[Chem. Pharm. Bull.
19(1) 80—86 (1971)]

UDC 543.544.4

An Improved Solvent Chamber for Thin–Layer Chromatography

Ryuzo Takeshita

Department of Public Health Pharmaceutics, The Institute of Public Health[1]

(Received June 30, 1970)

A special chamber for thin–layer chromatography was devised in which compounds can be developed after adequate equilibration of the chromatographic media with the vapor of solvent systems. A comparison was made between the patterns of mobilities of several compounds when the compounds were developed using this chamber and a conventional chamber. Using the chamber, a well reproducible *Rf* value was obtained even when a compound was developed with the multicomponent solvent system and under different conditions of temperature.

Introduction

Many workers[2–9] pointed out the importance, in order to obtain accurate *Rf* values in thin–layer chromatography, of saturating the chromatographic chamber with solvent vapor as completely as possible before the introduction of the chromatoplates. Saturation of the chamber is said to be effective in eliminating not only the so-called "edge effect,"[9] but also the variation of *Rf* values if the layers have a fairly consistent thickness. Therefore, many methods and equipments have been proposed to equilibrate the chromatographic media completely with the solvent vapor before commencing the development.[5–8] Although special chambers are available from various manufacturers of thin–layer equipment, there are no apparatus to satisfy these conditions completely. From this point of view, a new chamber was designed by which the chromatoplates can be suspended and raised or lowered without opening the chamber. When using this chamber, compounds will be developed after the chromatographic media are equilibrated with the solvent vapor.

Experimental

Chromatographic Chamber——An air–tight glass chamber, 12×22×25 cm, is equiped with a device by which any size of plates (20×20 cm, 5 plates; 5×20 cm, or 5×10 cm, 16 plates; and other plates having various length) can be suspended as shown in Fig. 1. The main part of the device is made of a kind of polyacetal resin and its construction is shown in Fig. 2.

A is a pipe which has a screw in the lower outside part and a little rod is driven through the pipe, A, so that a boss, B, is made in the inside of the pipe. Through a hole of approximately 2 cm in diameter at the center of the glass–cover of the chamber, the pipe, A, is fixed with two ring nuts, C and C′. D is a plate having 8 screwed holes, in which 10 cm long stainless–steel rods, E, with a diameter of 5 mm, are held, and is jointed to a rod, F. The lower part from (a) of the rod, F, is vertically grooved, the upper part from (a) being screwed. The rod, F, exactly fits the pipe, A, and is suspended from the top of the pipe, A, with a ring nut,

---

1) Location: *6–1, Shirokanedai 4–chome, Minato–ku, Tokyo.*
2) E.V. Truter, "Thin-Film Chromatography," Cleaver-Hume Press, London, 1963.
3) E. Stahl, "Dünschicht-Chromatographie," Springer-Verlag, Berlin, 1962.
4) K. Randerath, "Dunschicht-Chromatographie," Verlag Chemie, Weinheim, 1962.
5) E. Stahl, *Arch. Pharm.*, 292/64, 411 (1959).
6) M.S.J. Dallas, *J. Chromatog.*, **17**, 267 (1965).
7) M. Brenner and A. Niederwieser, *Experientia*, **17**, 237 (1961).
8) M. Brenner, A. Niederwieser, G. Pataki, and A.R. Fahmy, *Experimentia*, **18**, 101 (1962).
9) D. Braun and H. Greenen, *J. Chromatog.*, 7, 56 (1962).

G. The plates can easily be suspended from the stainless-steel rods with aids of paper clips as shown in Fig. 1. By turning the ring nut, G, the stainless-steel rods can be raised or lowered without trembling, since the boss slides freely along the groove. The rod, F, can be made longer by connecting some extention rods, H, between the plate, D, and the rod, F, and the thus rod elongated can suspend plates shorter than 20 cm in length.

