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Title of Study: EXPERIMENTAL PROJECTS IN PAPER CHROMATOGRAPHY  
FOR HIGH SCHOOL LABORATORY WORK

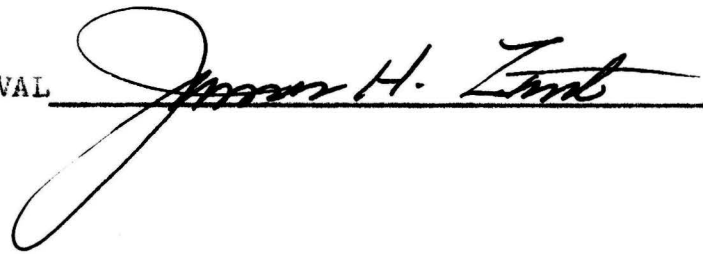
Pages in Study: 22 Candidate for Degree of Master of Science

Major Field: Natural Science

Scope and Method of Study: The use of paper chromatography is very wide spread in applied chemistry, but is seldom mentioned in high school text books. Because of this fact, a selection of several projects for high school laboratory use or class demonstration have been selected and checked by the writer in the laboratory.

The projects which were vivid in color and fast in reaction time were considered to be most useful for demonstration purposes. Projects number one, two, and four were most satisfactory.

ADVISER'S APPROVAL

  
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EXPERIMENTAL PROJECTS IN PAPER  
CHROMATOGRAPHY FOR HIGH SCHOOL  
LABORATORY WORK

By

HARLEY WILLIAM BROCK

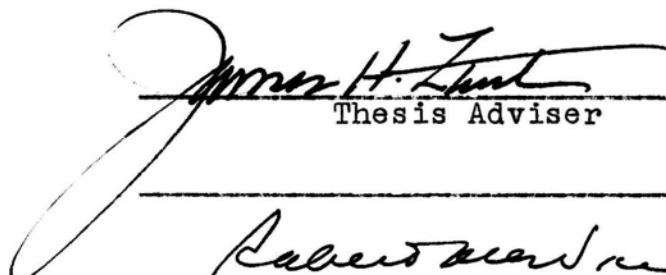
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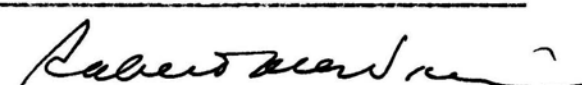
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EXPERIMENTAL PROJECTS IN PAPER  
CHROMATOGRAPHY FOR HIGH SCHOOL  
LABORATORY WORK

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## CHAPTER I

### PURPOSE OF THE REPORT

During the past twelve or fifteen years, the use of chromatography has become important in the field of applied chemistry. Its use has become almost commonplace, but its methods and techniques have seldom found a place in the elementary teaching curriculum. Laboratory manuals and guides have been written by Block, Brimley and Barrett, Pollard and McOmie, and others. There has been an innumerable number of periodical articles written concerning the subject. It is from this material that a few projects have been selected to form a representative cross-section of the application of paper chromatography. These projects have been conducted in the laboratory by the writer and were found to give satisfactory results. The writer was unable to find the subject of paper chromatography in any of several high school texts or laboratory manuals which he examined. It was because of the wide use of the subjects and the scarcity of the material in high school texts that this report was undertaken. It is hoped that teachers of chemistry and botany may find one or more of these projects applicable to his use.

This is by no means an attempt to introduce a course or part of a course in paper chromatography into an already over-crowded high school curriculum. It is a selected source

of simple projects which can and should be introduced to the student of chemistry or biology on the high school level. This introduction could be on a class basis or as a demonstration by the instructor.

Paper partition chromatography is the name given to a technique of analysis and/or preparation in which there is a dynamic partition or distribution of dissolved or dispersed materials between two immiscible phases, one of which is moving past the other. In this study there are a few common words which will receive a special meaning. When the word solution is used, it shall mean the material or materials which are to be separated. The solvent is the material in which the strip is dipped or the material which migrates up the paper strip. The developer is a material which is usually sprayed on the dried chromatogram to form a colored area and thus locate and identify the separated material.

