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STUDIES IN ADSORPTION CHROMATOGRAPHY

I. THE CHROMATOGRAPHIC BEHAVIOR OF A SERIES OF PHENOLS AND EVALUATION OF STREAK REAGENTS APPLICABLE FOR THEIR DETECTION

II. AN EVALUATION OF VANILLIN AS A COLOR PRODUCING AGENT FOR QUANTITATIVELY DETERMINING RESORCINOL

III. DETECTION OF RESORCINOL IN THE PRESENCE OF ITS ISOMERS

A THESIS

Presented to

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Master of Science in Chemistry

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Approved:

Date Approved by Chairman:

May 15, 1960

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SUMMARY

Adsorption chromatography is a very valuable tool for the isolation and characterization of organic compounds. Since the occurrence of colored compounds is less frequent than colorless compounds, the potential of chromatography for identification purposes cannot be fully realized without means of detecting colorless compounds. Colorless zones on a column may be detected by streaking with an appropriate reagent or series of reagents which will react to form a colored product. Streak reagents have been reported for several homologous series of organic compounds; however, no generally useful reagent has been reported for the detection of phenols. One purpose of the present investigation was to determine R values for a number of phenols, and to evaluate reagents which might be applicable for their detection.

A large number of phenols were chromatographed employing conventional techniques, the columns were extruded, and several streak reagents were evaluated for the detection of phenols. Of the reagents evaluated vanillin in sulfuric acid and 4-aminoantipyrine were the most effective. Both reagents possess great sensitivity; however, 4-aminoantipyrine is capable of detecting more phenols. Vanillin is specific for phenols, but 4-aminoantipyrine possesses the disadvantage that it will also detect some anilines. The combined use of the

two reagents and R values should be of great value for the detection and characterization of phenols.

Vanillin in sulfuric acid produced red, violet, and brown zones with resorcinol, catechol, and hydroquinone, respectively, when employed as a streak reagent in adsorption chromatography. The decided difference in the coloration of the zones suggested that vanillin in sulfuric acid might be employed to quantitatively determine the isomers in a mixture by a colorimetric procedure. Vanillin in sulfuric acid produces a very intense red coloration with aqueous solutions of resorcinol. The absorption maximum was determined to be 518 millimicrons. However, further investigation revealed that vanillin in sulfuric acid and aqueous solutions of catechol and hydroquinone also produced solutions with absorption maxima at 518 millimicrons. This alone rendered the method useless unless a separation could be effected. An attempt to isolate and characterize the product of the resorcinol, vanillin reaction failed.

Even though the vanillin reagent cannot be employed to quantitatively determine resorcinol, catechol, and hydroquinone in a mixture, it can be utilized in conjunction with adsorption chromatography to differentiate the isomers in a mixture. Vanillin and 4-aminoantipyrine were found to be very selective for resorcinol, and can be used to detect minute quantities of resorcinol in the presence of catechol and hydroquinone. The lower limit of detection for the reagents is five gamma.

PART I

THE CHROMATOGRAPHIC BEHAVIOR OF A SERIES OF PHENOLS AND EVALUATION OF STREAK REAGENTS APPLICABLE FOR THEIR DETECTION

CHAPTER I

INTRODUCTION

Chromatography was defined by Strain (1) as "an analytical technique for resolution of solutes, in which separation is made by differential migration in a porous media and migration is caused by flow of solvent." This very general definition is applicable to various types of chromatography. The present investigation concerns adsorption chromatography, and several common terms employed in it should be defined. The chromatographic process involves the distribution of a solute, called the adsorptive, between a stationary phase and a mobile phase. In adsorption chromatography the stationary phase is a solid adsorbent, and the mobile phase is an organic solvent or mixture of solvents. The mobile phase is commonly referred to as the developer. The ratio of the rate, or distance, a solute moves relative to the rate, or distance, the developer moves is characteristic of the solute for the particular adsorbent and developer employed. This ratio is called the R value.

Adsorption chromatography provides a very rapid and convenient method for the separation and characterization of organic compounds; it is particularly useful for the separation and characterization of isomers and homologues. Initially, chromatography was associated with colored compounds and was

consequently of limited value since the occurrence of colored compounds is much less than that of colorless compounds. The full potential of chromatography for identification purposes cannot be realized without means of detecting colorless compounds. The problem of detection presented by colorless compounds can be eliminated if a suitable reagent or series of reagents is available which will react with the adsorptive to form a colored product. Positions of zones on a column can be determined by developing the column, extruding, and streaking with an appropriate reagent or series of reagents. Ideally, a reagent should produce a characteristic color with each compound, but this result is seldom obtained. A combination of R value and color is helpful for identification.

Streak reagents for several homologous series have been reported (2, 3); however, no generally useful reagent has been reported for the detection of phenols. Several reagents commonly employed in spot tests and semimicro qualitative analysis were selected for investigation as possible streak reagents, among them the following: nitrous acid (4), 5-nitroso-8-quinolinol (5), ferric chloride (6), ceric nitrate (7), alkaline permanganate (3), chloranil (7), p-nitrobenzenediazonium tetrafluoroborate (8), vanadium-oxine (9), 4-aminoantipyrine (4, 10), vanillin (3), and phosphomocybdic acid (11).

Many methods of standardizing adsorbents have been proposed. Brockman and Schodder (12) determined the activity of alumina by the chromatographic behavior of several selected

dyes. Muller (13) developed a method of determining the activity of adsorbents by measuring the heat evolved by contact between solvent and adsorbent. Several investigators (13, 14) have observed the dependence of strength of adsorption of various grades of silicic acid on the water content. Adsorbents partially deactivated by the addition of water have been shown to afford better separation than the corresponding activated adsorbents. R values have little meaning unless a standardization, such as the R value of a dye or the moisture content of the adsorbent, is reported. Silicic acid and Florisil containing about 12.5 and 2.5 per cent water, respectively, were employed as adsorbents in the present investigation.

In a recent investigation Carlton (15) observed a very decided difference in coloration of the resorcinol, catechol, and hydroquinone zones when vanillin in sulfuric acid was employed as streak reagent. Hydroquinone and resorcinol are difficult to separate chromatographically; however, one of the compounds could possibly be detected in the presence of its isomer if a streak reagent was available which would produce different colors with the compounds.

One purpose of the present investigation was to determine R values of a number of phenols, and to evaluate reagents which might be applicable for their detection. Another purpose was to determine the feasibility of differentiating between hydroquinone and resorcinol employing adsorption chromatography and the appropriate streak reagents.

CHAPTER II

CHEMICALS AND EQUIPMENT

Chemicals

The following chemicals were obtained as Eastman White Label grade and were used without further treatment.

4-Aminoantipyrine hydrochloride

o-Bromophenol

p-Bromophenol

n-Butyl ether

4-Chlororesorcinol

2,4-Dinitrophenol

Eugenol

Hydroquinone monomethyl ether

2-Hydroxy-1,4-dimethyl benzene

4-Hydroxy-1,2-dimethyl benzene

4-Hydroxy-1,3-dimethyl benzene

5-Hydroxy-1,3-dimethyl benzene

o-Hydroxyacetophenone

p-Hydroxyacetophenone

β-Naphthol

5-Nitroso-8-quinolinol

o-Nitrophenol

m-Nitrophenol

p-Nitrophenol

Phenol

o-Phenylphenol

p-Phenylphenol

Pyrogallol

8-Quinolinol

Resorcinol

Salicylaldehyde

Salicylamide

Salicylic Acid

Thymol

2,4,6-Trichlorophenol

Vanillin

The following chemicals were obtained as Eastman Practical grade and were used without further treatment.

4-Chloro-2,6-dinitrophenol

o-Chlorophenol

p-Chlorophenol

m-Ethylphenol

p-Ethylphenol

Guaiacol

p-Hydroxyacetophenone

2,4,5-Trichlorophenol

The following chemicals were obtained as Eastman Technical grade and were used without further treatment.

Pentabromophenol

Pentachlorophenol

The following chemicals were obtained as Merck Reagent grade and were used without further treatment.

n-Butanol

Benzene

Chloroform

Sodium molybdate

Sodium phosphate dibasic

The following chemicals were obtained as J. T. Baker Reagent grade and were used without further treatment.

Ammonium hydroxide

Nitric acid

Sulfuric acid

The following chemicals were obtained as Eastman Practical grade and were recrystallized from chloroform.

Catechol

Hydroquinone

The following chemicals were obtained as Eastman Practical grade and were redistilled under reduced pressure.

o-Cresol

m-Cresol

p-Cresol

Picric acid.--J. T. Baker National Formulary grade picric acid containing ten per cent water was dried at 80° C for twelve hours.

α-Naphthol.--J. T. Baker Purified grade α-naphthol was recrystallized from water and dried in a vacuum desiccator.

Florisil.--100 Mesh Florisil was obtained from the Floridin Company, Tallahassee, Florida and ground in a ball mill for three hours. It was used without further treatment (see Table 1 for analysis).

Silicic acid.--Merck Reagent grade silicic acid was ground in a ball mill for three hours and then dried at 150° C for twelve hours. An appropriate amount of water was added to give the desired moisture content, and then the mixture was blended for three hours in a tumbling devise (see Table 1 for analysis).

Solutions

Phenol solutions.--0.01 Molar solutions of the phenols to be chromatographed were prepared in chloroform.

Alkaline permanganate.--A 0.0075 molar potassium permanganate solution in 0.25 molar sodium hydroxide was prepared.

