective solutions to a depth of 1 cm. A scale is provided for this purpose within the case. The humidity will tend to rise as capillary action begins. The air flow is therefore correspondingly regulated to maintain a 60% relative humidity for a period of thirty minutes. The air flow is then shut off and the strips removed and allowed to dry. Figure 2 shows the appearance of the thirteen dyes under the influence of one reagent. The strips are of course definitely colored and offer a much better contrast than is apparent in a black and white print. The amount of dye deposited on each strip is of the order of 0.1 to 1 gamma.

The following tables, abridged from several hundred experiments, show the capillary rise of the thirteen dyes as affected by the different reagents.

Capillary heights are expressed as centimeters of rise above the liquid surface; *viz.*, in the case of erythrosine in the presence of $AlCl_3$, the height of zero means no rise above the surface of the dye solution, although the strip may be colored at the lowest centimeter where it was immersed in the solution. It must be emphasized that the relative heights of dyes in any one series are of greater importance than the individual heights in centimeters. The latter will vary somewhat in different apparatus. Small variations in time, temperature and humidity will likewise have a slight effect upon the heights.

[1] Since this paper was written, the Department of Agriculture has added one more soluble food dye, the potassium salt of naphthol yellow S. Under the new act these dyes are designated as F. D. & C. dyes.

TABLE I.—EFFECT OF AQUEOUS SALT SOLUTIONS UPON CAPILLARY RISE.

Certified Dye.	Mol. Wt.	Color on Paper.	NaCl			Na_2SO_4	$BaCl_2$		$AlCl_3$		N. F. Soln. Al Acetate.
			M/1	*M*/10	*M*/100	*M*/10	*M*/10	*M*/100	*M*/10	*M*/100	*M*/4
Erythrosine	880	Brilliant Pink	1	1.5	5	1	1	2	0	0	1
Amaranth	604	Purplish Red	3.5	5.5	10.5	4	3.5	7	2.5	6.5	4.5
Ponceau 3R	494	Red	1.5	3.5	8.5	2	0	0	2.5	2.5	2.5
Ponceau SX	480	Red	4	6	10	4.5	3	5.5	2	3.5	3
Fast Green FCF	809	Green	9	12.5	13.5	12.5	7	10	5	7	8
Guinea Green B	691	Green	2	6	8.5	5	1.5	3	1.5	2	2
Light Green SFY	793	Pale Green	8.5	12	12.5	11.5	7.5	12.5	2.5	6	5
Indigotine	466	Violet Blue	2	5.5	9	3	2	5.5	2.5	6.5	4
Brilliant Blue FCF	793	Blue	6.5	11	13	9	5.5	7.5	4	5	6.5
Naphthol Yellow S	358	Yellow	6	9	11.5	6	7.5	10	4.5	8	7.5
Tartrazine	534	Yellow	5.5	8.5	12	7	7.5	10	4.5	9	8
Sunset Yellow FCF	452	Orange	4	7.5	10.5	4.5	6	9	3	6.5	7.5
Orange I	350	Orange	2.5	4	6	2.5	2.5	2.5	2	2.5	2

TABLE II.—EFFECT OF p_H UPON CAPILLARY RISE.

Certified Dye.	Hydrochloric Acid.					CH_3COOH	Dist. H_2O	NH_4OH	Sodium Hydroxide.	
	M/1	*M*/10	*M*/100	*M*/1000	*M*/10,000	*M*/100		*M*/100	*M*/1000	*M*/100
	p_H: 0.1	1	2	3	4	3.4	6	10.8	11.1	12.1
Erythrosine	0	0	0	5	11.5	7	11.5	12.5	7.5	10
Amaranth	3.5	5.5	5	13.5	13.5	15	14	14	13	14
Ponceau 3R	2	3.5	4.5	11	13	11.5	12.5	13.5	11.5	12.5
Ponceau SX	2.5	5	5	11.5	12.5	13	12.5	12.5	12	12.5
Fast Green FCF	11.5	9.5	10.5	14.5	14	16	14.5	13.5	13	13.5
Guinea Green B	11	6.5	3.5	6	12.5	4	11.5	12	11.5	12
Light Green SFY	13	13.5	13	12.5	12	15	12.5	12	11.5	11.5
Indigotine	3.5	5	5	11.5	13	13	13	13.5	12.5	12.5
Brilliant Blue FCF	10.5	8.5	9	13.5	13	15	13.5	13	12	12.5
Naphthol Yellow S	10.5	10	11	13	13	14	14	13	12.5	13
Tartrazine	7.5	9	11.5	13	13	15	13	13	12	12.5
Sunset Yellow FCF	5	7	9.5	12	12	14	12	12.5	12	12
Orange I	2	3.5	3	6.5	10	5.5	10	12	9.5	11

By running a control simultaneously with the unknown dye, identification will be facilitated. Such a control may consist of a single dye of the color of the unknown, or preferably a mixture of three dyes. A good combination is naphthol yellow, indigo and erythrosine, each in 0.1 millimolar concentration. After capillarization, the dyed strip will appear as a three-color zone pattern, yellow at the top, green in the middle and reddish at the bottom. Although there is an overlapping of colors at the bottom two-thirds of the color zone, the height of each of the three dyes can be sharply defined, and serves to gage the relative height of any certified dye that may be present in the unknown, and which has been capillarized from a separate beaker at the same time, and within the same apparatus.