## CHAPTER II

### A BRIEF HISTORY OF CHROMATOGRAPHY

The phenomenon of adsorption is not a recent discovery, as Scheele, the Swedish chemist, first noted it in 1773.<sup>1</sup> By 1785 it was being used commercially in the wine industry where wood charcoal was used to decolorize tartaric acid before crystallization and also for improving the color of so-called "rotten wines". The general process developed at that time is still in use, although in most cases, greatly modified and improved. In the sugar industry the syrup is clarified by the use of vegetable charcoal before crystallization. Since the early days in the petroleum industry, lubricating oils and gasolines have been decolorized and otherwise treated by passing through a bed of clay; gasoline has been recovered from vapors by the use of activated charcoal and use is being made of adsorption for separating and preparation of various fractions. The principles of the gas mask are handed down from about the 1850's, when it was found that the odors of sewer gas could be removed by passing them through charcoal.

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<sup>1</sup>Orsino C. Smith, Inorganic Chromatography (New York, 1953), p. 13.

The adaptation of the use of chromatography, as we know it today, starts with the work of Dr. Michael Tswett, a lecturer on botany at the University of Warsaw, who in a series of three articles published in 1906 first called attention to the possibilities of this phenomenon and devised a method for its use.<sup>2</sup> Tswett was a botanist and his work was with the coloring matters of plants. His method consisted of pouring a solution of the plant pigments in a light petroleum through a column of powdered calcium carbonate contained in a glass tube. The pigments were adsorbed on the top of the column, and as more solution percolated through the column, the zones became broader and a band formed which was yellowish at the top and greenish near the bottom. On further washing with pure light petroleum, one of the yellow pigments passed through the column while the other pigments were separated by selective adsorption at different levels of the column, and eventually formed into distinct zones. After allowing it to drain, the column of calcium carbonate was carefully pushed out of the tube and divided into sections containing the different pigments. From these sections the pigments were individually removed by extraction with alcohol. Dr. Tswett's really great contribution was the use of a developing material, which was often different from the original solvent, to separate mixtures on a column. The idea of a developing material was also used by S. K. Kvitka and D. T. Day

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<sup>2</sup>F. H. Pollard and J. F. W. McOmie, Chromatographic Methods of Inorganic Analysis (New York, 1953), p. 2.



with some success.

Even though Dr. Tswett receives credit for the use and explanation of chromatography, nine years before his fundamental paper appeared, Dr. V. R. Day used a long glass tube filled with fuller's earth through which he filtered crude green Pennsylvania oil.<sup>3</sup> Light gasoline appeared first in the filtrate. In a reverse experiment the oil was allowed to rise in the column by capillary forces and the light hydrocarbons rose more rapidly. Subsequent investigations by Gilpin and Cram, Gilpin and Bransky, and Gilpin and Schneeberger between 1908 and 1913, which were evidently made without the knowledge of Tswett's method, showed that if the oil is sucked by a pump upward through the column, the light aliphatic hydrocarbons are accumulated in a top section; aromatics and unsaturated compounds do not rise as high; and, finally, the amount of nitrogen and sulphur containing compounds gradually decreases from bottom to top because of "selective adsorption".

Dr. Tswett's first paper, contained a study of more than 100 adsorbents used in conjunction with several different solvents and a comparison of the efficiency of column and batch adsorption.<sup>4</sup> Tswett died prematurely in 1920 and his method was used only rarely during the following years.

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<sup>3</sup>L. Zechmeister, Progress in Chromatography 1938-1947 (London, 1950), p. 3.

<sup>4</sup>Edgar Lederer and Michael Lederer, Chromatography (New York, 1953), p. 1.

Its lack of use in early days was probably partly due to the belief of some investigators that the adsorption method altered the composition or structure of the adsorbed material, but it was principally due to the fact that the original papers were published in a botanical journal, and larger, more complete work was published in Russian.<sup>5</sup> There was sporadic uses made of the method during this interval, but it was not until the publication of Kabn and Lederer that the real revival of the method took place. This remarkable research, by which carotene of carrots was resolved into and isomers, causing an awakening of the importance of adsorption in the analytical and preparative fields and its use spread like wildfire.

In the last ten years, the development of partition chromatography, the introduction of efficient ion-exchange resins, improved methods of following the process of separations, and the micro-chromatographic methods of filter paper chromatography, have revolutionized organic research. Now no matter what the problem being investigated, in some form or other the organic chemist finds chromatography of value in his work.