4-Aminoantipyrine.--One gram of Eastman White Label grade 4-aminoantipyrine hydrochloride was dissolved in 50 milli-

liters of water and diluted with an equal volume of 1:1 ammonium hydroxide prior to use.

Ammonium vanadate.--C. P. ammonium vanadate was obtained from E. H. Sargent and a solution containing 200 to 300 milligrams per liter of vanadium was prepared.

Ceric nitrate.--Twenty grams of G. Frederick Smith Reagent grade ceric ammonium nitrate was dissolved in 50 millliliters of 2 molar nitric acid.

Chloranil.--Eastman Practical grade chloranil was obtained and a saturated solution prepared in dioxane.

Ferric chloride.--A four per cent solution was prepared by dissolving the appropriate amount of J. T. Baker Reagent grade anhydrous ferric chloride in water.

Phosphomolybdic acid.--2.0 grams of sodium phosphate dibasic, 5.0 grams of sodium molybdate, and 20.0 millliliters of concentrated nitric acid were added to enough water to give a total volume of 100 millliliters.

Potassium ferricyanide.--A two per cent aqueous solution was prepared from J. T. Baker Reagent grade potassium ferricyanide.

5-Nitroso-8-quinolinol.--One gram of Eastman White Label grade 5-nitroso-8-quinolinol was dissolved in 100 grams of sulfuric acid.

Nitrous acid.--One gram of J. T. Baker Reagent grade sodium nitrite was dissolved in 100 grams of sulfuric acid.

p-Nitrobenzenediazonium tetrafluoroborate.--p-Nitrobenzenediazonium tetrafluoroborate was prepared (16), and a one per cent aqueous solution was prepared. The solution is stable for only about two days, and a fresh solution should be prepared as needed.

8-Quinolinol.--Eastman White Label grade 8-quinolinol was obtained and a two and one half per cent solution was prepared in six per cent acetic acid.

Vanillin.--One gram of Eastman White Label grade vanillin was dissolved in 100 grams of sulfuric acid.

Equipment

Chromatographic tubes.--Borosilicate glass, chromatographic tubes, number one precision taper, were obtained from the Scientific Glass Company, Bloomfield, New Jersey.

Vacuum pump.--A Welch model 1404H vacuum pump was obtained from the W. W. Welch Scientific Company. Any good vacuum pump will suffice.

CHAPTER III

PROCEDURE

A conventional chromatographic apparatus was assembled in the following manner. A vacuum pump was connected through a three-way stopcock to a surge tank. The three-way stopcock allowed the vacuum pump to be vented when not in use, and the surge tank was employed to eliminate large fluctuations in pressure. A series of T-tubes was connected to the surge tank. Suction flasks, which supported the chromatographic tubes, were connected through three-way stopcocks to the T-tubes. A manometer was attached to one of the tubes.

The lower section of the chromatographic apparatus consisted of a tube with a Standard Taper 10/18 inner joint with a perforated glass insert. This tube was placed in a one-hole rubber stopper, and mounted in the suction flask. The chromatographic tube, or upper section of the apparatus, was provided with a Standard Taper 10/18 outer joint so that the two sections could be joined. The insert supported a cotton wad which in turn supported the adsorbent.

The adsorbents were stored in 250 milliliter wide mouth bottles provided with delivery tubes. The delivery tube consisted of a one-hole rubber stopper, through which was inserted a short section of ten millimeter inside

diameter glass tubing attached to a short piece of thin-walled rubber tubing closed with a pinch clamp.

The following procedure was used for filling the columns with adsorbent. A small wad of cotton was tamped into the bottom of the chromatographic tube. Adsorbent was delivered from the storage bottle into the tube by removing the pinch clamp, attaching the rubber tubing to the chromatographic tube, and shaking the bottle until the desired amount of adsorbent had been delivered to the tube. Uniform packing was achieved by tapping the tube vigorously with a dowel rod while applying the maximum vacuum supplied by the pump. Finally, the top of the column was leveled with a dowel rod without exerting pressure on the adsorbent. Columns of approximately equal lengths were desirable and were attained with practice.

With the vacuum still applied, a 0.5 milliliter sample of the solution to be chromatographed was pipetted onto the top of the packed column, and followed immediately by a small portion of the developing solvent. The phenol was worked onto the column with several small increments of developer, and finally the free space above the adsorbent was filled with developer. Development was allowed to proceed until the solvent reached the bottom of the column. The vacuum was disconnected by means of a three-way stopcock which simultaneously vented the suction flask. The chromatographic

tube was lifted from the support and tapped on a folded towel until the column of adsorbent was loosened. The column was then extruded onto a smooth surface such as a porcelain burette stand base with the aid of a dowel rod. The column was then streaked with the desired color producing reagent.

Most streak reagents employed in adsorption chromatography consist of a single solution; however, it is not uncommon to streak with one reagent, and then overstreak with another to complete the reaction. Of the streak reagents employed in this investigation only vanadium-oxine, and 4-aminoantipyrine consisted of two solutions. To utilize the vanadium-oxine reaction the column was first streaked with ammonium vanadate solution and then with 8-quinolinol. The 4-aminoantipyrine reaction was achieved by streaking with aqueous potassium ferricyanide, and then overstreaking with 4-aminoantipyrine reagent.

When the zone identifying a phenol appeared, measurements were made to determine the R value. A ruler graduated in millimeters was placed beside the column, and the column length and the distance from the top of the column to the lower edge of the zone were recorded. Also, the color and sensitivity of the reagents were noted so that the reagents could be evaluated. R values reported in the tables are the average of at least two determinations.

CHAPTER IV

DISCUSSIONS OF RESULTS

The R values obtained from the chromatography of the phenols are listed in Tables 2 through 5. R values listed in Tables 2 and 3 are those for which silicic acid was employed as adsorbent. Florisil was used as adsorbent for R values contained in Tables 4 and 5. Benzene and butyl ether were employed as developers for the R values listed in Tables 2 and 3 and Tables 3 and 5 respectively. Some pK_a values are also listed in Tables 2 and 4.

An examination of the tables will reveal that R values and acid strengths cannot be correlated directly. From Table 2, phenol and hydroquinone have the same pK value and yet there is a decided difference in the R values. Furthermore, the R values of catechol and picric acid are very similar, and yet the acid strengths differ by 8.60 pK units. Even though acid strengths and R values cannot be correlated directly, one cannot overlook the relationship between R values and the availability of the electron pairs on the phenolic oxygen atom.

The factors which influence adsorption are numerous, and to fully understand adsorption a knowledge of the roles of solvent, adsorptive, adsorbent, and their complex inter-

actions is essential. However, the behavior of many systems may be understood on the basis of steric effects, internal hydrogen bonding, inductive effects, and resonance effects.

Carlton and Bradbury (17) studied a series of phenols, and demonstrated that large ortho alkyl groups effectively blocked the approach of the phenolic group to the adsorbent. Steric hindrance has also been employed to explain large R values in ortho disubstituted benzenes (18) and anilines (2). In the present investigation the R values of o-cresol, o-chlorophenol, o-bromophenol, o-hydroxyacetophenone, and guaiacol were large as compared to the corresponding meta and para isomers, and is in complete agreement with the steric interpretation. α-Naphthol exhibits less adsorption than β-naphthol. This can be explained on the basis of steric hindrance if the carbon atom in the eight position of the naphthalene ring is considered to be equivalent to an ortho methyl group. Compare α-naphthol and β-naphthol with o-cresol and m-cresol, respectively. The spatial arrangement around the phenolic group is similar in the naphthol and the corresponding cresol, and the R values would be expected to be similar. This was found to be true.

Frequently an ortho substituted aromatic compound will exhibit an R value very near 1.00, and thus indicate little or no adsorption. In most of these cases adjacent groups are capable of internal hydrogen bonding, and thus the electrons

on the phenolic oxygen atom are not available for hydrogen bonding to the adsorbent. Such an effect is exhibited by salicylaldehyde and o-nitrophenol, and these compounds have R values of 0.90 and 1.00, respectively, when silicic acid and benzene are employed as adsorbent and developer.

The factors listed previously can usually be utilized to qualitatively predict the degree of adsorption a compound will exhibit provided only one group is capable of actively participating in the adsorption process. If two or more groups may participate in adsorption the task of predicting R values is more difficult.

Several compounds studied possessed R values which would not be expected, and will be discussed. o-Nitrophenol, m-nitrophenol, p-nitrophenol, 2,4-dinitrophenol, and 2,4,6-trinitrophenol have R values of 1.00, 0.21, 0.15, 0.70, and 0.11, respectively (silicic acid adsorbent and benzene developer). The large R value of o-nitrophenol is expected, and is in complete agreement with the internal hydrogen bonding interpretation. Also, the R values of m-nitrophenol and p-nitrophenol would be expected to be somewhat smaller than the R value of phenol provided the nitro group participates in adsorption. Adsorption by the nitro group and internal hydrogen bonding are sufficient to explain the R values of 2,4-dinitrophenol, however, the

very small R value of 2,4,6-trinitrophenol cannot be explained by these factors.

Hydrogen bonding is assumed to play a major role in the adsorption process. There are two ways a hydrogen bond may be formed between the adsorptive and the adsorbent. First, the adsorbent may act as hydrogen donor and the adsorptive as hydrogen acceptor, and secondly, the process may be reversed so that the adsorbent accepts a hydrogen donated by the adsorptive.

