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The Techniques of Thin Layer Chromatography and the Study of Carbohydrates Using Thin Layer Chromatography

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THE TECHNIQUES OF

THIN LAYER CHROMATOGRAPHY

AND

THE STUDY OF CARBOHYDRATES

USING THIN LAYER CHROMATOGRAPHY

by

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ABSTRACT

Refined Kraft pulp is found to contain D (+), Mannose, D (+) Xylose, and Glucose; and not to contain Galactose. Silia gel plates and Kieselguhr plates were used for the separation. Sodium hydroxide was the carrier solvent and the plates conditioned for thirty minutes.

The Kamag Vario-KS-Chamber is found to be a good effective laboratory tool. There are many areas where this technique can be used.

A detailed historical background is given for a basic background in thin layer chromatography, along with a complete experimental procedure of the use of the Vario-KS-Chamber.

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Principles of Thin Layer Chromatography

The Principles of Thin Layer Chromatography, according to A. A. Akhrem and A. I. Kuznetsova (1).

The technique of thin layer chromatography involves the following operations: on one side of a small glass plate a thin layer of sorbent is spread. As in paper chromatography, samples of compounds and their mixtures are brought onto the starting line and the edge of the plate below the starting line is dipped in a solvent system. As the liquid moves up the plate, the separation of the mixture takes place. The height of the rise of the liquid (the solvent front) is marked, the plate is dried and treated in a manner similar to a paper chromatogram to detect the substances as colored spots. The positions of the spots are marked as shown in Fig. 1; these correspond to the compounds under investigation and are located between the starting line and the solvent front. To determine the position of the spot the distance between the center of the spot and the starting line (segment AB) is measured. The distance from the solvent front to the starting line is then measured (segment AC). The ratio of the distance AB between the starting line to the center of the spot to the distance AC between the starting line to the solvent front is the constant R_f = AB/AC is typical for a given compound on a given sorbent and in a given system, and is a function of a number of factors: experimental technique, nature and activity of the sorbent, thickness of layer, nature of solvents, amount of substance brought onto the plate, the length of the path traversed by the solvent, the position of the starting line, and is almost independent of the temperature.
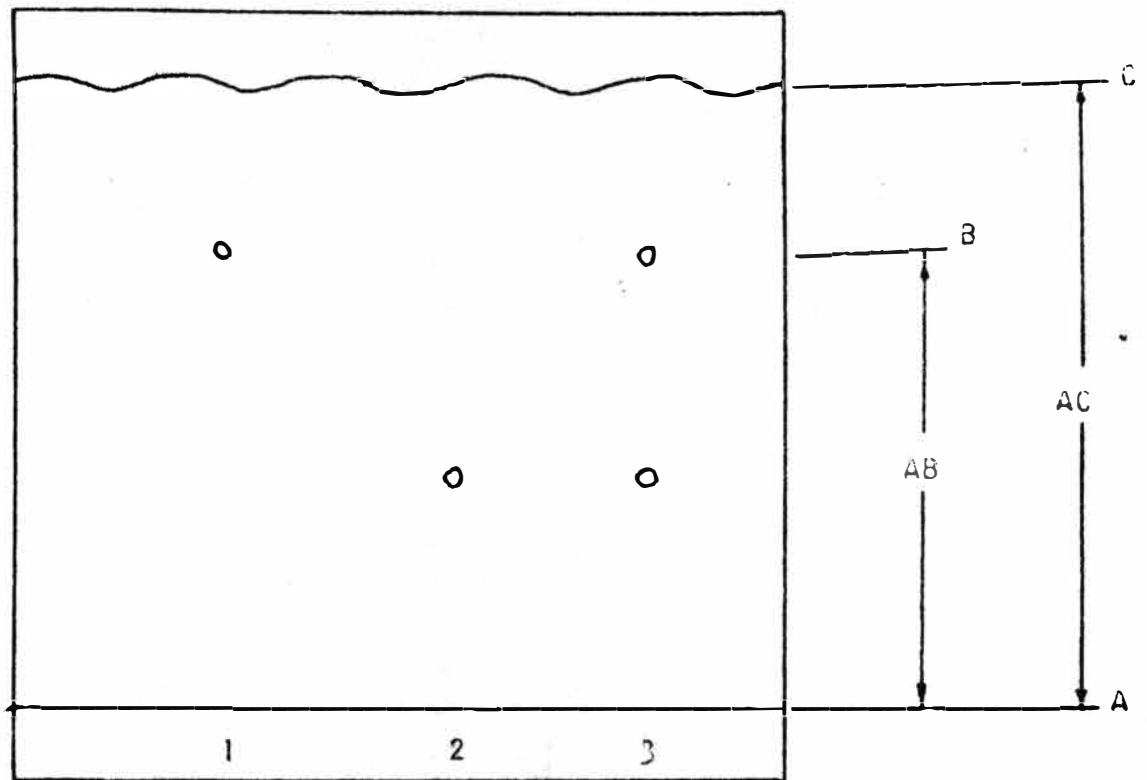
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FIG. 1