The application of modern ideals of paper chromatography to the separation of inorganic compounds, by R. P. Linstead, F. H. Burstall, and R. A. Wells at the Chemical Research Laboratory at Teddington, A. Lacourt at the

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<sup>5</sup>Ibid, p. 2.

University of Brussels, M. Lederer in Australia and F. H. Pollard and J. F. W. McOmie at the University of Bristol, has resulted in revolutionizing methods of analysis in organic chemistry.<sup>6</sup> The advantage of the new techniques over capillary analysis is that by a judicious selection of solvent under the right conditions, it is possible to separate many closely related inorganic compounds into zones several inches apart on strips of filter paper. Furthermore, methods have been devised for the location, identification, and quantitative estimation of the compounds separated. While filter paper can be used for micro-quantities of material, columns of cellulose serve to separate larger quantities in amounts suitable for preparative work. It is claimed that some of the material thus separated is better than spectrographically pure. Chromatography has thus become an important technique in inorganic chemistry as well.

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<sup>6</sup>F. H. Pollard and J. F. W. McOmie, *op. cit.*, p. 4.

## CHAPTER III

### GENERAL PROCEDURES

Chromatography has been defined by H. H. Strain<sup>1</sup> as an analytical technique for the resolution of solutes, in which separation is made by differential migration in a porous medium, and migration is caused by flow of solvents. The essential requirements for the process of paper chromatography are:

- (a) A strip of filter paper which is insoluble in the solvents used.
- (b) A solution of the substance to be separated.
- (c) A solvent which is used to irrigate the strip of filter paper after the solution of the substances has been placed on it. The solvent is known as the mobile phase.
- (d) A substance, called a developer, is usually needed to help locate the separated substances.

One of the great advantages of paper chromatography is the simplicity with which the chromatograms can be prepared. This method requires very little special equipment, and even that can usually be improvised. The general procedures

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<sup>1</sup>H. H. Strain, Chromatographic Adsorption Analysis (New York, 1942), p. 2.

described in this chapter will be of the ascending type. Only minute quantities of the materials will be necessary and results should be satisfactory with little difficulty if the minimum care is exhibited.

The filter paper can be an ordinary grade of Whatman No. 1 which permits a moderate flow. The filter paper should be approximately 25 cm in length. A pencil mark needs to be made about 5 cm from the end which is to be dipped into the solvent. Arrangements should be made to suspend the strips of filter paper in closed containers while the process is in operation. A large test tube (10 cm x 38 cm) or a milk bottle will serve well. A small amount of solution to be separated is placed at the marked spot on the filter paper. The amount of this solution will depend upon the substance to be chromatographed, but it should be round and about 2mm in diameter. A common eye-dropper will produce a spot far too large. If the head of a straight pin is dipped into the solution it will retain the approximate amount necessary. The spot should be thoroughly dried before the filter paper is dipped into the solvent. It is advisable to place a weight at the bottom of the strip of filter paper so that it can be kept taut. A double slot cut into the end will hold a piece of glass rod. The time which is required for the migration of the solvent will vary according to the particular experiment. After the proper time has elapsed, the strip of filter paper is removed, the highest point of solvent migration

marked, and thoroughly dried, sometimes in air and sometimes in an oven at specific temperatures. A developer is sometimes sprayed on the substances which have been separated. It is advisable to run a series of single substances to use as control in order to properly identify the members of a group.

Relative movements of the solutes are conveniently given in terms of  $R_f$  values, which is defined as:

$$R_f = \frac{\text{Distance of movement of solute.}}{\text{Distance of movement of solvent.}}$$

The  $R_f$  values of various solutes have been established for a given solvent by previous studies.

This method which has been described is very general and simple. Although its application in research is at times complex both in execution and in interpretation, probably no other laboratory technique with such versatility can be presented to the student in rudimentary form so simply and inexpensively. While all sorts of substances are separable by this means, the student may well begin with the separation of amino acids, which is historically its first use and probably its most important application. This method deserves to be included in the curriculum of the future chemist.

## CHAPTER IV

### EXPERIMENTAL PROJECTS

#### EXPERIMENTAL PROJECT I: Separation of Colors into Color Components.<sup>1</sup>

This basic demonstration describes paper chromatography in terms which are readily interpretable within the time covered in one lecture period. It requires no special solvents, developing agents, or equipment. The colors are common food colors which can be purchased at any grocery store.