Now consider 2,4,6-trinitrophenol. The phenolic hydrogen would be expected to be highly electronegative, and adsorption may occur by the formation of a hydrogen bond in which the adsorptive acts as hydrogen donor. 2,4,6-Trinitrophenol has a pK of 0.80, and is thus about 32 per cent ionized in aqueous solution. Although the compound will certainly be somewhat less ionized in the less polar developers, one must not overlook the possibility that the picrate ion is the adsorbing species. In each of the above cases steric hindrance by the large ortho-nitro groups is still operative, but in the latter case internal hydrogen bonding is eliminated. The unexpected adsorption exhibited by 2,4,6-trinitrophenol is probably due to a combination of these factors, and not simply adsorption by the nitro groups.

An examination of the tables will also reveal that in all combinations of adsorbents and developers the R values of

p-nitrophenol are less than the corresponding R values of m-nitrophenol. Both the phenolic group and the nitro group may be responsible for adsorption, and resonance effects may operate from the para position but not from the meta position. Apparently the adsorbability of the nitro group is enhanced to a greater extent than that of the phenolic group is decreased.

Several interesting factors are noted by comparing the R values listed in Tables 2 through 5 and the corresponding adsorbents and developers. Butyl ether is a much stronger developer than benzene as one would anticipate. Electron pairs of the ethereal oxygen compete with the electron pairs of the phenolic oxygen atom for hydrogens of the adsorbent. Silicic acid containing about twelve and a half per cent water and Florisil containing about two and a half per cent water have similar properties. However, Florisil exhibits a marked affinity for nitro compounds, particularly if an ortho-nitro group is present in the phenol. This enhanced affinity for ortho-nitro phenols can be explained by postulating a six membered chelate ring formed by joining oxygen atoms of the phenolic and nitro groups to the magnesium ion of Florisil. The formation of a six membered chelate ring will certainly take precedence over the alternate possibility of internal hydrogen bonding. Silicic acid does not offer the possibility of

chelate formation. Thus, internal hydrogen bonding operates and large R values result when this adsorbent is employed.

Compounds capable of forming five and six membered rings behave in a fashion similar to the ortho-nitrophenols, and support the chelate ring interpretation. Although Florisil is apparently a slightly stronger adsorbent than silicic acid, the decided decrease in R values on Florisil compared to silicic acid for compounds capable of forming five and six membered chelate rings cannot be ascribed only to this factor. The following compounds are capable of forming five or six membered rings and support the above interpretation; catechol, salicylaldehyde, eugenol, guaiacol, o-nitrophenol, 2,4-dinitrophenol, 2,4,6-trinitrophenol, and 4-chloro-2,6-dinitrophenol.

Several reagents were evaluated which may be applicable for the detection of phenols. Colors produced with the reagents are listed in Tables 6 through 11. Solutions of all phenols listed in the tables were chromatographed, extruded, and streaked with the various reagents. Blanks in the tables indicate that the corresponding phenols were not detected with the reagent at the concentration studied.

Several reagents employed are useful for studying R values, however, their use for identification purposes is limited by their ability to detect various classes of compounds. Alkaline permanganate and p-nitrobenzenediazonium tetrafluoroborate are members of this group. Alkaline

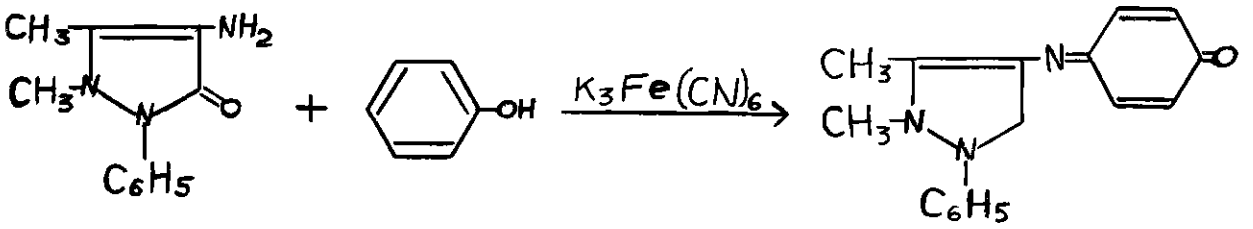
permanganate is reduced by any easily oxidizable compound; the zone appears brown over a purple streak. p-Nitrobenzenediazonium tetrafluoroborate is capable of coupling with most active phenols in the ortho or para positions. However, this reagent will also react with anilines under similar conditions to form diazoaminobenzenes or the rearranged aminoazobenzenes. Coupling reactions are enhanced in basic media, and overstreaking the p-nitrobenzenediazonium tetrafluoroborate reagent with 6 N sodium hydroxide increases the detection limits.

Vanillin in sulfuric acid is an excellent streak reagent for the detection of phenols. Most phenols containing electron donating groups are easily detected by this reagent, however, strong withdrawing groups inhibit the reaction. Vanillin in sulfuric acid is a specific reagent for phenols, and consequently is a very valuable reagent. The chemistry of the reaction is not fully understood, but is believed to consist of a condensation reaction with ultimate production of a quinone type product.

4-Aminoantipyrine has been employed as a color producing reagent for the quantitative estimation of very minute quantities of phenol in water (10). The sensitivity of the reagent is equally well suited for the detection of phenols in adsorption chromatography. Tables 6 and 7 reveal that 4-aminoantipyrine is more general than the vanillin reagent for the detection of phenols. Also, vanillin in

sulfuric acid produces a dark streak the entire column length when employed in conjunction with butyl ether developer. 4-Aminoantipyrine does not exhibit this undesirable effect. Aniline and the toluidines may be detected with the reagent, and, of course, this is a disadvantage. However, the reagent will not detect chloro, bromo, and nitroanilines, and consequently is generally a very excellent reagent for the detection of phenols.

Martin (10) outlines the following reaction for phenol and 4-aminoantipyrine.



The reagent will normally condense para to the phenolic group, however, if this position is occupied the reaction will take place in the ortho position.

Several other reagents were investigated and all were found to be of little or no use for detecting phenols. Phosphomolybdic acid is capable of detecting only a limited number of phenols, and the sensitivity of the reagent is very poor. Ceric nitrate is not a generally useful reagent for the detection of phenols as it is not very sensitive, and is also capable of detecting other classes of compounds such as anilines. Chloranil and vanadium-oxine were found to be of no

use for the detection of phenols. Nitrous acid may be employed for the detection of phenols; however, the reagent is very unstable and similar results can be obtained with the much more stable p-nitrobenzenediazonium tetrafluoroborate.

Ferric chloride will not detect many phenols, but has the advantage that it is specific for phenols which are capable of chelate formation. Purple to black colors are produced with catechol, salicylic acid, salicylamide, and pyrogallol.

Of the reagents evaluated vanillin in sulfuric acid and 4-aminoantipyrine were found to be excellent streak reagents for detection of phenols. 4-Aminoantipyrine is possibly the better of the two reagents as it will detect more phenols, possesses great sensitivity, and is almost specific for phenols. Vanillin is an excellent reagent also, and the combined use of the two reagents and R values will serve to identify most phenols.

APPENDIX

Table 1. Particle Size Distribution of Adsorbents

Silicic Acid

Screen Size (U. S.)	Weight of Adsorbent Retained on Screen from a 100 g. Sample
80	0.64 g.
100	17.60 g.
140	56.87 g.
170	14.44 g.
200	0.42 g.
325	2.12 g.
>325	7.04 g.

Florisil

Screen Size (U. S.)	Weight of Adsorbent Retained on Screen from a 100 g. Sample
80	14.23 g.
100	36.31 g.
140	30.91 g.
170	7.80 g.
200	2.34 g.
325	5.38 g.
>325	1.89 g.

Table 2. R Values and Some pK Values of a Series of Phenols on Silicic Acid Adsorbent with Benzene Developer

Compound	R	pK
Phenol	0.38	10.0
Catechol	0.12	9.4
Resorcinol	0.00	9.4
Hydroquinone	0.00	10.0
Pyrogallol	0.00	7.0
α-Naphthol	0.51	
β-Naphthol	0.40	
o-Cresol	0.47	10.20
m-Cresol	0.37	10.01
p-Cresol	0.36	10.17
m-Ethylphenol	0.41	
p-Ethylphenol	0.40	
2-Hydroxy-1,4-dimethylbenzene	0.56	
4-Hydroxy-1,2-dimethylbenzene	0.36	
4-Hydroxy-1,3-dimethylbenzene	0.53	
5-Hydroxy-1,3-dimethylbenzene	0.42	
Eugenol	0.65	
Thymol	0.69	
o-Nitrophenol	1.00	7.21
m-Nitrophenol	0.21	8.00
p-Nitrophenol	0.15	7.16

Table 2. (continued)

Compound	R	pK
2,4-Dinitrophenol	0.70	4.0
2,4,6-Trinitrophenol	0.11	0.80
o-Bromophenol	0.70	
p-Bromophenol	0.41	
Pentabromophenol	1.00	
o-Chlorophenol	0.72	
p-Chlorophenol	0.37	
2,4,5-Trichlorophenol	0.82	
2,4,6-Trichlorophenol	0.72	
Pentachlorophenol	0.78	
4-Chlororesorcinol	0.11	
4-Chloro-2,6-dinitrophenol	0.45	
Guaiacol	0.63	
Hydroquinone monomethyl ether	0.23	
o-Hydroxyacetophenone	0.76	
p-Hydroxyacetophenone	0.13	
Salicylic acid	0.15	
Salicylamide	0.15	
Salicyaldehyde	0.90	
p-Hydroxybenzaldehyde	0.14	