Separation of a Mixture on a Plate

with a Thin Film of Sorbent

A   - Starting Line

B   - Center of Spots

1, 2 - Individual Substances

3   - Mixture of 1 and 2

For the sake of reliability in the identification of substances the determination of R_f is often supplemented by the use of markers. A known substance (Marker) is chromatogramed on the plate together with the mixture to be separated and the position of the spots on the chromatogram is expressed as the ratio R_s between the R_f of the unknown substance and the value of the R_f of the marker:

$$R_s = \frac{R_f}{R_f \ marker}$$

The separation of mixtures on a thin layer of sorbent may be attained by adsorption, partition and ion exchange chromatography. This classification is based on the nature of the forces acting between the dissolved substances and the solid or the liquid phases with which they come in contact.

Adsorption chromatography is based on the sorption of the dissolved substance by the surface of the solid phase.

In partition chromatography the substances are distributed between two liquid phases, one of which is stationary.

Ion exchange chromatography is based on the formation of ionic compounds between the dissolved substances and the electrically charged groups of the sorbent.

In practice these processes almost never take place singly. Thus, for instance, adsorption chromatography is accompanied by partition chromatography if the separation takes place on low activity sorbent in systems containing water; partition chromatography is accompanied by adsorption chromatography if the substances being separated have an affinity to the carrier sorbent. Ion exchange chromatography is almost invariably accompanied by adsorption phenomena.

Adsorption Chromatography

Fundamental Principles

Adsorption chromatography, according to Akhrem and Kuznetsova, is based on the sorption of the dissolved substance by the surface of the sorbent. The chromatographic separation is accompanied by a continuous process of sorption and disorption of the dissolved substance on the surface of the sorbent. When the number of particles adsorbed on the surface per unit time is equal to the number of particles leaving the surface, or, in other words, when the rate of sorption is equal to the rate of desorption, adsorption equilibrium has been attained. The most general case of this process is gas adsorption.

Langmuir expressed the dependence between the amount of the gas adsorbed at constant temperature and its concentration in the surrounding space in the form of the following equation:

$$n = \frac{swc}{1 + wc}$$

where n is the amount of the gas adsorbed per unit area, s is the maximum amount of gas which may be adsorbed by the surface, w is the adsorption coefficient and c is the concentration of the gas.

The curve which expresses the dependence between the amount of the adsorbed gas and the gas in the surroundings after adsorption equilibrium at constant temperature has been attained, is called adsorption isotherm. A similar relationship for the case of adsorption from solution may be expressed by the adsorption isotherm equation of Freundlich:

$$n = ac^b$$

where n is the amount of the substance adsorbed on unit surface, c is the concentration of the substance in solution, and a and b are magnitudes which are constant for the given adsorbent-adsorbate system.