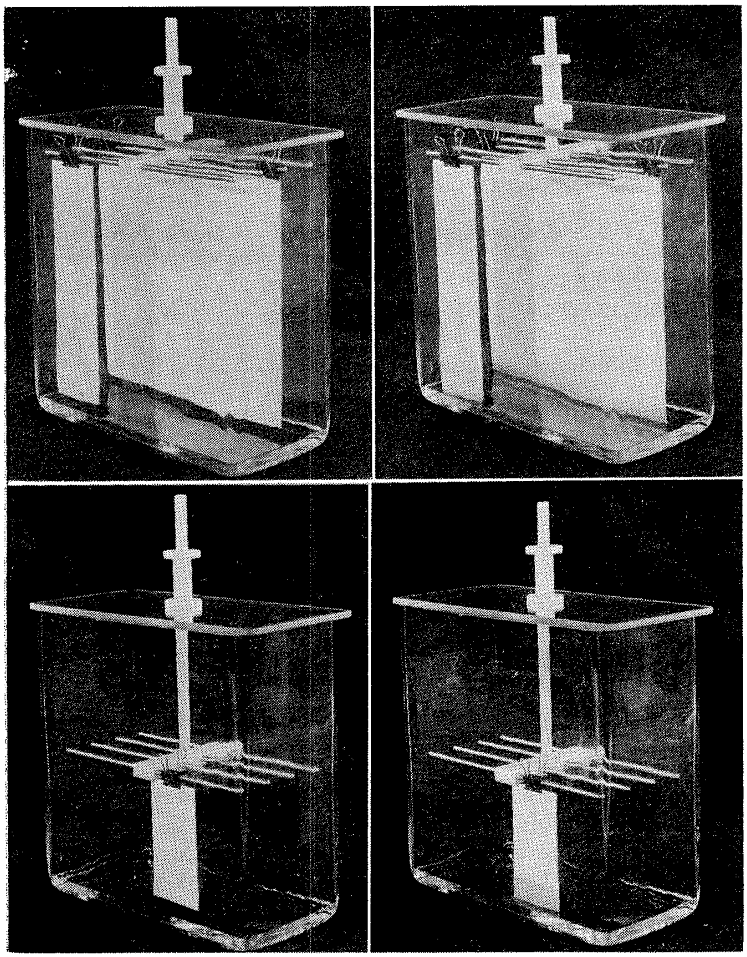
(a) (b)

Fig. 1. Chromatographic Chamber (Reproduced through the Courtesy of Shigemi Standard Joint Ind. Co., Tokyo) which can be used to equilibrate Thin-layer Chromatoplates prior to Development

a) in raised position b) in a lowered position

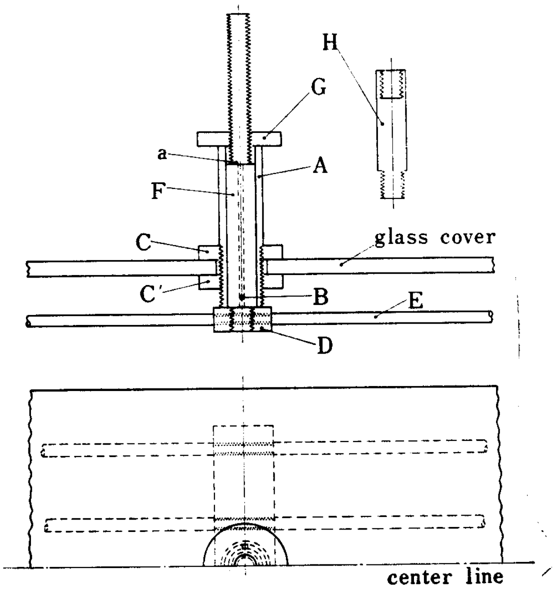

Fig. 2. Half Plans of the Device for suspending the Chromatoplates

Adsorbent——All the plates used as the adsorbent were ready-made plates; TLC-plates silica gel (without fluorescence indicator, 5×20 cm) were obtained from E. Merck AG, Darmstadt, and Polyamide UA Plate (5×10 cm) from Wako Pure Chemical Ind. Tokyo. The former plates were dried for 30 min at 110° and the latter for 30 min at 70°.

