CENTRIFUGAL PAPER CHROMATOGRAPHY AND SOME OF ITS APPLICATIONS

JAMES A. LOWERY AND JAMES E. CASSIDY

American Cyanamid Company, Pearl River and Stamford Laboratories, Pearl River, N.Y. and Stamford, Conn. (U.S.A.)

(Received June 19th, 1963)

Separations by paper chromatography frequently require many hours, yet this technique is very useful for the separation of many organic mixtures. Often a suitable developing system is not known, and appreciable time may be required in the evaluation of various solvents. Thus paper chromatography suffers from a time disadvantage when compared to thin layer and vapor phase chromatography.

To overcome these disadvantages centrifugal force[1] has been employed as a means of obtaining within minutes instead of hours paper chromatographic separation of mixtures. The advantages and disadvantages have been reported for several centrifugal paper chromatographic units[2–10].

The major limitation of commercial units is poor performance for separations requiring volatile solvents despite the use of a pre-equilibrated chamber. Other mechanical limitations are irregularities of flow of solvent from the feed systems and paper disintegration.

EXPERIMENTAL

Unit

A centrifugal unit (Fig. 1) was designed and built at the Pearl River Laboratories to eliminate these defects. The unit has no development chamber; instead, the paper is pressed between two metal plates. A small hole in the center of the upper plate allows the solvent to be fed to the paper. Four screws are used to attach the upper plate securely to the face plate.

The feed nozzle, which is positioned manually, supplies a continuous stream of solvent (0.8–1.5 ml/min). The nozzle, which is constructed of stainless steel, has an inner solvent chamber divided by a sintered stainless steel filter of 10 mμ porosity. A thin stream of liquid is delivered as a result of the solvent being forced through the orifice (0.008 in. diameter) by an air pressure mechanism. Both the filter and orifice disc are fitted securely using teflon gaskets to ensure tight seals and inertness to solvents.

Operational procedure

The sample is centrally spotted on a circular chromatographic paper, and the chromatogram developed with 4 ml of solvent using a pressure of 4 p.s.i. The air pressure is not turned on until a constant speed of 900 r.p.m. is obtained. The rotating head is stopped when all of the solvent has been delivered. The paper is hung in a ventilated hood to dry, and the zones of the chromatogram are then located with the appropriate detection reagents.