In the ideal case the partition function is linear and the adsorption isotherm is a straight line. In practice the spots diffuse on the chromatogram and the equilibrium between sorption and desorption is not immediately established. For this reason the adsorption isotherm is not in fact linear, but a curve such as that represented in Fig. 2, and the spots on the chromatogram are sharply delineated on the top and diffuse out on the bottom in the shape of "tails" jutting out towards the starting line. The tails are the smaller, the less the amount of the substance applied to the starting line.
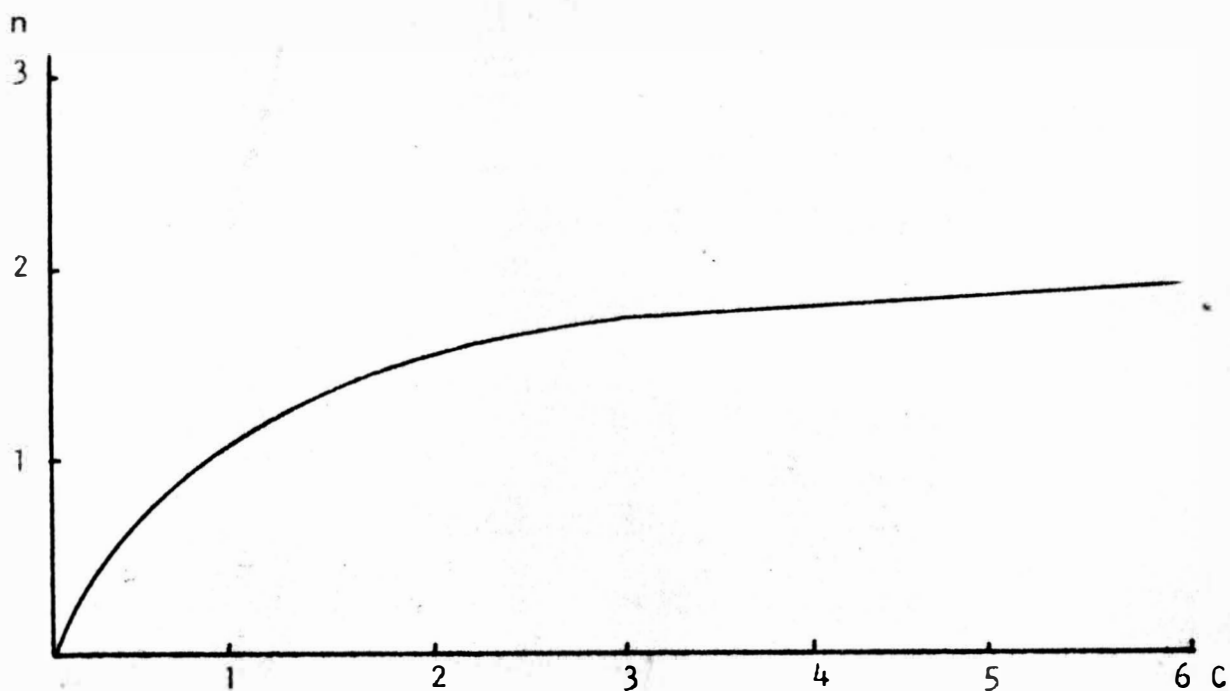
FIG. 2

Adsorption Isotherm from Solution

The amount of Substance n, adsorbed by unit surface
is plotted on the ordinate; the Concentration C of
substance in solution is plotted on the abscissa.

Selection of Sorbents and Solvents

For an efficient separation of a mixture the choice of a sorbent of a suitable quality and activity is very important. It must be remembered that certain sorbents, e.g., aluminum oxide, silica gel, and other sorbents may produce side reacting during the separation. The criteria for the selection of a sorbent are preductable only to a limited extent. In practice the worker will begin with the properties of the compounds to be separated: their solubility (hydrophilic, hydrophobic), and the content and the nature of their functional groups.

If the substance has only a weak affinity to the sorbent, active layers and weakly polar solvents are used, such as appears at the beginning of the eluotropic series (Table 1). If, on the contrary, the substance is strongly adsorbed, weakly active sorbents and strongly polar solvents are employed.

Stahl proposed the following simple device for the selection of the experimental conditions for adsorption chromatography on a thin layer of sorbent (Figure 3). The shaded triangle rotates around an axis; one of its corners shows the compounds to be separated, the other shows the required activity of the sorbent, the third shows the solvent.

In principle all sorbents which are used in column chromatography can be employed in thin layer chromatography. Up till now the following sorbents have been used: silica gel, aluminum oxide, Kieselguhr, calcium hydroxide, magnesium silicate, florisil, Celite, Plaster of Paris, cellulose, and a few other sorbents.

Solvents employed in thin layer chromatography should be pure. The purification and the drying of the solvents are carried out by methods usually employed in other kinds of chromatography.

TABLE I

ELUCTROPIC SERIES OF STAHL (3)