The identification of a dye by this method is more often an indication rather than a positive proof of identity. However, while it is possible that two dyes may rise to the same height under the influence of a specific reagent, an examination of the tables will show that no two dyes behave the same for all reagents. It is of course not necessary to run a large series of experiments utilizing every reagent listed in order to identify the dye. The following represents a selection of reagents which will give the optimum differentiation:

For Red Dyes: Solution Aluminum Acetate, N. F. (Approximately $M/4$ or 5% aluminum acetate).
A better differentiation of ponceau 3R and SX is obtained by the use of $M/10$ NaCl.

For Yellow and Orange Dyes: $M/10$ Na_2SO_4. A better differentiation of naphthol yellow S and tartrazine is obtained with $M/1$ HCl.

For Blue and Green Dyes: $M/10$ Na_2SO_4.

An examination of the tables will show the following additional points of interest. Increase of salt concentration lowers the capillary rise. Increase of ion valence lowers the capillary rise. Between p_H 4 and 10, there is only a slight change in the capillary rise. However, exceptions exist for acetic acid and for ammonia. The greater volatility of these reagents, as compared with the other acids and bases, plays a rôle in modifying the rise of some of the dyes.

In addition to the reagents listed, reducing and oxidizing agents, such as sodium hydrosulfite and sodium persulfate were tried but gave little satisfaction. In general the blue and green dyes of the triphenylmethane class resisted bleaching action by these reagents. Likewise no advantages could be found in using the surface-active agents, such as sodium lauryl sulfate. These caused distortion or a general leveling of the capillary rises. An attempt to adapt filter paper strips previously impregnated with electrolytes, as applied by Clarke and Hermance (21) in their inorganic spot-testing technique, gave distorted images when applied to the capillary analysis of the dyes.

As a confirmatory test, the dyed strip may be examined and compared with a known dye by reflected light in a spectrophotometer. Investigations are at present under way to determine absorption maxima for the certified dyes at different concentrations, since no such data on spectral reflection curves are at present available in the literature. The spectral transmission properties of seven of the permitted dyes have been published (22) and curves for the other permitted dyes are at present being prepared by Evenson of the Color Certification Section of the Department of Agriculture. A more positive spectrophotometric identification can be made by the method of absorption ratios (23). This is the ratio of extinction coefficients of the dye solution at two specific wave-lengths at both sides of the maximum. Whether this method can be adapted to reflection measurements of dyed paper is at present being investigated.

When a mixture of several dyes is present, the problem becomes more complex. As can be visualized from an examination of Fig. 2, the presence of more than one dye in solution will give a blend of colors corresponding to the respective dyes.

It was reasoned however that if the differentiated capillary heights could be maintained, and the lower colored part of each strip could by some manner be eliminated, then a mixture of two dyes could be determined without interference. This was finally accomplished by the development of a method of successive capillarizations. That is, after the first thirty minute capillarization is completed, the colored strip is allowed to dry and again capillarized, under similar conditions of humidity, temperature and time, by immersion into a beaker containing a solution of appropriate reagents without any dissolved dye.

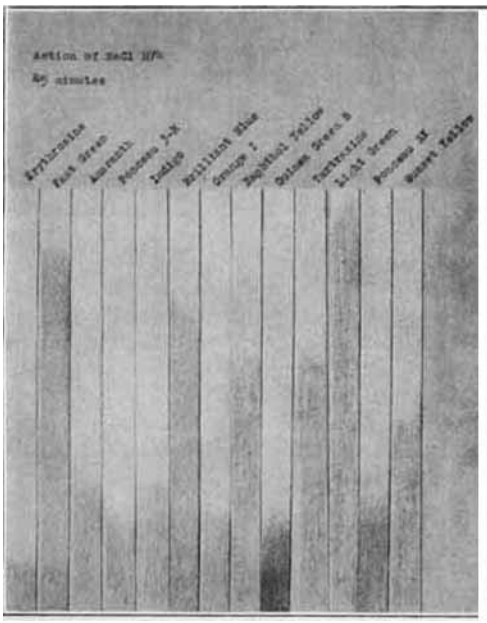

Fig. 2.