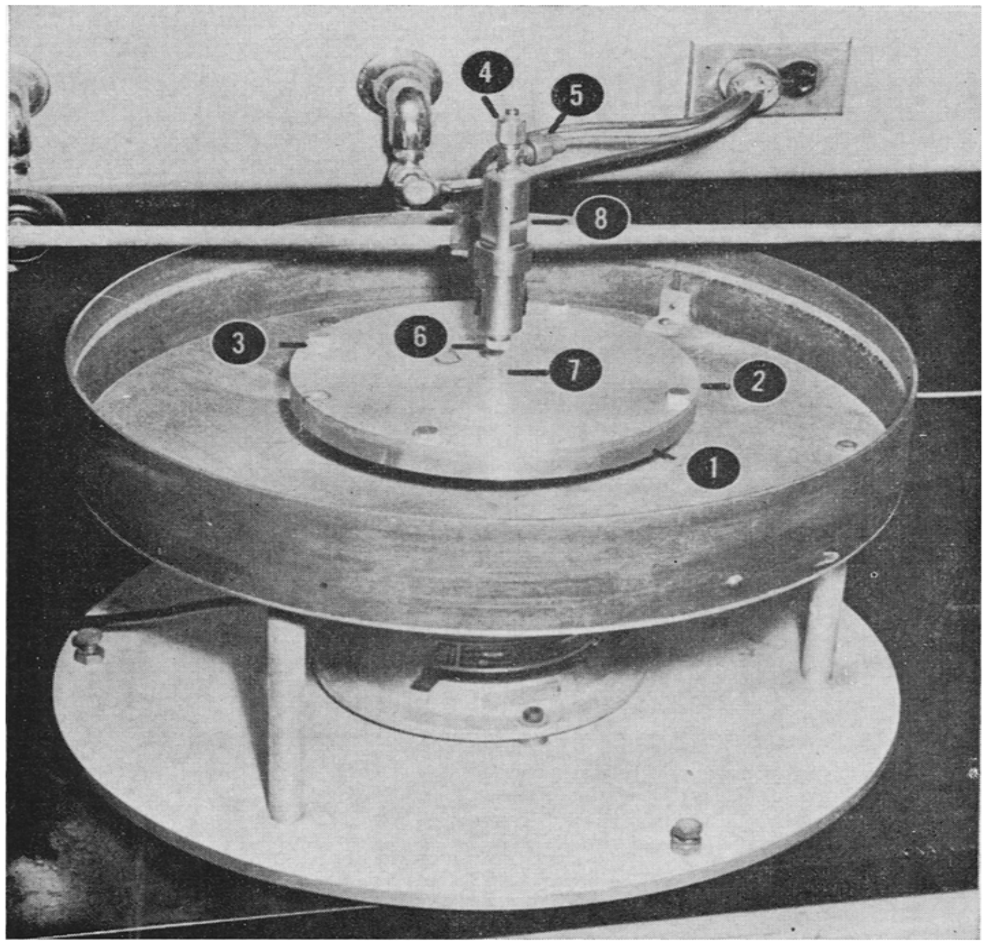

Fig. 1. 1 = Lower plate. 2 = Upper face plate. 3 = Peripheral bolt (4). 4 = Removable cap. 5 = Air line. 6 = Drilled disc. 7 = Solvent access hole. 8 = Solvent feed assembly.

Paper

The paper used was Schleicher & Schüll 470. Circles of 19 cm diameter were cut from sheets. Treated as well as untreated papers were used in this investigation.

Treatment consisted of dipping the circular paper in an appropriate coating solution and then drying. Aqueous buffers used were 5.0 % oxalic acid having a pH of 1.0, and 0.1 *M* sodium dihydrogen phosphate adjusted to a pH of 2.0 with phosphoric acid. For the Zaffaroni type development the paper was treated with a 5 % solution of acetamide in acetone; for inverse-phase separations the paper was impregnated with 5 % Dow Corning Silicone Fluid No. 550 in chloroform.

Data

Seven organic mixtures were selected to evaluate the unit as a means of obtaining rapid paper chromatographic separation using relatively volatile and very volatile solvent systems. Conventional paper chromatographic procedures has previously been developed for each mixture. A comparison of R_F values determined by conventional and centrifugal chromatography was thought to be a suitable method of evaluating this unit.

In most cases (Table I) the R_F values determined by centrifugal chromatography

TABLE I

COMPARISON OF R_F VALUES BY CENTRIFUGAL AND STANDARD CHROMATOGRAPHY

Compounds	Solvent system			
	R_F Cent.*	R_F St.	R_F Cent.*	R_F St.
	Methyl ethyl ketone–0.1 N NH_4OH			
1. Steroids				
Triamcinolone (9α-fluoro-11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione)	0.93	0.83		
Triamcinolone 21-monohemisuccinate	0.32	0.17		
Triamcinolone 16,21-dihemisuccinate	0.13	0.02		
Development time	180 sec	4 h		
Detection: Blue tetrazolium[11], plain paper.				
	n-Butanol–ethanol–water (4:1:1)		*n-Butanol–acetic acid–pyridine–water (4:1:1:2)*	
2. Urea and related compounds				
Urea	0.53	0.30	0.60	0.43
Guanylurea hydrochloride	0.42	0.17	0.51	0.53
Cyanamide	0.93	0.69	0.95	0.70
Dicyandiamide	0.67	0.43	0.73	0.52
Development time	240 sec	16.5 h	240 sec	16.5 h
Detection: Nitroprusside[12], plain paper.				
	Acetone–acetonitrile–water (14:1:20)			
3. Nematocide				
O,O-Diethyl O-2-pyrazinyl phosphorothioate	0.63 with solvent front	0.33		
Sodium pyrazinol	200 sec	0.82		
Development time		4 h		
Detection: Iodine vapor; inverse phase, paper treated with silicone; chromatograms somewhat irregular.				
	Aq. ammonia–ethanol–n-propanol (3:6:1)		*Acetone–benzene–formic acid–water (10:10:2:6) (upper layer used)*	
4. Organic acids				
Malic acid	0.51	0.34	0.23	0.11
Maleic acid	0.51	0.37	too diffuse	0.41
Fumaric acid	0.69	0.44	0.85	0.75
Development time	240 sec	15.5 h	140 sec	4.5 h
Detection: 0.2 % ethanol solution of bromophenol blue containing 3 % $HgCl_2$, plain paper.				
	Benzene–hexane–methanol (10:10:1)			
5. Pesticide				
Dimethoate (O,O-dimethyl-S-methyl-carbamoylmethyl phosphorodithioate)	0.40	0.35		
O,O,S-Trimethyl phosphorodithioate	nothing	solvent front		
Development time	200 sec	4 h		
Detection: Iodine vapor and 2,6-dibromo-N-chloro-*p*-benzoquinone imine[13]; paper treated with acetamide; chromatograms somewhat irregular.				