SERIAL NUMBER	SOLVENT	SERIAL NUMBER	SOLVENT
1	HEXANE	7	ETHER
2	HEPTANE	8	ETHYL ACETATE
3	CYCLOHEXANE	9	PYRIDINE
4	CARBON TETRACLORIDE	10	ACETONE
5	BENZENE	11	ETHANOL
6	CLOROFORM	12	METHANOL
		13	WATER
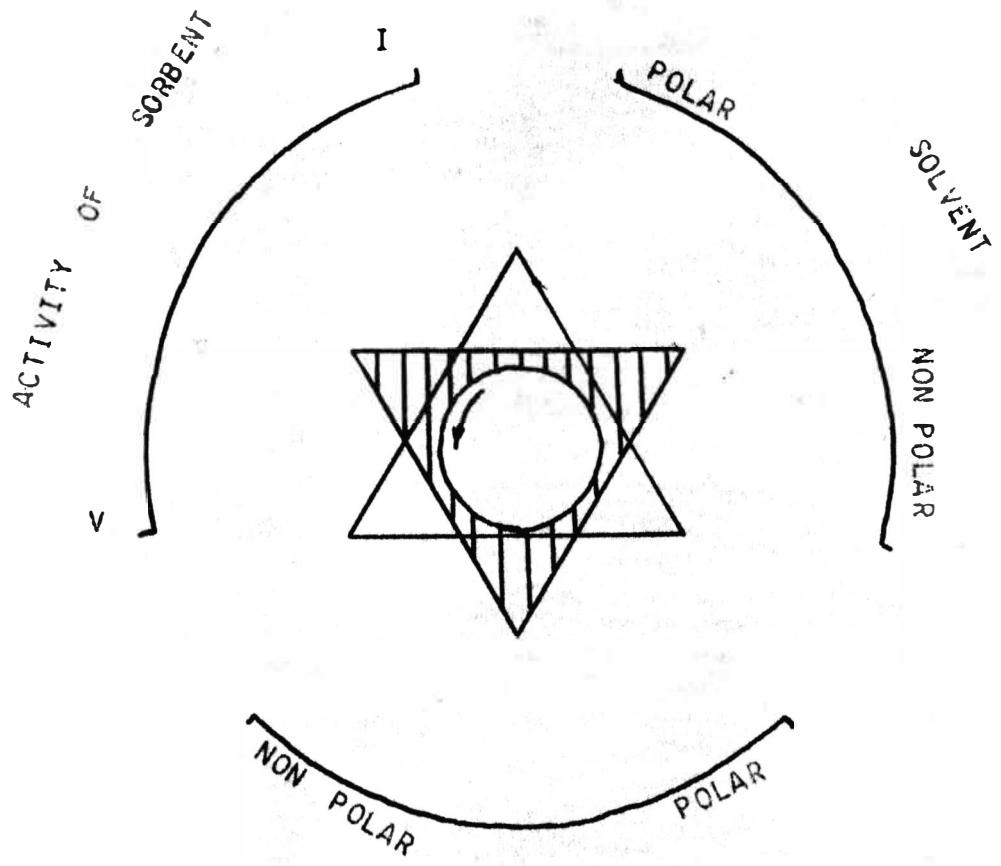
SUBSTANCES TO BE SEPARATED

FIG. 3

Selection of experimental conditions for thin layer chromatography.
Roman numerals indicate activity according to Brockmann.

Mixtures may be separated by means of one solvent but the usual practice is to employ mixtures of two, three, or even four solvents. The solvent system must be freshly prepared for each plate since the component ratio in the solvent mixture changes as a result of the separation.

In choosing the solvent for thin layer adsorption chromatography any of the known eluotropic series, giving the sequence of eluting capacities of the solvents can be used. Stahl proposed the eluotropic series given in Table 1.

It has been recommended that the solvent be selected by the micro-circular technique, the principle of which is the following: to a glass plate with a sorption layer a few spots of the substance are applied in the usual way, 2 cm away from each other. After a few minutes the solvent or solvent system tested is applied to the center of each such spot with a thin capillary. The dried chromatogram is revealed; the individual components of the mixture are then seen as concentric circles. The most successful separation of the mixture indicates the solvent or solvent system to be chosen.

Dependence of the Adsorption Capacity
of a Substance on its Structure

Saturated hydrocarbons are adsorbed to a small extent or are not adsorbed at all. The introduction of double bonds, especially conjugated double bonds, increases the adsorbability of the compound. Functional groups intensify the adsorbability to an even greater extent.

The adsorptive capacity of the functional groups increases in the following sequence: $CH = CH$, OCH_3 , $COOR$, $C = O$, CHO , SH , NH_2 , OH , $COOH$[3]. If more than one substituent is present, this sequence changes.

Partition Chromatography

Fundamental Principles

This section according to Akhrem and Kuznetsova.

Partition chromatography is based on the partition of a substance between two non-miscible liquids in both of which it is soluble, when one of the liquids (stationary phase) is retained by a suitable inert solid carrier. The substances to be separated should be more soluble in the stationary phase. The solvent, the so-called mobile phase, moves through the stationary phase and extracts the substances on the plate. During the development each substance is distributed between the fixed and the mobile phases until an equilibrium state is attained, the equilibrium constant depending on the solvents chosen and on the nature of the substances being separated. The constant σ is called the partition coefficient of Nernst:

$$\sigma = \frac{c_1}{c_2} = \text{const.}$$

Where c_1 and c_2 are the concentrations of the substance in the two phases.