The solvent consists of a denatured alcohol-water mixture of 2 parts alcohol to 8 parts of water.

The filter paper strips are about 20 cm in length. Test tubes and corks of appropriate size are used.

A spot of each color, usually 4, is applied to the strip of filter paper and allowed to dry. The strips are dipped into the solvent and the process is allowed to begin. It will require only about 30 minutes to complete the operation, but it is advisable to observe the migration of the solvent continuously, as the colors are visible at all times

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<sup>1</sup>Evelyn S. Kritchensky and David Kritchensky, "A Simple Lecture Demonstration of Paper Partition Chromatography," Journal of Chemical Education, July, 1953, p. 370.

and one can actually see the colors separate into their component parts. After complete separation occurs there is no further developing to be done.

The colors will separate as follows: yellow into only yellow; green into leading blue and trailing yellow; blue into leading blue and trailing magenta; red into leading pink and trailing carmen.

The experiment is not quite ideal in that the colors tend to streak, but the resolution is rapid and dramatic and serves well to illustrate the principles of paper partition chromatography.



EXPERIMENTAL PROJECT 2: Separation of Amino Acids.<sup>2</sup>

The amino acid solutions are prepared by dissolving 5 mg each of glycine, d-l methionine, and l-leucine in 15 ml of water. The three solutions may be mixed in equal parts to form a test solution.

The solvent is prepared by mixing 100 ml of n-butanol, 25 ml of glacial acetic acid, and 125 ml of water in a separatory funnel. Shake mixture well, allow it to settle, and discard the lower layer. The remaining upper layer is the usable solvent.

The 4 strips of filter paper should each be about 30 cm in length marked about 3 cm from the lower end.

Put a spot of each solution, about 2 cm in diameter on the marked spot. Allow the spot to become thoroughly dry.

Place about 25 ml of solvent in each test tube, dip the end of the filter paper into it about 1 or 2 cm and place a stopper in the test tube. Allow the process to proceed about an hour and a half or until the solvent has migrated approximately 20 cm. Remove the strips and place them in the air to dry.

During the waiting period prepare a solution of .2 percent solution of ninhydrin in butanol which has been saturated with water. Spray the strips with this developer so that they are damp. Place the strips for 5 or 10 minutes

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<sup>2</sup>Thomas B. Gage, Carl D. Douglas, and Simon H. Wender "A Simplified Laboratory Experiment in Paper Partition Chromatography," Journal of Chemical Education, March, 1950, p. 159.

in an oven that is at 105°C. Note the pink or lavender zones where the amino acids are concentrated.

R<sub>f</sub> values as established by Gage, Douglas, and Wender<sup>7</sup> are: glycine .17; methionine .44; leucine .70.

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<sup>7</sup>Ibid.

EXPERIMENTAL PROJECT 3: Separation of Sugars.<sup>3</sup>

The sugar solutions are prepared by dissolving 5 mg each of glucose, fructose, rhamnose, sucrose, raffinose and rutin in separate test tubes of 10 ml of water. Stir until well dissolved.

The solvent is a mixture of 5 ml of glacial acetic acid, 25 ml of water, and 110 ml of n-butanol. About 20 ml of solution is used for each sugar.

The strips of filter paper, about 30 cm in length, are spotted with the sugar solution. It is necessary to apply the sugar solution 3 or 4 times to the filter paper allowing the spot to thoroughly dry each time. The filter strip is then placed in a test tube containing the solvent and allowed to progress for approximately 2 hours. However, better separation could be achieved by allowing the strips to remain in the solvent overnight.

Dry the strips until the acetic acid odor is absent. Spray them with a developer containing .93 gm of aniline and 1.66 gm of phthalic acid in 100 ml of water-saturated n-butanol. Heat the strips at 105°C in an oven with interior illumination and a window through which the order of appearance of the spots can be observed. At this temperature glucose and rhamnose spots appear first, fructose considerably later, and sucrose and raffinose spots remain colorless.

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<sup>3</sup>A. R. Patton, "Further Student Experiments with Paper Chromatography," Journal of Chemical Education, October, 1950, p. 574.