Reagents——*p*-Dimethylaminoazobenzene (Butter Yellow), as a tester for activity of silica gel plates, and 3-*tert*-butyl-4-hydroxynisol (BHA) were obtained from Wako Pure Chemical Ind. Tokyo. BHA was recrystallized from hexane. Caffeine obtained from Iwaki Pharm. Co., Tokyo, was recrystallized twice from benzene. These compounds were used for silica gel thin-layer chromatography. Benzoic acid, sorbic acid, and dehydroacetic acid obtained from Tokyo Chemical Ind. Co., were recrystallized from ethanol and subsequently from benzene. The standard dyes of Naphthol Yellow S, tartrazine, and Sunset Yellow FCF were distributed by National Institute of Hygienic Sciences. Seven acidic compounds were used for polyamide thin-layer chromatography.

The follwoing solutions were prepared as the testers in thin-layer chromatography; 0.2% benzene solution of Butter Yellow, 1% acetone solution of BHA, 2% EtOH solutions of dehydroacetic acid and benzoic acid, 0.2% EtOH solution of sorbic acid, 1% $CHCl_3$ solution of caffeine, and 0.2% aqueous solutions of Naphthol Yellow S, Tartrazine, and Sunset Yellow FCF. An aliquot (0.2—0.5 μl) of each solution was applied on the thin-layer plates. The solvent systems (A—F) are listed in Table I. All the solvents used in this experiment are of analytical grade.

Common Application of Compounds and Development of Chromatoplates——An aliquot of a test solution was spotted with a micropipette on a line 1.5 cm from the lower edge of the plate. The thin-layer chromatoplates were developed by either of the following developing manners (a) or (b).

Development (a): The plate was suspended from the stainless-steel rod with a clip, controlled by turning the ring, G, so that the lower edge of the plate would be immersed in the solvent to a depth of about

Table I. Solvent System for Thin-Layer Chromatography

a) Silica gel plates

Compound	Solvent system		
	Symbol	Component	Ratio (v/v)
Butter Yellow	A	ethyl acetate	
Butyrated hydroxyanisol	B	benzene–acetone	9:1
Caffeine	C	ethyl acetate–methanol–acetic acid	8:1:1

b) Polyamide plates

Compound	Solvent system		
	Symbol	Component	Ratio (v/v)
Butyrated hydroxyanisol	D	methanol	
Benzoic acid, Dehydroacetic acid Sorbic acid	E	benzene–acetic acid	40:1
Naphthol Yellow S, Sunset Yellow FCF, Tartrazine	F	butanol–pyridine–conc. NH_4OH–H_2O	0.5:1:6:4

0.5 cm, and then the placed in the chamber, the inside of which had partially been lined with filter paper soaked in the solvent.

Development (b): After the plate was suspended from the rod according to the above manner and subsequently controlled by revolving the ring so that the plate would not be immersed in the solvent, the plate was held over the solvent in the chamber under the same condition as in (a). After being allowed to stand for a definite time, the plate was lowered by the ring without opening the glass-cover of the chamber so that the lower edge of the plate was immersed in the solvent to a depth of about 0.5 cm.

As soon as the solvent had exactly travelled a desired distance from the starting line, the plate was removed rapidly and dried as quickly as possible with a stream of warm air or by holding the plate between two pieces of filter paper.

The following experiments were carried out by the use of these procedures for the application of compounds and development of chromatoplates.

Procedure 1: After spotting 0.5 μg of Butter Yellow on each of silica gel plates, the plates were separated into some groups; one group of plates was developed with solvent system A according to the developing manner (a), and the other groups were developed with the same solvent after being suspended for 5 to 20 min at 5 min intervals according to the developing manner (b). Development was carried out at 20±1° until the solvent front had travelled 6 cm from the starting line.

Procedure 2: After spotting 0.5 μg of Butter Yellow on each of silica gel plates, they were separated into some groups; one group of plates was developed with solvent system A according to the developing manner (a), and the other groups were developed with the same solvent after being suspended for 15 min over the solvent according to the developing manner (b). Development was carried out at 20±1° until the solvent front had travelled to 6 cm at 1 cm intervals from the starting line.