As the result of the different values of the coefficients σ the substances will move more or less rapidly on the plate and thus become separated from each other.

The value of R_f in the ideal case may be calculated from the distribution σ by the expression:

$$\frac{1}{R_f} = 1 + K$$

Where K is a constant whose value will depend on the carrier sorbent.

The actual value of R_f obtained on the chromatogram will not always be in agreement with the theoretical calculations, since it is difficult to allow for the sorption of the substances on the carrier.

Selection of Sorbents and Solvents

The following substances have been used as carriers of the station-
ary phase in thin layer chromatography: silica gel, Kieselguhr, plaster
of Paris, Celite 55 and cellulose.

When selecting solvents for partition chromatography, both liquid
phases should always be taken into account. The solvent is chosen by
using any of the known series in which the solvents are arranged in the
order of their tendency to form hydrogen bonds. Stahl proposes to use
for this purpose the mixotropic series of Hecker. The series is headed
by hydrophilic compounds, the so-called polar compounds with the most
conspicuous tendency to form hydrogen bonds, whereas hydrophobic com-
pounds or nonpolar compounds are placed at the bottom of the series.
When separating polar compounds by partition chromatography, water usually
serves as the stationary phase while the mobile phase consists of a less
polar organic solvent, immiscible with water, to which water is added, or
which is saturated with water.

To separate hydrophobic (nonpolar) substances reversed phase thin
layer partition chromatography is employed. In this case the carrier
layer is saturated with a lipophilic substance such as undecane, paraf-
fin oil, tetradecane, silicone oils of various viscosities, etc. The
mobile phase consists of polar organic solvents, which are usually
saturated with the stationary phase. Sometimes not the amount of the
polar solvent is saturated, but only a part of it; the remainder is added
without saturation. This is made in order to prevent any phase separa-
tion on the plate.

In order to separate substances of a medium-polar character a
nonvolatile polar liquid such as formamide, or polyethylene glycol (2)

is taken as the stationary phase. The mobile phase consists of nonpolar liquids, beginning with chloroform and ending with heptane, saturated with the stationary phase.

The amount of the solvent (stationary phase), which is required to saturate the plate should be accurately determined.

Ion Exchange Chromatography

Technique

This section according to Heftmann (3).

The techniques employed in ion exchange chromatography are quite
similar to those in adsorption and partition chromatography. For example,
conventional ion exchange resins are used in columnar systems like other
sorbents, and ion exchange papers are used in the same manner as filter
paper is in paper chromatography.

Ion exchange is used primarily for the separation of ionic species,
i.e., for the separation of anions from cations, ionic from nonionic
species, cations, and anions. Because of the high fixed ion concentra-
tion within the structure of most ion exchange resins, these materials
function as concentrated electrolyte solutions and are therefore usable
as "salting-in" agents for the chromatographic separation of nonelectro-
lytes such as alcohols and aldehydes.

Classification

The chromatographic separation of various ionic and nonionic species
by ion exchange depends upon several factors. Although most separations
are based on differences in selectivity between the various ionic species
entering into ion exchange reactions, ion exchange to materials can be
used without exchange of ions. Applications of ion exchange to chroma-
tography may be classified according to the basic principles involved as
follows: (a) ionic charge differences, (b) ion exchange selectivity,
(c) specific adsorptive effects, (d) Donnan membrane equilibria, and
(e) "salting-in" or "salting-out" effects.

Ionic Charge Differences

Since most ion exchange materials are not amphoteric, separations of ionic from nonionic species are achieved without difficulty. These applications present a most favorable situation, where some species are capable of being sorbed, while others cannot be sorbed by the exchanger at all. The analyst only needs to avoid overloading the exchanger with the ionic species to be sorbed by taking into account the capacity of the exchanger.

Ion Exchange Selectivity

Most chromatographic separations by ion exchange are based upon differences in selectivity of the exchanger for the ions to be separated. The factors that govern the selectivity of an exchanger for a particular ion include (a) valence, (b) ionic radius, (c) concentration, (d) nature of the exchanger, and (e) solvent. The ease of separation in ion exchange chromatography depends upon the magnitude of the differences in selectivity. Ions differing in valence or size are usually separated without difficulty. Ions of similar size and valence may also be separated by ion exchange chromatography, but the experimental conditions require more careful control. In many such instances differences in selectivity may be magnified by the addition of citrates in the case of the rare earths and of hydrocloric acid in the case of the transition elements may be quoted as examples.

