Effect of solvent volume and solvent equilibration time on Whatman filter paper No. 20

The significance of paper chromatography as a research tool lies primarily in the reproducibility of the R_F value for a solute–solvent–paper system. Studies by MEINHARD[1], BATE-SMITH[2], KOWKABANY AND CASSIDY[3], CASSIDY[4] and other investigators show the effect on R_F values of variations in temperature, equilibrium, solvent–paper systems, and related factors. CLAYTON[5], studying solvent volume, defined "critical volume" as the volume of solvent needed to saturate a chamber under specific conditions. Using Whatman filter paper No. 1, CLAYTON[5] found no significant differences in the R_F values of amino acids with pre-equilibration during 2 to 4 h.

LANIGAN AND WALTON[6], using WHATMAN filter paper No. 20, in the same solvent system as CLAYTON[5], reported marked deviation in the R_F values of 16 common amino acids with pre-equilibration of the chamber and paper during 1 to 3 h, whereas they had no significant variation using Whatman papers No. 1 and 4. These investigators[6] related the reproducibility of R_F values using Whatman No. 20 paper to the distance traveled by the developer front.

The present work was undertaken to study the effect of critical volume and pre-equilibration on Whatman filter paper No. 20 employing conditions similar to those of CLAYTON[5].

Experimental

Solutions of alanine, arginine, aspartic acid and glycine were 0.1 M in glass distilled water, adjusted to neutrality with potassium hydroxide. The amino acids were detected at 1 and 2 μmoles.

One batch of Whatman No. 20 paper was used, in the machine direction, as supplied by the manufacturer. Ascending chromatograms were developed overnight until the solvent front traveled a minimum distance of 24 cm, using a solvent system of n-butanol–acetic acid–water (25:6:25, v/v) at 26 $\pm$ 2°. A pyrex chromatojar, 12 $\times$ 12 $\times$ 24 in., having a 56.5-liter capacity was used in all experiments. The filter paper, 23 $\times$ 55 cm, was equilibrated[7] for 18 to 24 h in the presence of freshly prepared solvent unless otherwise stated. Critical volume was 10 to 12 ml of solvent per liter-capacity of the chamber[5].

Developed chromatograms were thoroughly dried in a hood and the solvent front was visualized by U.V. illumination. Amino acids were detected with ninhydrin in ethanol by the method of PATTON AND CHISM[8].

Results and discussion

A series of more than 50 chromatograms was run to determine the conditions giving reproducible R_F values with Whatman No. 20 filter paper. The results shown in Tables I and II are average values of several determinations.

Varying the solvent volume (Table I) gave maximum R_F values, reproducible within 0.02[2], with a critical solvent volume of approximately 570 ml. This result showed that the distance traveled by the solvent front was not the variable giving inconsistent results with Whatman No. 20 filter paper as reported by LANIGAN AND WALTON[6]. Further, this effect showed a variation from CLAYTON[5], who reported minimum R_F values using critical volume with Whatman No. 1 filter paper.

TABLE I

EFFECT OF SOLVENT VOLUME ON THE R_F VALUES

Compound	$R_F \times 100$						
	60 ml*	*168 ml*	*504 ml*	*504** ml*	*616 ml*	*728 ml*	*Critical volume*
Alanine	22	25	27	22	30	28	29
Arginine	9	12	15	14	17	17	17
Aspartic acid	10	13	19	17	18	19	19
Glycine	13	17	21	18	21	21	21

* Equilibration, 1 h; 18 h equilibration, all other solvent volumes.
** Second use of solvent; first use, all other solvent volumes.

The data in Table I also show that the R_F values determined for this solvent-paper system varied with both equilibration and solvent volume. At low solvent volumes an extended period of equilibration increased the R_F value by 0.03 to 0.04. After 18 h of equilibrium additional increases were obtained ranging from 7–24 %.

The increase in R_F value due to equilibration alone is shown in Table II. This effect of equilibrium on Whatman No. 20 paper may also be seen by a comparison of Tables I and II. The values determined at critical volume with no equilibration were approximately 50 % of those obtained with 1 h of equilibration and 1/9 of the solvent volume.

TABLE II

THE EFFECT OF EQUILIBRATION ON R_F VOLUMES AT CRITICAL SOLVENT VOLUME

Compound	$R_F \times 100$		*Increase in R_F* (%)*
	No equilibration	*Period of equilibration 18 h*	
Alanine	11	29	158
Aspartic acid	6	19	215

* Increase in R_F above $\pm$ 0.02.

SMITH[9], in discussing chromatographic solvents, states that equilibration is not necessary when the less volatile solvents are used and that the latter are adequate for two consecutive runs under controlled conditions. These factors were investigated using a solvent volume of 504 ml in consecutive overnight development with Whatman No. 20 paper equilibrated for 18 h (Table I). The R_F values obtained for the first use of the solvent were within a difference of 0.02 when compared to those of critical volume (Table I). In the second use of the solvent, the R_F values of alanine and glycine decreased 11 and 5 %, respectively, compared to both the initial R_F value and that with critical volume; the R_F value of arginine decreased 6 % compared to that with critical volume. These variations in the R_F values were similar to those observed with a change from 168 ml of solvent to critical volume.

The results shown in Table II may be compared favorably to the study of

BENTLEY AND WHITEHEAD[10] who reported that the R_F values of amino acids rose (for their solvent–paper system) with an increase in the water content of the solvent. The three-fold increase in the R_F values with equilibration (Table II) indicated that the "water of hydration" for the solvent–paper system employed in this study was high, and that omitting equilibration with Whatman No. 20 paper lowered the water-rich phase. This resulted in a different solvent composition as the moving solvent was dehydrated.

The data obtained in this study indicate a functional difference between Whatman No. 20 filter paper and the commonly employed Whatman filter papers No. 1 and 4. It is recommended that specific conditions be established for Whatman No. 20 paper for each solute–solvent–chamber-temperature system. It is also recommended that Whatman No. 20 paper be equilibrated for 18 to 24 h in the presence of freshly prepared solvent prior to irrigation with a critical volume of solvent.

Acknowledgements

This study was supported by Research Grant AI 06192-02, National Institutes of Health, United States Public Health Service. The authors thank S. MARA WALTON for her assistance in this study.

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Received May 2nd, 1966

J. Chromatog., 25 (1966) 483–485

Chromatographic spray for the identification of tyrosine, histidine and their amines

In the course of estimating histamine in plant tissue, need arose for the specific detection of histamine on paper chromatograms. STEFANOVIÉ, CIRKOVIÉ AND BRESJANAC[1] described a colorimetric test for the detection of histamine in injection solutions. The method involved the coupling of histamine with diazotised *p*-aminobenzoic acid in alkaline solution. It was found that this test could be modified for use as a spray reagent to detect histamine on paper chromatograms.