According to the same procedure as for Butter Yellow, 2 μg each of BHA and 5 μg of caffeine on silica gel plates, and 2 μg of BHA, 4 μg each of benzoic acid and dehydroacetic acid, and 0.5 μg of sorbic acid, Naphthol Yellow S, Tartrazine, and Sunset Yellow FCF was spotted on polyamide plates, each plate was developed using one of the solvent systems (B—E), listed in Table I.

Procedure 3: After spotting 0.5 μg of Butter Yellow on each of silica gel plates, they were separated into some groups. Each group was developed with solvent system A, having been suspended over the solvent which kept at 10±1° or 20±1° for 15 to 30 min at 5 min intervals acccrding to the developing manner (b). Development was carried out until the solvent front had travelled 6 cm.

By the same steps as above, 0.5 μg of sorbic acid was spotted on each of polyamide plates, and the plates were developed with solvent system E.

Detection of Spots on the Chromatograms——The spots of dehydroacetic acid, sorbic acid, and benzoic acid were observed under ultraviolet (UV) ray (2536 Å), and those of BHA and caffeine were visualized by placing the developed chromatoplates in I_2 vapor.

Result and Discussion

It is known that care should be taken to control the nature of the adsorbent, the developing solvent system, the nature and amount of sample applied, saturation of the chamber, the multicomponent solvent system, temperature, and so on,[2–9] in order to obtain the reproducible

Rf value in thin-layer chromatography. As a way to saturate the chamber with solvent as completely as possible before commencing the development, lining the inside walls of the chamber partially with filter paper soaked into the solvent and shaking the chamber containing the solvent are conventionally carried out.

When using the chromatographic chamber proposed in this study, any plates of from 5 to 20 cm in length can be raised or lowered without opening the cover of the chamber. This thereby allows adequate equilibration of the chromatographic media with the solvent vapor prior to development.

In order to prevent variation in *Rf* values resulting from the nautre of the plates, ready-made plates, which have a fairly consistent thickness and whose activities are kept consistent by drying them at a constant temperature for a constant time, were used in the present experiments.

When the chromatoplates were developed with solvent system A according to the procedure 1, equilibration of the chromatographic media with the solvent vapor for a shorter than 10 min induced a weak so-called "edge effect" at the initial stage of development. As shown in Fig. 3, when Butter Yellow was developed after the chromatographic media had been equilibrated with the solvent vapor for a longer than 15 min before commencing the development, the *Rf* value of the compound on the chromatograms became constant and the variation in the *Rf* value was below ±0.01.

Judging from these results, equilibration of the chromatographic media with the solvent vapor was completely established by suspending the plate for 15 min without immersing it into solvent, although it is natural that the time required for complete saturation of the chamber would be related to the constitution of the solvent system, properties of the constitutional solvents, and capacity of the chamber.

In order to know how successfully the reproducible *Rf* values would be obtained using the chamber, several compounds which are different in polarity were selected and developed on either silica gel or polyamide plates with the solvent systems consisting of one or more kinds of solvent.

When Butter Yellow was developed with the solvent system A by the developing manner (b), migration distance of the compound was proportional to that of the solvent. When the compound was developed with the same solvent by the developing manner (a), migration distance of the compound was marked longer at the initial stage of development than that obtained by the developing manner (b), because the solvent vaporized from the plates, especially from the solvent front. As soon as the vapor saturation was established during the development, migration distance of the compound became proportional to that of the solvent. The *Rf* values obtained thereby were always higher, as is well known,$^{6)}$ than those obtained by the developing manner (b) (Fig. 4a).