These 2 spots can be developed by heating above 120°C. Rutin can be observed as a yellow area under ultra-violet light. The  $R_f$  value of the fructose is only slightly higher than that of the glucose, but it is easily differentiated from glucose because it appears distinctly later and is a different shade of brown. Rhamnose separates widely from other sugars.

EXPERIMENTAL PROJECT 4: Separation of Plant Pigments.<sup>4</sup>

Place a few grams of fresh or frozen spinach in a mortar, add about three volumes of acetone and macerate. Filter with suction and discard filtrate. Place the residue in the mortar and grind again. By dipping an applicator stick into the acetone solution, apply spots repeatedly to a strip of filter in duplicate until each spot is an intense green. Dry thoroughly after each application.

Place strips of filter paper in test tubes containing about 20 ml of petroleum ether, such as Skellysolve B as the solvent. As this chromatogram requires less than 10 minutes it is advisable to watch the progress continuously.

In order of increasing  $R_f$  values, the colored areas which separate are chlorophylls, xanthophylls, and a gray area sometimes appears which is due to decomposed chlorophylls.

This experiment can be repeated with carrots or tomato catsup.

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<sup>4</sup>Ibid.

EXPERIMENTAL PROJECT 5: Separation of Chloride, Bromide, and Iodide.<sup>5</sup>

The solutions are prepared by putting 1 gram each of the sodium salts in 10 ml of water. The three solutions are mixed in equal proportions to form the unknown solution.

A solvent may be prepared containing 90 ml of pyridine and 10 ml of water. An alternate solvent which may also be used is prepared by mixing 80 ml of acetone and 20 ml of water.

The filter paper strips are spotted with the solution, allowed to dry, and placed in a test tube containing one of the solvents. The migration of the solvent is allowed to continue for about 3 hours or until it has reached a height of about 20 cm.

After the strips are removed and thoroughly dried in air they are sprayed with a developer consisting of a mixture of 2 percent silver nitrate and fluorescein solution. The fluorescein solution is prepared with a 5 percent solution of 8-hydroxyquinoline in 60 percent ethanol. After drying, the halides appear as characteristic dark spots. The chloride is dark brown or black in natural light and under ultra-violet light as a dark band on a fluorescent background. The bromide is also a dark brown or black under natural light, but is located as the middle spot of the group. The iodide is yellow and located in the lead.

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<sup>5</sup>F. H. Burstall, G. R. Davies, R. P. Linstead, and R. A. Wells, "Inorganic Chromatography on Cellulose. Part II," Journal of Chemical Society, January, 1950, pp. 516-528.

EXPERIMENTAL PROJECT 6: Separation of Nickel, Manganese, Cobalt and Zinc.<sup>6</sup>

The solutions are prepared by putting 1 gram of each of the chloride in 10 ml of water. The solution is made acid by adding 1 or 2 drops of dilute hydrochloric acid. An unknown solution may be prepared by combining equal parts of the four solutions.

A solvent is prepared by mixing 5 ml of water, 100 ml of acetone, and 8 ml of 6-N hydrochloric acid.

The filter paper strips are spotted with the solution, thoroughly dried, and dipped into the solvent. It requires about 3 hours for the solvent to migrate about 20 cm. After the strips are removed and thoroughly dried, they are exposed to an ammonia vapor and then sprayed with the developer; alizarin, rubeanic acid in alcohol. The developer is prepared by dissolving a .1 percent solution of alizarin red S in water, and a .5 percent rubeanic acid in 96 percent ethanol.

Nickel will appear as a blue spot near the starting point, manganese is yellow and above nickel. Cobalt is next in order with brown spot, and zinc is in the solvent front.

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<sup>6</sup>Ibid.

## CHAPTER V

### SUMMARY

The projects included in this report have been carried out in the laboratory by the writer. Some of them were very impressive in their results due to the rapidity of the process and the colorations obtained. Other of the projects were valuable in that they accomplished a purpose, but were slow in operation and the material was colorless. In order that a project be used for the purpose as outlined in the problem, it should be fairly rapid and colorful. The separation of color into color components, separation of amino acids, and separation of plant pigments are projects which will do just this. The other projects would not be very impressive to the high school student.

The projects of this report are intended to be supplemental material for presentation to a high school class. The writer feels that there is a definite need to introduce this very important phase of chemistry into the existing high school curriculum. This report is made in hopes that it will be of value to the teacher in meeting the aforesaid need.



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