(continued on p. 470)

TABLE I (*continued*)

Compounds	Solvent system			
	R_F Cent.*	R_F St.	R_F Cent.*	R_F St.
	Methyl ethyl ketone satd. with water			
6. Tetracyclines				
a. Anhydro-tetracycline	0.20	0.41		
Tetracycline	0.00	0.25		
Development time	210 sec	15 h		
Detection: Ultraviolet radiation and ammonia vapor; paper treated with oxalic acid.				
	n-Butanol satd. with water			
b. 6-Demethyl-tetracycline	0.73	0.52		
A Mannich type product of 6-demethyl-tetracycline and lysine	0.00	0.05		
Development time	185 sec	16 h		
Detection: Ultraviolet radiation and ammonium vapor; paper treated with phosphate buffer.				

* Average of several values.

were greater than those determined by the conventional method. However, results are comparable.

The best resolutions were obtained for steroids, tetracyclines, amino acids and urea derivatives. Separations were not as good for the remaining mixtures. The two bands for O,O-diethyl O-2-pyrazinyl phosphorothionate and sodium pyrazinol were not well defined; this phenomenon is probably due to the pretreatment of the paper with acetamide. The bands for the organic acids were diffuse and difficult to detect. Inasmuch as the detection reagent for these acids was an acid–base indicator, both development systems gave trouble since one contained formic acid and the other ammonia. Even though overnight drying prior to spraying did not correct this difficulty completely, the zones could still be detected against the background.

Detection was not difficult with a thinner paper such as Whatman No. 3; however, a continuous flow of solvent could not be maintained without flooding this paper. The high volatility of O,O,S-trimethyl phosphorodithioate resulted in it being removed completely from the paper even though the paper was pressed between two plates.

Because of these generally favorable results this unit was used to follow a Mannich base reaction. Lysine hydrochloride (183 mg) was mixed with 6-demethyltetracycline (430 mg) in methylcellosolve and the basicity of the mixture was adjusted with 0.1 *N* sodium hydroxide to effect solution. The rate of reaction was followed qualitatively by centrifugal chromatography. A nearly complete reaction was indicated at the end of 21 min (Table I, 6b).

Other organic mixtures that were separated were solutions of amino acids and FD & C standard dyes (Table II). For the separation of the amino acids the motor was run at $^1/_3$ speed and 6.5 ml of solvent was used instead of 4 ml. Good separations were obtained for both these mixtures.

TABLE II

SEPARATIONS BY CENTRIFUGAL CHROMATOGRAPHY

Amino acids	R_F *value*
Leucine	0.95
Proline	0.62
Histidine	0.42

Detection: ninhydrin, plain paper.
Developing solvent: *n*-butanol–acetic acid–water (50:12:50).
Developing time: 140 sec.