Specific Adsorptive Effects

In certain cases separations by means of ion exchange chromatography are based upon specific adsorption. For example, phenols are adsorbed by

anion exchangers by van der Walls forces, and borate salts of anion exchangers may be employed for the chromatography of sugars capable of forming borate complexes.

Selection of the Suitable Chromatographic Method

This section according to Akhem and Kuznetsova.

In the selection of a suitable method the prime consideration must be the structure of the substances to be separated.

In thin layer chromatography the technique most often employed is that of adsorption chromatography, which is better understood and technically simpler.

Both adsorption and partition chromatography may serve to separate substance differing in the nature, number and character of their polar and nonpolar constituents, structural isomers, stereoisomers, and substances with other structural differences. However, only partition chromatography which involves solubilities of substances in the mobile phase is capable of separating the homologs of higher fatty alcohols, aldehydes and acids.

Ion exchange chromatography may be employed for the separation of substances which carry different overall charges on their ionized groups under the conditions of the separation (for the given pH value and salt concentration).

The so-called molecular sieves have been lately used for the separation of mixtures of high molecular substances, e.g., mixtures of amino acids, oligopeptides, and proteins.

Carbohydrates

The carbohydrate and sugar sections according to Akhem and Kuznetsova.

The separation of hydrophilic compounds, e.g., carbohydrates, by thin layer chromatography, can be effected both on modified Kieselguhr-plaster of Paris and silica gel-plaster of Paris layers and on cellulose-plaster of Paris layers.

Sugars

A number of sugars were separated on Kieselguhr-plaster of Paris layer impregnated with 0.02M sodium acetate. The conditions for separation of the sugars and the R_f values for ascending development on various sorbents (4) are given in Table 1. Separation of sugars can also be achieved on a silica gel-plaster of Paris layer, saturated with boric acid. To prepare a layer, 6 ml 0.1N boric acid are added to 4 g silica gel. The development is then conducted with acid solvent systems Table 2.

The quantitative analysis of sugars, described in the paper, consists of the following operations: the spots are scraped off the plate, eluted, oxidized by 0.05 or 0.01N potassium bichromate in 70% sulfuric acid and the residual bichromate is back-titrated.

The separation of these carbohydrates was also conducted on a layer, prepared from an aqueous suspension of MN300 cellulose powder. The layer was dried for 10 minutes at 100°. Monosaccharides can be readily separated on this layer by two-pass development with ethyl-pyridine-water (2:1:2). Mannose and arabinose are preferably separated by water-saturated phenol containing 1% of ammonia (Table 1).

A number of carbohydrates have also been separated on plaster of Paris layer (30-60 mesh), dried in the air for 20 hours (Table 2).

TABLE II

R_f values of a number of sugars on various sorbent layers

Solvent Systems:

    I - 65 ml ethyl acetate + 35 ml isopropanol-water (2:1)

    II - Benzene-methanol-acetic acid (1:3:1)

    III - Methyl-ethyl-ketone-methanol-acetic acid (3:1:1)

    IV - Ethyl acetate-pyridine-water (2:1:2)

    V, VI, VII - Chloroform-methanol (19:2), (19:3), (19:5)

COMPOUND	Kieselguhr Plaster of Paris saturated with .02M sodium acetate	Silica Gel Plaster of Paris, saturated with IN Boric Acid		Cellulose	Plaster of Paris		
	SOLVENT SYSTEM						
	I	II	III	IV	V	VI	VII
Arabinose	0.28	0.62	0.42	1.11	0.63	0.75	----
Xylose	0.39	0.59	0.39	1.25	0.58	0.88	----
Ribose	0.49	----	----	1.42	0.79	0.86	----
Rhamnose	0.62	0.67	0.52	1.52	0.88	0.95	----
Glucose	0.17	0.63	0.42	1.00	0.27	0.77	----
Galactose	0.18	0.55	0.32	0.90	0.30	0.40	----
Mannose	0.23	0.58	0.32	1.09	0.33	0.62	----
Fructose	0.25	0.52	0.31	----	0.48	0.91	----
Sorbose	0.26	0.51	0.24	----	0.73	0.95	----
Lactose	0.04	0.56	0.25	----	----	----	0.46
Multose	0.06	----	----	----	----	----	0.26
Saccharose	0.08	0.63	0.29	----	----	----	0.61

The separation of sugars by ascending development may be carried
out also on a cellulose powder-plaster of Paris layer, prepared from
cotton cellulose. Better results can be obtained on this layer by the
descending technique when a number of sugars are chromatogrammed by the
Matthias method.   Optimum separation was attained in the systems
N-butanol-pyridine-water (10:3:3) and N-butanol-25% ammonia-water
(16:1:2). Mannose, arabinose, lyxose, and ribose have been separated
by the systems phenol-butanol-acetic acid-water (5:5:2:10).