Table 3. R Values of a Series of Phenols on Silicic Acid Adsorbent with Butyl Ether Developer

Compound	R
Phenol	0.87
Catechol	0.59
Resorcinol	0.35
Hydroquinone	0.32
Pyrogallol	0.26
α-Naphthol	1.00
β-Naphthol	0.85
o-Cresol	1.00
m-Cresol	0.88
p-Cresol	0.86
m-Ethylphenol	0.89
p-Ethylphenol	0.90
2-Hydroxy-1,4-dimethylbenzene	0.93
4-Hydroxy-1,2-dimethylbenzene	0.87
4-Hydroxy-1,3-dimethylbenzene	0.96
5-Hydroxy-1,3-dimethylbenzene	0.87
Eugenol	0.90
Thymol	1.00
o-Nitrophenol	1.00
m-Nitrophenol	0.87
p-Nitrophenol	0.78

Table 3. (continued)

Compound	R
2,4-Dinitrophenol	0.62
2,4,6-Trinitrophenol	0.10
o-Bromophenol	0.94
p-Bromophenol	0.85
Pentabromophenol	1.00
o-Chlorophenol	0.91
p-Chlorophenol	0.88
2,4,5-Trichlorophenol	1.00
2,4,6-Trichlorophenol	1.00
Pentachlorophenol	1.00
4-Chlororesorcinol	0.56
4-Chloro-2,6-dinitrophenol	0.29
Guaiacol	0.82
Hydroquinone monomethyl ether	0.73
o-Hydroxyacetophenone	1.00
p-Hydroxyacetophenone	0.34
Salicylic acid	0.62
Salicylamide	0.30
Salicylaldehyde	1.00
p-Hydroxybenzaldehyde	0.46

Table 4. R Values and Some pK Values of a Series of Phenols on Florisil Adsorbent and Benzene Developer

Compound	R	pK
Phenol	0.36	10.0
Catechol	0.00	9.4
Resorcinol	0.00	9.4
Hydroquinone	0.00	10.0
Pyrogallol	0.00	7.0
α-Naphthol	0.43	
β-Naphthol	0.34	
o-Cresol	0.46	10.20
m-Cresol	0.34	10.01
p-Cresol	0.34	10.17
m-Ethylphenol	0.40	
p-Ethylphenol	0.37	
2-Hydroxy-1,4-dimethylbenzene	0.51	
4-Hydroxy-1,2-dimethylbenzene	0.32	
4-Hydroxy-1,3-dimethylbenzene	0.46	
5-Hydroxy-1,3-dimethylbenzene	0.35	
Eugenol	0.28	
Thymol	0.66	
o-Nitrophenol	0.30	7.21
m-Nitrophenol	0.17	8.00
p-Nitrophenol	0.10	7.16

Table 4. (continued)

Compound	R	pK
2,4-Dinitrophenol	0.00	4.0
2,4,6-Trinitrophenol	0.00	0.80
o-Bromophenol	0.54	
p-Bromophenol	0.36	
Pentabromophenol	0.10	
o-Chlorophenol	0.61	
p-Chlorophenol	0.39	
2,4,5-Trichlorophenol	0.30	
2,4,6-Trichlorophenol	0.26	
Pentachlorophenol	0.10	
4-Chlororesorcinol	0.09	
4-Chloro-2,6-dinitrophenol	0.00	
Guaiacol	0.44	
Hydroquinone monomethyl ether	0.22	
o-Hydroxyacetophenone	0.32	
p-Hydroxyacetophenone	0.00	
Salicylic acid	0.00	
Salicylamide	0.00	
Salicylaldehyde	0.17	
p-Hydroxybenzaldehyde	0.05	

Table 5. R Values of a Series of Phenols on Florisil Adsorbent with Butyl Ether Developer

Compound	R
Phenol	0.87
Catechol	0.20
Resorcinol	0.40
Hydroquinone	0.38
Pyrogallol	0.00
α-Naphthol	0.95
β-Naphthol	0.83
o-Cresol	0.92
m-Cresol	0.88
p-Cresol	1.00
m-Ethylphenol	0.89
p-Ethylphenol	0.86
2-Hydroxy-1,4-dimethylbenzene	0.96
4-Hydroxy-1,2-dimethylbenzene	0.88
4-Hydroxy-1,3-dimethylbenzene	0.96
5-Hydroxy-1,3-dimethylbenzene	0.89
Eugenol	0.51
Thymol	1.00
o-Nitrophenol	0.41
m-Nitrophenol	0.59
p-Nitrophenol	0.39

Table 5. (continued)

Compound	R
2,4-Dinitrophenol	0.00
2,4,6-Trinitrophenol	0.00
o-Bromophenol	0.76
p-Bromophenol	0.82
Pentabromophenol	0.12
o-Chlorophenol	0.86
p-Chlorophenol	0.84
2,4,5-Trichlorophenol	0.14
2,4,6-Trichlorophenol	0.34
Pentachlorophenol	0.11
4-Chlororesorcinol	0.42
4-Chloro-2,6-dinitrophenol	0.00
Guaiacol	0.64
Hydroquinone monomethyl ether	0.64
o-Hydroxyacetophenone	0.52
p-Hydroxyacetophenone	0.25
Salicylic acid	0.00
Salicylamide	0.10
Salicylaldehyde	0.28
p-Hydroxybenzaldehyde	0.30

Table 6. Colors Produced with Vanillin Reagent and Various Phenols

Compound	Silicic Acid-Benzene	Silicic Acid-Butyl Ether	Florisil-Benzene	Florisil-Butyl Ether
Phenol	Gray		Yellow	
Catechol	Violet	Brown	Violet	
Resorcinol	Red	Red	Red	Violet
Hydroquinone	Brown	Green-brown	Brown	
Pyrogallol	Gray	Red-brown	Red	
α-Naphthol	Blue-green		Violet	
β-Naphthol	Gray	Green	Violet	
o-Cresol	Violet		Red-orange	
m-Cresol	Violet		Red-orange	
p-Cresol	Violet			
m-Ethylphenol	Purple	Brown	Yellow-orange	Orange
p-Ethylphenol	Purple	Green	Yellow-orange	
2-Hydroxy-1,4-dimethylbenzene	Red-orange	Brown	Red-brown	Violet
4-Hydroxy-1,2-dimethylbenzene	Violet	Brown	Red-brown	Violet
4-Hydroxy-1,3-dimethylbenzene			Yellow	
5-Hydroxy-1,3-dimethylbenzene	Red			
Eugenol	Violet	Brown	Red-brown	Violet
Thymol	Red-orange	Red	Red	
o-Nitrophenol				

Table 6. (continued)

Compound	Silicic Acid-Benzene	Silicic Acid-Butyl Ether	Florisil-Benzene	Florisil-Butyl Ether
m-Nitrophenol				
p-Nitrophenol				
2,4-Dinitrophenol				
2,4,6-Trinitrophenol				
o-Bromophenol				
p-Bromophenol	Violet			
Pentabromophenol				
o-Chlorophenol				
p-Chlorophenol	Gray			
2,4,5-Trichlorophenol				
2,4,6-Trichlorophenol				
Pentachlorophenol				
Guaiacol	Violet	Green		
Hydroquinone monomethyl ether	Brown	Green	Pink	
o-Hydroxyacetophenone				
p-Hydroxyacetophenone	Violet			
Salicylic acid				
Salicylamide				
Salicylaldehyde	Violet			
p-Hydroxybenzaldehyde				

Table 7. Colors Produced with 4-Aminoantipyrine and Various Phenols

Compound	Silicic Acid-Benzene	Silicic Acid-Butyl Ether	Florisil-Benzene	Florisil-Butyl Ether
Phenol	Red	Red	Red	Red
Catechol	Brown	Purple	Brown	Purple
Resorcinol	Red-brown	Red-brown	Red-brown	Red
Hydroquinone		Purple	Red-brown	Red-brown
Pyrogallol	Red-orange	Purple	Red-brown	
α-Naphthol	Red-brown	Brown	Purple	Green
β-Naphthol	Orange	Green	Red	Red-brown
o-Cresol	Red	Orange	Red	Red
m-Cresol	Red	Orange	Red	Red
p-Cresol	Pink	Yellow	Red	
m-Ethylphenol	Red	Orange	Red-brown	Red-brown
p-Ethylphenol	Pink		Red-brown	Red-brown
2-Hydroxy-1,4-dimethylbenzene	Orange	Orange	Red-brown	Red-brown
4-Hydroxy-1,2-dimethylbenzene	Blue-gray		Red-brown	Red-brown
4-Hydroxy-1,3-dimethylbenzene				Red-brown
5-Hydroxy-1,3-dimethylbenzene	Purple	Purple	Purple	Purple
Eugenol		Green		Red-brown
Thymol				
o-Nitrophenol				

Table 7. (continued)

Compound	Silicic Acid-Benzene	Silicic Acid-Butyl Ether	Florisil-Benzene	Florisil-Butyl Ether
m-Nitrophenol	Red-brown			
p-Nitrophenol				
2,4-Dinitrophenol				
2,4,6-Trinitrophenol				
o-Bromophenol	Red	Red	Red-brown	Red-brown
p-Bromophenol	Red	Violet	Red-brown	Red-brown
Pentabromophenol	Blue-green		Pink	Red-brown
o-Chlorophenol	Red	Red	Red-brown	Red-brown
p-Chlorophenol	Red	Violet	Orange	Red-brown
2,4,5-Trichlorophenol	Orange	Orange	Pink	Red-brown
2,4,6-Trichlorophenol	Red-brown	Orange	Pink	Red-brown
Pentachlorophenol	Blue-green		Pink	Red-brown
Guaiacol	Red	Red	Red-brown	Red-brown
Hydroquinone monomethyl ether	Red	Red-brown	Red	Red
o-Hydroxyacetophenone		Orange	Pink	Red-brown
p-Hydroxyacetophenone				
Salicylic acid				
Salicylamide	Red	Red	Red-brown	Red
Salicylaldehyde			Pink	Pink
p-Hydroxybenzaldehyde				