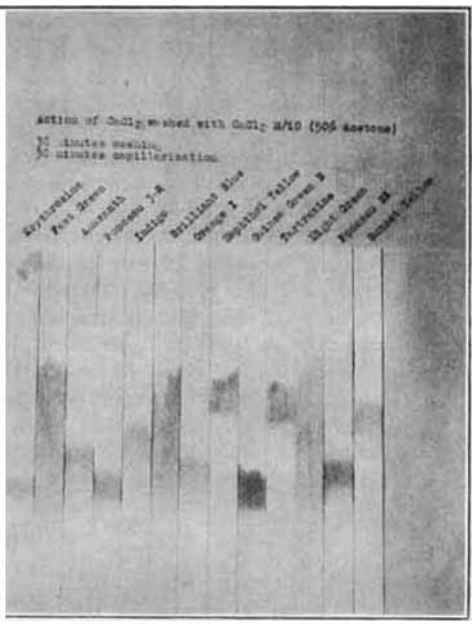

Fig. 3.

The reagent which thus far has been found to give the most successful results consists of $M/10$ $CaCl_2$ in 50% acetone. This second capillary procedure will be referred to as a washing process since the dye on the lower part of the strip is washed up by the capillary action of the colorless reagent. The dye forms a concentrated zone of color of relatively small vertical width. Figure 3 shows the result of a preliminary capillarization from solutions containing their respective dyes and $M/10$ $CaCl_2$, followed by a washing or second capillarization with a colorless solution of $M/10$ $CaCl_2$ in 50% acetone. It can readily be visualized by an inspection of the dyed strips that if several dyes were present, such as ponceau 3R and sunset yellow, or a mixture of naphthol yellow S, indigotine and erythrosine, that discrete bands of color would appear on the strip after the washing process, each at a different level from the others. Their identities can be confirmed by spectrophotometric examination.

The $CaCl_2$-acetone reagent was selected because it obviates certain difficulties which occur if other washing media are used. If water alone is used the heights of the final narrow zones are not sufficiently differentiated. However for mixtures of certain dyes this may prove effective. If an electrolyte such as Na_2SO_4 is used as the washing medium the dyes tend to become mordanted to the strip and are not washed up. If acetone and Na_2SO_4 are used, then the dye is released from the paper and is washed slowly upward. The only disadvantage of this combination is that the sodium sulfate tends to crystallize out on the upper part of the paper, due to the poor solvent action of the acetone, and an uneven or distorted color band is produced, obscuring the identification of the dye. However, by using a deliquescent electrolyte such as $CaCl_2$ in 50% acetone, no crystallization of salt occurs on the strip, and fairly even bands of color are obtained for the majority of the dyes.

Although as many as five dyes in admixture have been determined by this method, no attempt has been made to work out all possible combinations in which two to thirteen dyes might be present. There would be no point in attempting to separate a mixture of all thirteen dyes, since it is realized that dyes other than the certified ones might be present. There is not enough space on a capillary strip to permit clear-cut zones of all possible dyes that might be present. Even with a fewer number of dyes it is realized that there might be interferences. For example the blue dyes are difficult to wash up and tend to interfere in many cases with the separation of other dyes. It may be that other reagents than the one suggested will act as better washing agents.

The method of successive capillarizations is however subject to broader application. If for example, erythrosine and fast green were present in a mixture, the second capillarization would result in an overlapping of red and blue zones at the bottom of the strip, as can be visualized from an inspection of the two strips at the extreme left of Fig. 3. However, by cutting off the bottom centimeter of the color zone and attaching it to a new white capillary strip, a third capillarization with appropriate reagent, such as 50% acetone in water, without electrolyte, would cause the erythrosine to be washed up from the green dye which would be left in a pure state at the bottom. Although this process may at first sound somewhat lengthy, it must be remembered that each capillarization takes but thirty minutes, so that the total time involved is still far shorter than is required with other methods now in use.

Another method of treating mixtures is to select for the first capillarization some reagent from the tables, which will best differentiate the suspected dyes, then dry the dyed strips, dip them for an instant in $M/10$ $CaCl_2$ and wash them up with $CaCl_2$-acetone reagent. The method is at present being studied with reference to the detection of non-permitted dyes present as impurities in the certified dyes; *viz.*, martius yellow in naphthol yellow S.

Although some inferences may be drawn concerning correlation between capillary rise and chemical constitution of the dyes, particularly the number of SO_3Na groups present, conclusions will be deferred until a greater number of dyes have been studied.

SUMMARY.

1. The method of capillary analysis has been adapted to the identification of the thirteen water-soluble permitted food dyes when present individually.

2. A method of multiple capillarizations has been evolved to aid in the separation and identification of mixtures of these dyes.

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