Mono-, Di- and Trisaccharides

Mono-, di, and trisaccharides were separated on Kieselguhr-plaster
of Paris and silicagel-plaster of Paris and silica gel-plaster of Paris
layers, and also on these layers impregnated with 0.1% boric acid or
0.02M sodium acetate. A successful separation was achieved of a mixture
containing one part of raffinose to a hundred parts of saccharose on
silica gel-plaster of Paris layer with the system N-propanal-ethyl-acetate-
water (7:2:1), using the Matthias method. This sorbent also served to
separate di- and trisaccharides: saccharose, D- mellibiose, maltose,
lactose, melezitose and raffinose in the system butanol-acetone-water
(4:5:1) and butanol-acetic acid-water (4:5:1).

Carbohydrate Derivatives

Qualitative and quantitative analysis of mixtures of saccharose and
its ethers and raffinose was also conducted on silica gel-plaster of Paris
layer. The best system for the development of the ethers proved to be
toluene-ethyl acetate-95% ethanol (10:5:5). To detect the spots the
plate was sprayed with concentrated sulfuric acid and heated at 110°.

For quantitative determination the spots were eluted from the plates; the composition of the mixture was determined by the colorimetric method.

Acetyl and benzoyl derivatives of sugars were separated on silica-gel-plaster of Paris layer. The best solvent system for acetylated derivatives proved to be benzene-methanol (95:5). Under these conditions mixtures of anomers are easily separated. As a rule, acetyl derivatives with 1.5-transconfigurations move faster than their anomers.

Detection. The spots were detected by spraying the chromatogram with silver nitrate-ammonia and sodium methylate. To do this three solutions were prepared: (1) 0.3% solution of silver nitrate in methanol; (2) methanol saturated with ammonia; (3) 7% solution of sodium in methanol. These solutions were combined in the ratio of (5:1:2) before use.

Thin layer chromatography on non-bounded alumina layer of grade II activity was used to separate certain derivatives of monosaccharides in the systems benzene-chloroform and benzene-methanol, taken in various ratios. The spots were detected by spraying with sulfuric acid.

Objective

The objective of this study is to present a brief but thorough history of the different types of thin layer chromatography and to show how the thin layer chromatography equipment at Western Michigan University is to be used. The historical background section makes a very good reference for understanding the basic principles of thin layer chromatography.

Bleached refined Kraft pulp was used and the study includes the identification of some of the simple sugars of the pulp, by the technique of thin layer chromatography. Four sugars were tested: D (+) Mannose, Galactose, D (+) Xylose, and Glucose. Each was tested under two different plate types: silica gel and Kieselguhr.

Experimental Procedure

Preparation of a Sample

A sample of bleached Kraft pulp was used. The following steps were taken prior to any chromatography work (5):

1. The sample was dried at 49° so as to form a dry mat. If this step was not taken, the effectiveness of the sulfuric acid would be lessened due to dilution factor.

2. Seventy-two percent sulfuric acid was added at about 3 milliliters of acid for every 0.35 g of dried pulp.

3. The solution was then mixed, and let stand at a constant temperature of 30° C for one hour.

4. Water was then added, 84 milliliters for every 3 milliliters of sulfuric acid added earlier.

5. Na_2CO_3 was added to neutralize the effect of the acid.

6. The solution was filtered and concentrated.

Two different sorbents were used: silica gel and Kieselguhr. They are both excellent sorbents for carbohydrate chromatography and will give a good basis of study. Four chromatograms were to be made for each sorbent, summing up eight for the study.

Use of the Camag Varia-KS-Chamber

1. Scrape the plates so that three sides of the plate are scraped 2 centimeters from the edge.

2. It is advisable at this time to mark the lines A and C on the plate with a pencil. (See Fig. 1). Line A will be marked 1.5 to 2.5 centimeters from the edge of the unscraped side. This is where the sample will be placed (next step). Line C is the solvent endpoint; it should be as far away from A as possible. A good idea is to put it a specific distance from line A, so as to make calculations easier.

3. Next, scrape off sorbent as to create strips so that the samples can have no effect on each other. A plate scraper apparatus is provided in the lab.