Table 8. Colors Produced with *p*-Nitrobenzenediazonium Tetrafluoroborate and Various Phenols

Compound	Silicic Acid-Benzene	Silicic Acid-Butyl Ether	Florisil-Benzene	Florisil-Butyl Ether
Phenol	Red-brown	Yellow	Red-brown	Brown
Catechol	Red-brown	Red-brown	Red-brown	
Resorcinol	Orange	Orange	Red-brown	Orange
Hydroquinone	Orange-brown	Red-brown	Red-brown	
Pyrogallol	Red-brown	Red-brown	Red-brown	
α-Naphthol	Red-orange	Red-brown	Red-brown	Brown
β-Naphthol	Orange	Orange	Red-orange	Orange
o-Cresol	Yellow-orange	Yellow	Red-brown	Brown
m-Cresol	Orange	Yellow	Red-brown	Red-brown
p-Cresol	Yellow-orange	Yellow	Orange	Orange
m-Ethylphenol	Orange	Orange	Red-brown	Orange
p-Ethylphenol	Orange	Yellow	Orange	Orange
2-Hydroxy-1,4-dimethylbenzene	Red-orange	Yellow-orange	Brown	Brown
4-Hydroxy-1,2-dimethylbenzene	Yellow-orange	Yellow	Orange	Orange
4-Hydroxy-1,3-dimethylbenzene	Orange	Yellow	Red-brown	Orange
5-Hydroxy-1,3-dimethylbenzene	Orange	Orange	Red-brown	Orange
Eugenol	Orange	Yellow-orange	Brown	Orange

Table 8. (continued)

Compound	Silicic Acid-Benzene	Silicic Acid-Butyl Ether	Florisil-Benzene	Florisil-Butyl Ether
Thymol	Orange	Yellow	Brown	Red-brown
o-Nitrophenol			Red-orange	
m-Nitrophenol				Orange
p-Nitrophenol				
2,4-Dinitrophenol				
2,4,6-Trinitrophenol				
o-Bromophenol	Orange	Yellow	Purple	Red
p-Bromophenol	Orange		Brown	Orange
Pentabromophenol			Red	Orange
o-Chlorophenol	Orange	Yellow	Red	Red
p-Chlorophenol	Orange		Brown-orange	Yellow
2,4,5-Trichlorophenol	Orange		Violet	
2,4,6-Trichlorophenol			Brown	
Pentachlorophenol				
Guaiacol	Orange	Yellow-orange	Purple	Brown
Hydroquinone monomethyl ether	Violet	Orange	Red-brown	Orange
o-Hydroxyacetophenone			Yellow-orange	
p-Hydroxyacetophenone			Red-brown	
Salicylic acid				

Table 8. (continued)

Compound	Silicic Acid-Benzene	Silicic Acid-Butyl Ether	Florisil-Benzene	Florisil-Butyl Ether
Salicylamide	Orange	Yellow	Brown	Orange
Salicylaldehyde			Red-orange	
p-Hydroxy-benzaldehyde			Red-brown	

Table 9. Colors Produced with p-Nitrobenzenediazonium Tetrafluoroborate Overstreaked with 6 N Sodium Hydroxide and Various Phenols

Compound	Silicic Acid-Benzene	Silicic Acid-Butyl Ether	Florisil-Benzene	Florisil-Butyl Ether
Phenol	Violet	Red	Brown	Purple
Catechol	Gray	Purple	Blue-black	
Resorcinol	Purple	Purple	Blue-black	Blue-green
Hydroquinone	Gray		Brown	Purple
Pyrogallol	Purple	Purple	Brown	
α-Naphthol	Purple	Purple	Green	Blue-green
β-Naphthol		Violet	Green	Purple
o-Cresol	Violet	Violet	Brown	Purple
m-Cresol	Violet	Violet	Brown	Purple
p-Cresol	Violet	Purple	Brown	Purple
m-Ethylphenol	Violet	Violet	Black	Purple
p-Ethylphenol	Purple	Purple	Purple	Purple
2-Hydroxy-1,4-dimethylbenzene	Violet	Purple	Brown	Purple
4-Hydroxy-1,2-dimethylbenzene	Violet	Purple	Brown	Purple
4-Hydroxy-1,3-dimethylbenzene	Purple	Purple	Black	Purple
5-Hydroxy-1,3-dimethylbenzene	Purple	Red-brown	Brown	Purple
Eugenol	Purple	Purple	Brown	Purple
Thymol	Purple	Purple	Black	Blue-green
o-Nitrophenol				

Table 9. (continued)

Compound	Silicic Acid-Benzene	Silicic Acid-Butyl Ether	Florisil-Benzene	Florisil-Butyl Ether
m-Nitrophenol			Violet	Red-brown
p-Nitrophenol				
2,4-Dinitrophenol				
2,4,6-Trinitrophenol				
o-Bromophenol	Red-brown	Red	Purple	Violet
p-Bromophenol	Purple	Violet	Purple	Purple
Pentabromophenol	Purple	Violet	Red	
o-Chlorophenol	Red-brown	Red	Purple	Violet
p-Chlorophenol	Purple	Violet	Purple	Purple
2,4,5-Trichlorophenol	Purple		Purple	Violet
2,4,6-Trichlorophenol	Purple			Violet
Pentachlorophenol	Purple			
Guaiacol	Purple	Red-brown	Purple	Purple
Hydroquinone monomethyl ether	Purple	Purple	Blue-green	Blue-green
o-Hydroxyacetophenone	Purple	Violet	Red	
p-Hydroxyacetophenone			Brown	
Salicylic acid	Purple	Violet		
Salicylamide	Orange	Red-brown	Purple	Red-brown
Salicylaldehyde	Purple		Brown	
p-Hydroxybenzaldehyde				

Table 10. Colors Produced with Phosphomolybdic Acid and Various Phenols

Compound	Silicic Acid-Benzene	Silicic Acid-Butyl Ether	Florisil-Benzene	Florisil-Butyl Ether
Phenol			Blue-gray	
Catechol	Blue-gray	Gray	Green	Green
Resorcinol			Green	Blue-green
Hydroquinone	Blue-gray	Blue-green	Blue-green	Blue-green
Pyrogallol	Blue-gray	Blue-green	Green	Green
α-Naphthol	Blue-gray		Blue-green	Blue-green
β-Naphthol	Gray		Blue-green	Blue-green
o-Cresol				Blue-green
m-Cresol				
p-Cresol				
m-Ethylphenol				
p-Ethylphenol				
2-Hydroxy-1,4-dimethylbenzene			Green	
4-Hydroxy-1,2-dimethylbenzene			Green	
4-Hydroxy-1,3-dimethylbenzene			Green	
5-Hydroxy-1,3-dimethylbenzene			Blue-green	Blue-green
Eugenol				Green
Thymol				
o-Nitrophenol				

Table 10. (continued)

Compound	Silicic Acid-Benzene	Silicic Acid-Butyl Ether	Florisil-Benzene	Florisil-Butyl Ether
m-Nitrophenol				
p-Nitrophenol				
2,4-Dinitrophenol				
2,4,6-Trinitrophenol				
o-Bromophenol				
p-Bromophenol				
Pentabromophenol				
o-Chlorophenol				
p-Chlorophenol				
2,4,5-Trichlorophenol				Green
2,4,6-Trichlorophenol				
Pentachlorophenol				
Guaiacol			Red-brown	
Hydroquinone monomethyl ether	Blue-gray	Blue-green	Blue-green	Blue-green
o-Hydroxyacetophenone				
p-Hydroxyacetophenone	Blue-gray			
Salicylic acid				
Salicylamide				
Salicylaldehyde				Blue-green
p-Hydroxybenzaldehyde	Blue-green			

Table 11. Colors Produced with Ceric Nitrate and Various Phenols

Compound	Silicic Acid-Benzene	Silicic Acid-Butyl Ether	Florisil-Benzene	Florisil-Butyl Ether
Phenol	Red-brown		Red-brown	
Catechol	Red-brown	Red-brown	Brown	Green
Resorcinol	Red-brown		Red-brown	Red-brown
Hydroquinone				
Pyrogallol	Red-brown	Brown		Red-brown
α-Naphthol	Red-brown		Red-brown	Orange
β-Naphthol	Red-brown		Red-brown	Orange
o-Cresol			Red-brown	
m-Cresol			Red-brown	
p-Cresol				
m-Ethylphenol	Red-brown	Yellow-orange	Red-brown	Red-brown
p-Ethylphenol		Yellow-orange	Red-brown	
2-Hydroxy-1,4-dimethylbenzene				
4-Hydroxy-1,2-dimethylbenzene				
4-Hydroxy-1,3-dimethylbenzene				
5-Hydroxy-1,3-dimethylbenzene	Red-brown		Red-brown	
Eugenol			Red-brown	
Thymol			Red-brown	
o-Nitrophenol			Red-brown	

Table 11. (continued)