FD & Standard dyes	R_F *value*
FD & C Red No. 4	0.42
FD & C Green No. 1	0.62
FD & C Yellow No. 8	0.99

Detection: paper treated with phosphate buffer.
Developing solvent: *n*-butanol saturated with water.
Developing time: 185 sec.

DISCUSSION

The evaluation of this unit indicates the advantage of two contiguous plates. Because of this modification very regular solvent fronts are formed even when solvents such as acetone, benzene, or methyl ethyl ketone are used. Usable chromatograms can also be obtained with pretreated paper. Since there is no development chamber, no pre-equilibration with the solvent system is necessary. All separations reported in Tables I and II were obtained using dried paper. In a few cases the paper was equilibrated in a separate chamber with the solvent system before development. The resulting chromatograms showed more diffuse bands than those obtained with dry paper.

Even with a known developing system, separation by this technique is not always successful. However, a large number of chromatograms can be obtained in a short time making possible an evaluation of a series of solvent systems in order to determine the feasibility of separation by the standard paper chromatographic technique. Though the ratios of R_F values for centrifugal chromatography to those for the standard technique are not constant, results are comparable. In this investigation the R_F ratios (R_F Cent./R_F Std.) vary only from 1 to 2, with but one exception and that involved the use of methyl ethyl ketone as solvent. If a mixture of compounds tends to travel with the solvent by centrifugal chromatography and resulting R_F values are 0.8–0.9, separation might be achieved by employing the standard technique since R_F values will usually be lower.

Qualitative results are quickly obtained when complete separation is achieved by this accelerated technique. R_F values are reproducible if the same operational procedure is carefully followed. Even quantitative work should be possible using

this technique. By off center spotting and subsequent development, one half of the paper can be sprayed to locate the bands of the various components and with this pattern the other half marked, cut out, extracted and analyzed.

The unit reported in this investigation does have certain drawbacks. It can be improved by using a motor with a governor and a tachometer. The feed system occasionally shows stream irregularities and stoppages in spite of the built-in filter. This difficulty can usually be eliminated by cleaning the orifice disc periodically and filtering the solvent system through a very fine glass filter before transferring to the nozzle. The thicker chromatographic paper required takes longer to dry, and if an acid is used in the developing solvent, detection with an acid–base indicator is difficult. In spite of these difficulties, the unit has two distinctive advantages: a drastic reduction in the time required for paper chromatography and the ability to use volatile solvents for developing systems.

MODIFICATION OF A NEW COMMERCIAL UNIT

A new commercial centrifugal chromatographic unit was introduced about the time this investigation was completed. This unit has a tachometer, a governor controlled $^1/_8$ h.p. motor (300–700 r.p.m.), and a closed development chamber containing a rotating plate on which the chromatographic paper is placed. The important innovation of this unit is a new automatic feed system which replaces a constant flowing stream of earlier commercial units. The solvent is delivered automatically by an electronically activated solvent metering system consisting of a timed relay and a graduated buret equipped with a solenoid valve for intermittent rather than continuous flow.

This new unit was modified in the Stamford Laboratories by fitting a second plate over the one in the development chamber. Good separation of mixtures of dicyandiamide, guanidine, and biguanide and mixtures of malic, maleic, and fumaric acids (Table III) have been achieved with this modified unit. Separations of the nitrogen compounds can be obtained without the second plate, but they tend to be irregular and the bands more diffuse. Separation and detection of the organic acids was possible using the modified commercial unit whereas difficulties were encountered using the one built in the Pearl River Laboratories (Table I). Succeess was achieved by using (1) a slower feed, (2) reduction of motor speed, and (3) thinner paper. This second plate must be used with developing solvents such as benzene and acetone; otherwise no chromatogram is obtained even though the chamber is equilibrated with developing solvent beforehand. To insure uniform development of chromatograms with such solvents, great care must be taken to seat evenly the upper plate.

Two other problems were resolved using this modified unit: (1) mixture of trace amounts of cyanamide and dicyandiamide, and (2) reaction mixtures taken from an autoclave initially charged with dicyandiamide. Resolutions of both mixtures were successfully carried out.