4. Now put a wick in the solvent trough as shown in the manual. Do not add any solvent. Pull the handle to lower solvent wick.

5. Next add sulfuric acid to the acid trough and put it in the Kamag.

6. Next put the samples on the plate on line A. Then put the plate in the Kamag sorbent side down with the samples at the wick end. Clamp down the plate. The plate must now condition for a predetermined time depending on sorbents, solvents and type of sample.

7. After the prescribed time, fill the solvent trough two-thirds full, then push in the handle to raise the solvent wick. It should make contact across the plate.

8. After the solvent line reaches line C take off the plate and spray it with sulfuric acid. Now you can read the plate and measure the R_f values.

9. The solvent and sulfuric acid must both be changed between each plate.

Data and Discussion

The data of all experimentation is given in Table III.

The experiment was run using plates coated with silica gel, and Kieselguhr reagents. Four plates were made for each. On each plate was the unknown (derived from pulp sample) and four known sugars: D (+) Mannose, Galactose, D (+) Xylose, and Glucose.

The solvents used for this experiment were:

1. For the silica gel plates ethyl acetate 65%-isopropyl alcohol-water (2:1) 35%.

2. For the Kieselguhr plates methanol-benzene-acetic acid (3:1:1).

The acid used in the acid trough was fifty percent sulfuric and the plates were sprayed with fifty percent sulfuric acid. The plates were conditioned for thirty minutes at twenty-five degrees celsius. The plate was then run for sixty minutes, at twenty-five degrees celsius.

The spots were hard to see and quite hard to measure, but were consistent throughout the plates.

The laboratory was not difficult and worked out to be a good test. A lot can be learned from thin layer chromatography.

TABLE III

DATA

SAMPLE	TRIAL	PLATE TYPE	R_f	MATCH UNK.
D (+) Mannose	1	Silica Gel	.38	No
D (+) Mannose	2	Silica Gel	.25	Yes
D (+) Mannose	3	Silica Gel	.20	Yes
D (+) Mannose	4	Silica Gel	.30	No
D (+) Mannose	1	Kieselguhr	.33	Yes
D (+) Mannose	2	Kieselguhr	.87	No
D (+) Mannose	3	Kieselguhr	.27	Yes
D (+) Mannose	4	Kieselguhr	.40	Yes
Galactose	1	Silica Gel	.33	No
Galactose	2	Silica Gel	.44	No
Galactose	3	Silica Gel	.40	No
Galactose	4	Silica Gel	.30	No
Galactose	1	Kieselguhr	.25	No
Galactose	2	Kieselguhr	.83	No
Galactose	3	Kieselguhr	.50	No
Galactose	4	Kieselguhr	1.00	No

TABLE I II (Cont.)

DATA

SAMPLE	TRIAL	PLATE TYPE	R_f	MATCH UNK.
D (+) Xylose	1	Silica Gel	.75	Yes
D (+) Xylose	2	Silica Gel	.25	Yes
D (+) Xylose	3	Silica Gel	.20	Yes
D (+) Xylose	4	Silica Gel	.33	Yes
D (+) Xylose	1	Kieselguhr	.34	Yes
D (+) Xylose	2	Kieselguhr	.93	No
D (+) Xylose	3	Kieselguhr	.28	No
D (+) Xylose	4	Kieselguhr	.50	Yes
Glucose	1	Silica Gel	.20	No
Glucose	2	Silica Gel	.33	Yes
Glucose	3	Silica Gel	.18	Yes
Glucose	4	Silica Gel	.37	Yes
Glucose	1	Kieselguhr	.41	No
Glucose	2	Kieselguhr	1.00	Yes
Glucose	3	Kieselguhr	.70	Yes
Glucose	4	Kieselguhr	.50	Yes

Conclusions

One of the objectives of this thesis was the identification of some of the simple sugars of Kraft pulp. The sugars were: (1) D (+) Mannose, (2) Galactose, (3) D (+) Xylose, and (4) Glucose.

Three of these sugars were identified as being in the sample. D (+) Mannose, D (+) Xylose and Glucose were all identified, Galactose was not.

Both the Silica Gel plates and the Kieselguhr plates proved satisfactory, giving the same results.

I think a wood chemistry 333 project using the Kamag Varia-KS-Chamber would be beneficial not only to the student, but his report could also be beneficial to the paper department at Western Michigan University.

Much more work could be done on the Kamag Varia-KS-Chamber in the form of another Bachelor's Thesis or Master's Thesis. Two dimensional thin layer chromatography, for example, would be an excellent thesis topic.

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