Compound	Silicic Acid-Benzene	Silicic Acid-Butyl Ether	Florisil-Benzene	Florisil-Butyl Ether
m-Nitrophenol	Red-brown		Red-brown	Brown
p-Nitrophenol	Red-brown			Brown
2,4-Dinitrophenol				
2,4,6-Trinitrophenol				Brown
o-Bromophenol	Red-brown	Brown	Red-brown	Red-brown
p-Bromophenol	Red-brown		Red-brown	
Pentabromophenol				
o-Chlorophenol	Brown	Brown	Red-brown	Red-brown
p-Chlorophenol	Red-brown		Red-brown	Red-brown
2,4,5-Trichlorophenol				
2,4,6-Trichlorophenol				
Pentachlorophenol				
Quaiacol	Red	Red-brown	Brown	Red-brown
Hydroquinone monomethyl ether				
o-Hydroxyacetophenone			Red-brown	
p-Hydroxyacetophenone	Red-brown	Yellow	Red-brown	Brown
Salicylic acid	Red-brown	Yellow-orange	Brown	Red-brown
Salicylamide		Red-brown		Red-brown
Salicylaldehyde			Orange	
p-Hydroxybenzaldehyde			Red-brown	Brown

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PART II

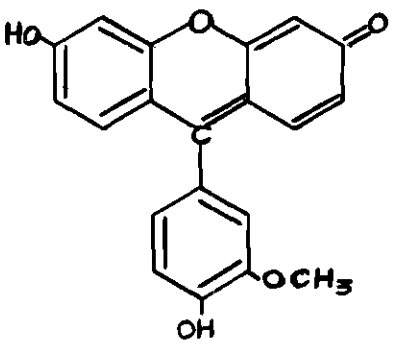
AN EVALUATION OF VANILLIN AS A COLOR PRODUCING AGENT FOR QUANTITATIVELY DETERMINING RESORCINOL

CHAPTER I

INTRODUCTION

LeRosen, Moravek, and Carlton (1) reported that vanillin in sulfuric acid could be employed as a streak reagent for detection of unsubstituted phenols. In a recent investigation by Carlton (2) vanillin in sulfuric acid was observed to produce red, violet, and brown zones with resorcinol, catechol, and hydroquinone, respectively. The decided difference in coloration of the various zones suggested the possibility of quantitatively determining one of the compounds in the presence of its isomers by a colorimetric procedure.

Isolation and characterization of the colored reaction product would give an insight into the vanillin reaction, and allow choice of optimum conditions for a colorimetric procedure. Also, necessary conditions may be determined for vanillin in sulfuric acid to react with substituted phenols. Sen and Sinha (3) prepared a condensation compound of resorcinol and vanillin and reported the product 2' methoxy 3' hydroxy benzein.



The resorcinol-vanillin reaction was chosen for investigation because the color intensity of the resorcinol zone was much greater than that of the other two zones, and a condensation compound of resorcinol and vanillin in sulfuric acid had been reported.

The purpose of the present investigation was to determine the feasibility of developing a colorimetric procedure based on the vanillin reaction for quantitatively determining resorcinol, catechol, and hydroquinone in a mixture, and to attempt to isolate the product of the vanillin-resorcinol reaction.

CHAPTER II

EXPERIMENTAL

The product of the resorcinol-vanillin reaction had a very large molar extinction coefficient, and thus only a very small amount of resorcinol was required for determination. The initial problem encountered was to devise a method of isolating a very small quantity of resorcinol accurately and reproducibly. A dilute solution of resorcinol in chloroform was prepared, the volume and concentration of which were chosen to require an amount of resorcinol large enough to permit accurate weighing on an analytical balance. A solution containing 150 milligrams per liter was found to be convenient. A suitable aliquot of this solution was pipetted into a volumetric flask and the solvent evaporated. Reproducible results were not obtained when the solvent was removed by heating. The solvent was also removed by passing a jet of air over the solution; however, reproducible results were not achieved.

Although the problem of isolating a very small amount of resorcinol was not solved, attention was directed to finding a convenient method of developing the color between vanillin in sulfuric acid and resorcinol. Practically no coloration resulted when solid resorcinol and the vanillin reagent were mixed. The color intensity was enhanced by heating, however, this was not suitable for an analytical procedure as it was

not reproducible. Addition of vanillin in sulfuric acid to an aqueous solution of resorcinol produced a very intense red coloration. The color intensity varied inversely with the amount of water present, and an excess of water prevented color formation. Furthermore, the color intensity varied directly with the vanillin concentration even though an excess was present. The use of an aqueous resorcinol solution was not only an excellent method of color development, but also eliminated the necessity of evaporating the solvent.

To determine the analytical applicability of the method solutions were prepared by adding one, two, and three milliliters of a standard resorcinol solution (.1502 g/l) to 25 millliliter volumetric flasks and developing the color by adding a measured volume of vanillin in sulfuric acid. The volume of resorcinol should be no more than three millliliters; a convenient concentration of vanillin is 0.25 per cent by weight. The solutions were allowed to cool twenty to thirty minutes and then diluted to the mark with sulfuric acid. The spectrum in the visible region was scanned and the absorption maximum determined to be 518 millimicrons (see Figure 1). Optical densities of the various solutions were determined at this wavelength. The above procedure was followed to develop the color in a series of solutions which contained one, two, and three millliliters of resorcinol solution and also contained the same amount of water. The absorption maximum of a solution of vanillin in sulfuric acid and water was determined to be

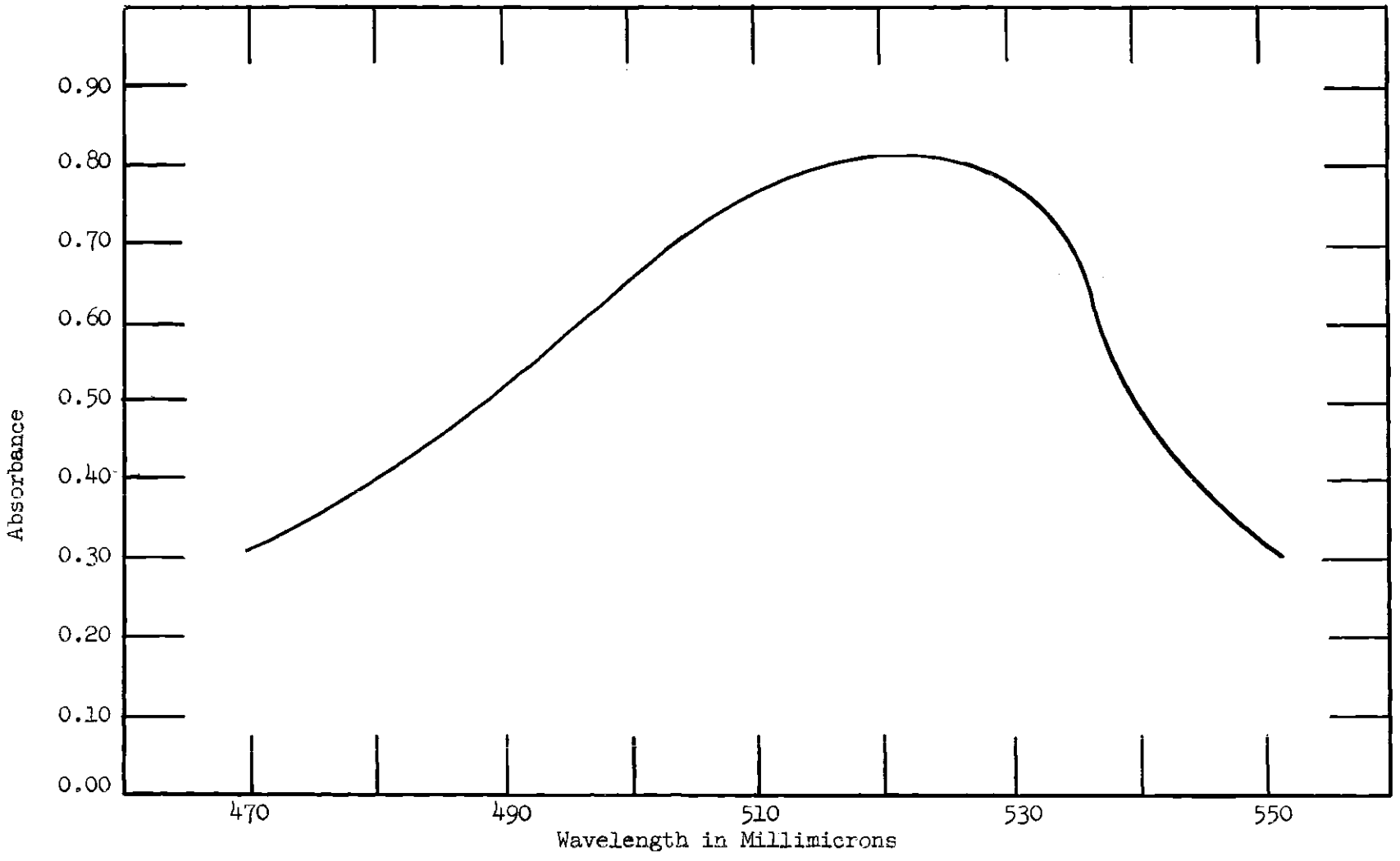


Figure 1. Absorption Spectrum of Aqueous Resorcinol - Vanillin in Sulfuric Acid

518 millimicrons also. The color was developed in aqueous solutions of catechol and hydroquinone, and the absorption maxima determined. A Beckman DU spectrophotometer with quartz cells was employed for all of the above measurements.