Quantitative as well as qualitative results were requested for the second problem. This was readily accomplished by off center spotting of duplicate samples. One half of the developed chromatogram was used for band detection, and then using this as a pattern the bands of the other half were located and then extracted. The materials extracted were then analyzed by ultraviolet spectrophotometry. For

TABLE III

SEPARATIONS USING THE MODIFIED COMMERCIAL UNIT

Mixture of dicyandiamide, biguanide and guanidine (200 μg)

	R_F value	
	No upper plate, chamber saturated with solvent	*With upper plate*
Dicyandiamide	0.61	0.61
Guanidine	0.41	0.43
Biguanide	0.28 irregular development	0.28 regular development

Off center spotting, 700 r.p.m.; 6 ml added in 1400 sec.
Paper: Schleicher & Schüll 470.
Developing solvent: butanol–ethanol–water (4:1:1).
Detection: nitroprusside[12], off center spotting.

Mixture of malic, maleic and fumaric acids (250 μg of each)

	R_F value
	With upper plate
Malic acid	0.12
Maleic acid	0.61 very diffuse
Fumaric acid	0.97

Off center spotting, 600 r.p.m.; 2.5 ml added in 1900 sec.
Paper: Whatman No. 3.
Developing solvent: acetone–benzene–formic acid–water (10:10:2:6) (used upper layer).
No chromatographic development without upper plate.
Detection: bromophenol blue, off center spotting.

example, the recovery of 193 μg of pure guanyl-O-methyl isourea was found to be 190 μg by this technique. By conventional paper chromatography 20 h were required to obtain equivalent results, by this centrifugal technique only 2 h were required.

Reproducible chromatograms have consistently been obtained with this intermittent feed system and not once has stoppage or irregular delivery of solvent occurred. Modifying the unit by the addition of a second plate has made it very versatile and useful for chromatographic development with volatile solvents. Following the progress of microbiological reactions as well as organic reactions should be possible.

SUMMARY

Greater applicability of centrifugal chromatography can be obtained by placing the paper between two metal plates instead of in a development chamber. Several

examples of the usefulness of such an arrangement are reported. With this modification incorporated in the design of a commercial unit centrifugal chromatography should drastically reduce the time required to obtain separations on paper. As a consequence paper chromatography will no longer suffer from a time disadvantage when compared with thin layer and gas liquid chromatography.

REFERENCES

1 G. CARONNA, *Chim. Ind. (Milan)*, 37 (1955) 113.
2 H. J. MCDONALD, E. W. BERMES, JR. AND H. G. SHEPHERD, *Naturwiss.*, 44 (1957) 9.
3 H. J. MCDONALD, E. W. BERMES, JR. AND H. G. SHEPHERD, *Chromatog. Methods*, 2, No. 1 (1957) 1.
4 H. J. MCDONALD AND L. V. MCKENDELL, *Naturwiss.*, 44 (1957) 616.
5 H. J. MCDONALD, L. V. MCKENDELL AND E. W. BERMES, JR., *J. Chromatog.*, 1 (1958) 259.
6 J. A. ANDERSON, *J. Chromatog.*, 4 (1960) 93.
7 J. R. TATA AND A. W. HEMMINGS, *J. Chromatog.*, 3 (1960) 225.
8 M. PAVLICEK, J. ROSMUS AND Z. DEYL, *J. Chromatog.*, 7 (1962) 19.
9 G. M. CHRISTENSEN AND W. SWOR, *J. Chem. Educ.*, 39 (1962) 347.
10 J. S. MATHEWS AND M. DE LOS ANGLES CERVANTES, *J. Chromatog.*, 9 (1962) 195.
11 N. P. CHERONIS AND H. STEIN, *J. Chem. Educ.*, 33 (1956) 120.
12 J. E. MILKS AND R. H. JAMES, *Anal. Chem.*, 28 (1956) 846.
13 J. J. MENN, W. R. ERWIN AND H. T. GORDON, *J. Agr. Food Chem.*, 5 (1957) 601.