The possibility of developing the color directly on silicic acid was investigated. A chloroform solution of resorcinol was placed on a silicic acid column. The column was developed with butyl ether, extruded, streaked with vanillin in sulfuric acid to detect the zone. The zone was isolated and appropriate amounts of water and vanillin reagent added to develop the color. Another method involved extraction of the resorcinol with water and centrifuging to separate the silicic acid.

The following procedure was utilized in an attempt to prepare the product of the resorcinol, vanillin reaction. A sample of 6.6 grams of dried resorcinol and 4.6 grams of vanillin were intimately mixed and added little by little to about ten milliliters of concentrated sulfuric acid. The mixture was heated for one hour at 110° centigrade. The mass was allowed to cool to room temperature, treated with ice water, filtered, and washed with cold water. The solid was dissolved in sodium hydroxide solution, and then reprecipitated with dilute hydrochloric acid. The reprecipitation step was repeated several times, and finally the solid material was washed with water and dried in a vacuum desiccator. A solid reaction product was difficult to obtain when the indicated

amount of sulfuric acid was employed. The procedure was modified so that a minimum of sulfuric acid was employed, and was added to the mixture of resorcinol and vanillin.

An attempt to recrystallize the material from ethanol failed. The solid material was recovered by evaporation of the solvent. The material could be recovered by the addition of water to the ethanol solution, but an amorphous mass always resulted. The original product was extracted with ethanol for several days in a soxlet extractor. The solvent was evaporated and a solid material recovered from the flask. Visible and ultraviolet spectra were determined for the materials which remained in the flask and in the thimble. The absorption maximum in the visible region was determined to be 440 millimicrons when sulfuric acid was employed as solvent. The material was chromatographed on Florisil with ethanol developer.

A large volume of colored solution of the aqueous resorcinol, vanillin reaction was prepared in the following manner. A mixture of 1.7 grams of resorcinol and 1.2 grams of vanillin were dissolved in 60 milliliters of water and 200 milliliters of sulfuric acid, respectively. The solutions were mixed carefully and allowed to cool. The mixture was diluted to about a liter with water, and extracted with butanol. The red material was effectively extracted with butanol, and both the original mixture and the butanol layer were noted to fluoresce. The layers were separated and a solution of sodium bicarbonate

added to neutralize any sulfuric acid present in the butanol layer. Part of the red material was soluble in the bicarbonate layer, however, only the bicarbonate layer was noted to fluoresce. The butanol layer was washed with water several times, and the solvent was removed by passing a jet of air over the solution. The visible spectrum of the resultant red amorphous material in sulfuric acid possessed absorption maxima at 440 and 518 millimicrons. The bicarbonate layer was acidified with sulfuric acid and then extracted with butanol. The butanol was then washed with water and the material recovered by removing the solvent. The visible spectrum of the resultant material also possessed maxima at 440 and 518 millimicrons. The height of the absorption maximum at 440 millimicrons was found to increase at the expense of the maximum at 518 millimicrons when the sulfuric acid solutions were allowed to stand for a week or more at room temperature.

A colored solution of the resorcinol, vanillin reaction was prepared as described previously, and then extracted with butanol. The fluorescent compounds were separated chromatographically employing Florisil as adsorbent and ethanol as developer. The column was extruded and the various zones isolated. The red material was removed from the column with water, and the yellow fluorescent substances were removed with ethanol. All materials were amorphous. The red material was recovered from the aqueous solution by evaporating the solvent at 35° centigrade. A red amorphous material was recovered

which was soluble in alcohols and water. This material was treated with boiling chloroform to remove any unreacted resorcinol or vanillin. After heating the material was found to be insoluble in alcohols, contained sulfur, and melted above 300° centigrade.

CHAPTER III

DISCUSSION OF RESULTS

The color reaction between vanillin in sulfuric acid and resorcinol was dependent on several factors which are not consistent with ideal analytical procedures. The color intensity varied with the amounts of water, sulfuric acid, and vanillin present in the reaction mixture. The inverse variation of color intensity with the amount of water present is reasonable if the reaction is of the condensation type. However, this did not explain the variation of color intensity when sulfuric acid was the only variable. Color intensity of the reaction between solid resorcinol and vanillin in sulfuric acid was increased by heating. Similarly, the intensity of color produced in the aqueous resorcinol, vanillin reaction was probably dependent on the amount of heat produced by the reaction of sulfuric acid and water. Since the heat produced in the reaction was determined by the amounts of sulfuric acid and water present the color intensity was also dependent on the same factors.

Optical densities were determined at the absorption maximum, and plotted versus concentration for varying amounts of resorcinol. The plot was curved upward, Figure 2. Investigation indicated that the color intensity was dependent on the amounts of water present. Also, vanillin in sulfuric acid and

water produced a slight absorption maximum at 518 millimicrons. Optical densities were determined for a series of solutions containing the same amount of water and corrected for the absorption produced by the vanillin reagent. A plot of optical density versus concentration yielded a curve which was approximately linear, Figure 2.

Aqueous solutions of catechol and hydroquinone did not produce different colors with vanillin in sulfuric acid as anticipated. All three isomers reacted with vanillin in sulfuric acid to produce solutions with identical absorption maxima and, of course, this rendered the method useless as a quantitative technique for the determination of one of the compounds in the presence of its isomers. However, the method could be employed for quantitatively determining the compounds if a separation could be effected.

Even though a chromatographic separation of resorcinol and hydroquinone could not be effected, the possibility of developing the color directly on silicic acid was investigated. This method would be applicable if a separation could be effected. The following objectional features rendered the method useless. Silicic acid became suspended in the sulfuric acid solution and thus interferred with optical density measurements. Butyl ether was difficult to remove from the column and reacted with sulfuric acid to produce an interferring color. Several developing solvents were employed but discolored in sulfuric acid and were of no use. Resorcinol was separated

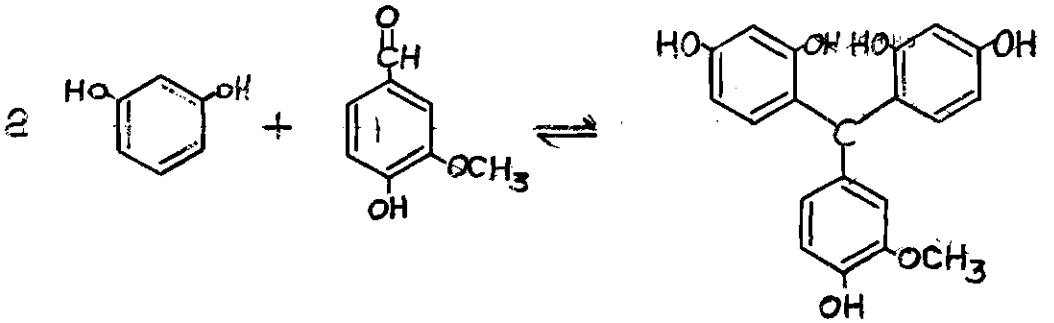
from silicic acid by addition of water and centrifuging, but butyl ether was present and reacted with sulfuric acid.

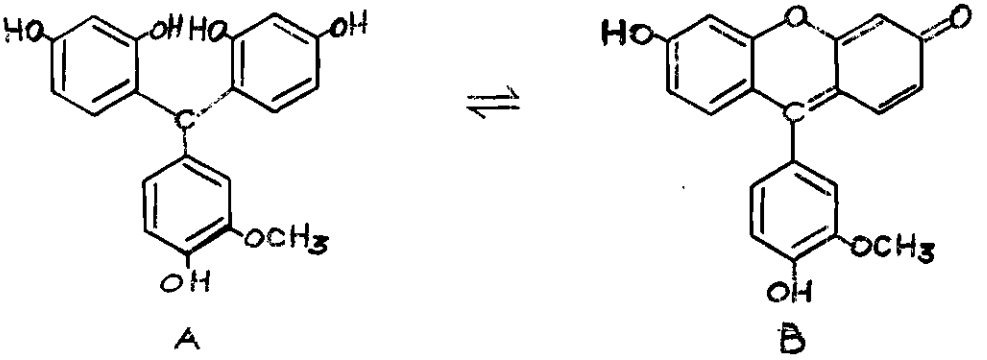
The procedure of Sen and Sinha (3) for the preparation of a condensation product of vanillin and resorcinol was followed in an attempt to characterize the color producing product of the reaction. The product could not be recrystallized as reported. The solubility seemed to increase on heating but would not crystallize on cooling. The absorption maximum in the visible region was 440 millimicrons and did not correspond to the maximum observed when an aqueous solution of resorcinol was treated with vanillin in sulfuric acid.

The conditions employed for developing the color of an aqueous resorcinol solution by the addition of vanillin in sulfuric acid were not as severe as the ones outlined by Sen and Sinha. A large volume of this solution was prepared and a red amorphous material recovered by extracting the solution with butanol and evaporating the solvent with a jet of air. The visible spectrum had absorption maxima at both 440 and 518 millimicrons, and indicated that the amorphous material contained the product prepared employing severe conditions as well as another substance or substances.

Several substances were separated chromatographically from the butanol extract employing Florisil as adsorbent and ethanol as developer. The following separation resulted: red zone R .00, yellow fluorescent zone R .08, yellow fluorescent zone R .20, and a very intense yellow fluorescent zone R .93. The materials were recovered, however, all were amorphous.

The absorption peak at 518 millimicrons was noted to decrease and the peak at 440 millimicrons to increase if the solutions of the red amorphous material in sulfuric acid were allowed to stand at room temperature for a week or more. This indicated that the major product of the aqueous resorcinol, vanillin reaction was converted to the product prepared when more severe conditions were employed. The shift in the spectrum can possibly be explained by postulating the following two step condensation. The product (A) of the first step is postulated to be the material which absorbs at 518 millimicrons, and compound B corresponds to the material with absorption maxima at 440 millimicrons.





A B

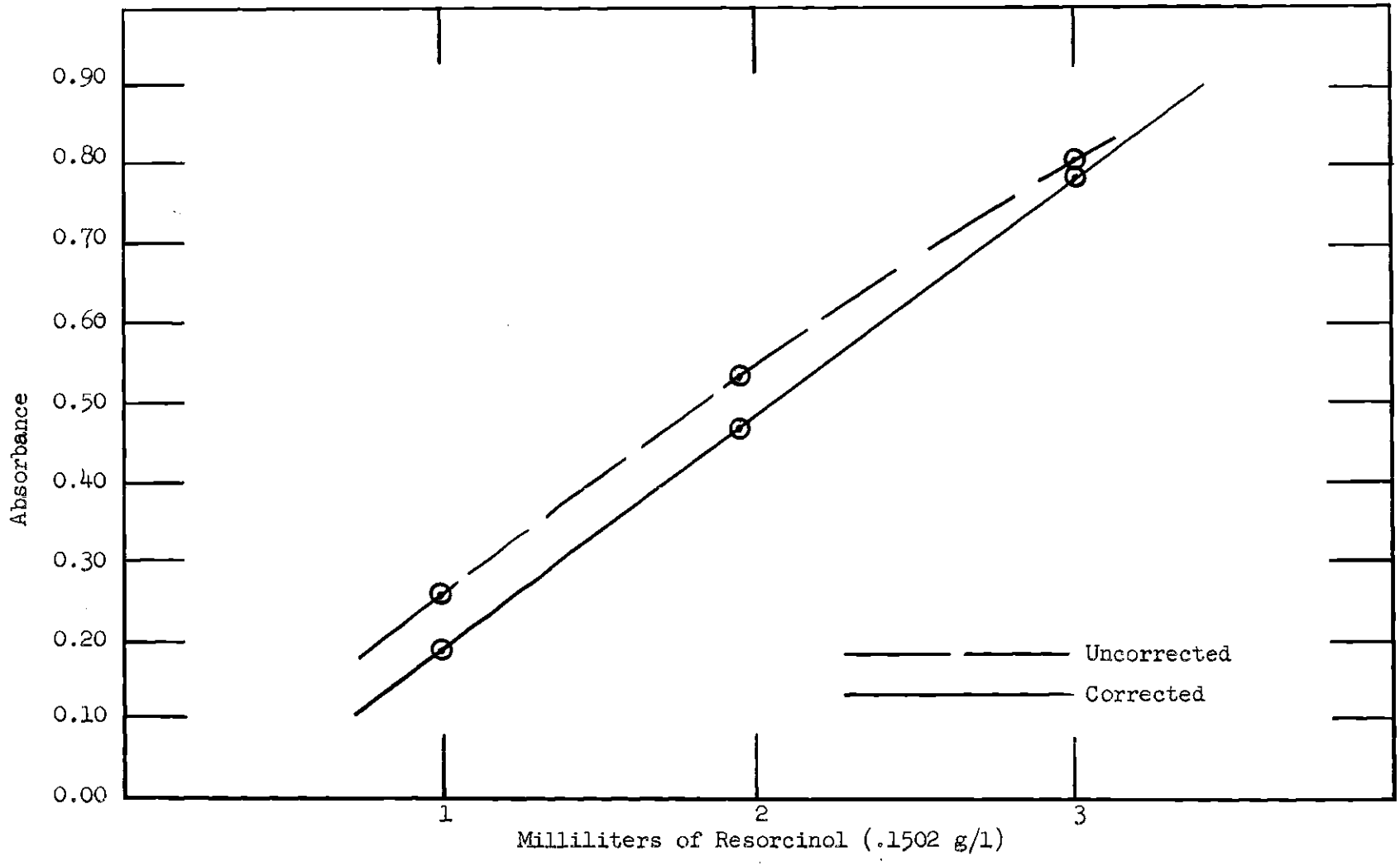


Figure 2. Absorbance Versus Milliliters of Resorcinol (.1502 g/l)

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PART III

DETECTION OF RESORCINOL IN THE PRESENCE OF ITS ISOMERS

CHAPTER I

INTRODUCTION

Frequently an analyst is confronted with the problem of detecting a trace amount of a substance present in a mixture as an impurity. More specifically, the problem is often the detection of a minute amount of a compound in the presence of its isomers. A preliminary investigation (1) demonstrated that vanillin in sulfuric acid produced red, violet, and brown zones with resorcinol, catechol, and hydroquinone, respectively, when employed as a streak reagent in adsorption chromatography.

The purpose of the present investigation was to determine the feasibility of employing vanillin as a reagent for detection of resorcinol in the presence of its isomers. Also, several other reagents were evaluated.

CHAPTER II

CHEMICALS, EQUIPMENT, AND PROCEDURE

Chemicals

The following chemicals were obtained as Merck Reagent grade and were used without further treatment.

Benzene

Chloroform

The following chemicals were obtained as Eastman White Label grade and were used without further treatment.

4-Aminoantipyrine hydrochloride

n-Butyl ether

Resorcinol

Vanillin

The following chemicals were obtained as Eastman Practical grade and were recrystallized from chloroform.

Catechol

Hydroquinone

The following chemical was obtained as J. T. Baker Reagent grade.

Potassium ferricyanide

Silicic acid and Florisil were prepared as in Part I of this thesis.

Solutions

4-Aminoantipyrine.--One gram of Eastman White Label grade 4-aminoantipyrine hydrochloride was dissolved in 50 milliliters of water and diluted with an equal volume of 1:1 ammonium hydroxide prior to use.

p-Nitrobenzenediazonium tetrafluoroborate.--p-Nitrobenzenediazonium tetrafluoroborate was synthesized (2) and a one per cent aqueous solution was prepared.

Potassium ferricyanide.--A two per cent aqueous solution was prepared from J. T. Baker Reagent grade potassium ferricyanide.

Vanillin.--One gram of Eastman White Label grade vanillin was dissolved in 100 grams of sulfuric acid.

Equipment

The equipment used was the same as that described in Part I of this thesis.

Procedure

R values listed in Table 12 indicate that catechol can possibly be separated chromatographically from resorcinol and hydroquinone. The table further indicates that a separation can be effected with either Florisil or silicic acid adsorbent when butyl ether is employed as developer. In order to determine which adsorbent was best suited for the separation a solution of resorcinol saturated with catechol and hydroquinone was prepared and chromatographed in the conventional manner. The columns were extruded and streaked with suitable reagents

to detect the various zones. Silicic acid was found to offer a better separation and will be employed as adsorbent in this investigation.

Since catechol can be separated from its isomers chromatographically the task of the streak reagent is reduced to differentiating resorcinol and hydroquinone. A standard solution of resorcinol saturated with hydroquinone was prepared in chloroform. A suitable amount was chromatographed on a silicic acid column, the column was extruded, and streaked with the reagents to be evaluated. A series of less concentrated solutions of resorcinol saturated with hydroquinone was prepared by diluting an appropriate aliquot of the standard with chloroform saturated with hydroquinone. Convenient amounts of these solutions were chromatographed, extruded, and streaked with the reagents to be evaluated. These columns were compared to a column which was prepared by chromatographing an equal volume of a saturated solution of hydroquinone in chloroform. Progressively dilute solutions were chromatographed and streaked until the reagent was not capable of distinquishing the resorcinol solution from a saturated solution of hydroquinone. The lower limit of detection of each reagent was determined in this manner.

CHAPTER III

DISCUSSION OF RESULTS

Vanillin and 4-aminoantipyrine can be utilized as streak reagents in conjunction with adsorption chromatography to differentiate resorcinol, catechol, and hydroquinone in a mixture. Table 12 reveals that catechol can possibly be separated from its isomers chromatographically employing either Florisil or silicic acid as adsorbent and butyl ether as developer. Catechol can be separated from its isomers in dilute solutions with either adsorbent, however, streaking occurs when a saturated solution of catechol in chloroform is chromatographed on Florisil. Notice that the catechol zone is above the other zones when Florisil is employed as adsorbent, and streaking of the catechol zone prevents separation of the isomers. Streaking does not occur on silicic acid columns, and if it did occur no interference would result as the catechol zone is below the other zones on the column. Silicic acid possesses a decided advantage over Florisil and is employed for this separation. Catechol can be characterized by its R value, and the streak reagent need only differentiate resorcinol and hydroquinone.

Vanillin and 4-aminoantipyrine are capable of differentiating resorcinol and hydroquinone. Vanillin produces red and brown zones with resorcinol and hydroquinone, respectively.

4-Aminoantipyrine produces a red-brown zone with resorcinol and a purple zone with hydroquinone. The lower limit of detection for each reagent is five gamma.

Combination of adsorption chromatography for the separation and characterization of catechol, and the use of either vanillin or 4-aminoantipyrine to differentiate resorcinol and hydroquinone should prove to be of great value for the detection and estimation of resorcinol in the presence of its isomers.

Table 12. R Values of the Dihydroxy Benzenes on Florisil and Silicic Acid Adsorbents with Butyl Ether Developer

Compound	Silicic Acid	Florisil
Catechol	.59	.20
Resorcinol	.35	.40
Hydroquinone	.32	.38

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