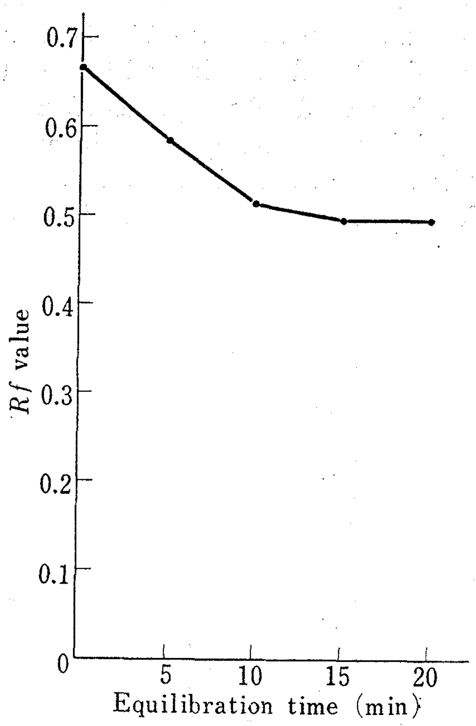

Fig. 3. Relationship between Equilibration Time of Silica gel Plates of Butter Yellow and Its *Rf* Value

solvent system: ethyl acetate
temperature: 20±1°

As shown in Fig. 4b—d, the same results were also obtained when BHA and caffeine were developed with the solvent system B and C, respectively, on silica gel plates, and BHA with solvent system D on polyamide plates.

It was found, however, that when dehydroacetic acid, sorbic acid, or benzoic acid was developed with the solvent system E on polyamide plates before equilibration of the chromatographic media was not completely established according to the developing manner (a), the compounds showed interesting patterns (Fig. 4e) in their mobilities at the initial stage of development. Sorbic acid and benzoic acid once slowed down in migration speed for a short time at the initial stage to show a marked lower *Rf* values than those obtained by the developing manner (b). On the other hand, dehydroacetic acid always showed higher *Rf* values than those obtained in such an equilibration, and this is in contrast to the patterns of sorbic acid and benzoic acid.

When Naphthol Yellow S, Tartrazine, or Sunset Yellow FCF was developed with a more polar solvent system than solvent system E, the compounds showed the same tendency on the chromatograms as benzoic acid and sorbic acid (Fig. 4f).

It is assumed that the reason why these compounds showed lower *Rf* values was the formation of a different constitution of the solvent system on the chromatographic media at the initial stage of the development, because vaporization of the more volatile component in the solvent systems before the vapor saturation had been accomplished in the chamber. When the development was commenced after adequate equilibration of the chromatographic media with the solvent vapor according to the developing manner (b), highly reproducible *Rf* values were obtained for all the compounds tested without regard to the migration distance of the solvent, and will be able to be obtained even if the starting line be lower than 1 cm from the lower edge of the plates.

It is said that variations in temperature have little effect on thin-layer chromatography, and the fact is in contrast to the situation with paper chromatography.[8] When Butter Yellow was developed on silica gel plates at 10° and 20° according to the procedure 3, the *Rf* values obtained gradually approached a constant value as shown in Fig. 5. It was necessary for obtaining accurate *Rf* values to suspend the plates for a more prolonged time at 10° than at 20° without immersing them in the solvent. The *Rf* value were constant when the compound was developed after the plates had been suspended for 20 min at 10° or for 15 min at 20° without

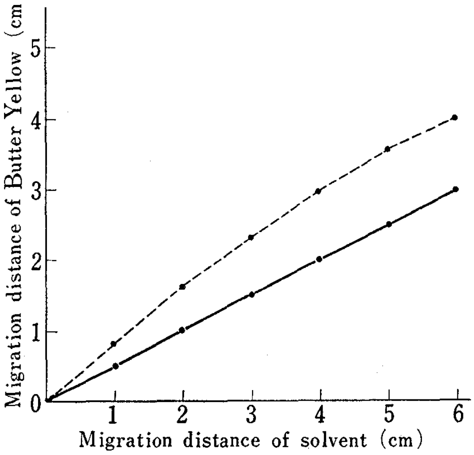

Fig. 4-a. Mobility of Butter Yellow showing the Effect of Prior Equilibration

plate: silica gel
solvent system: ethyl acetate
------: unequilibrated chromatogram
——: chromatogram equilibrated over solvent for 15 min

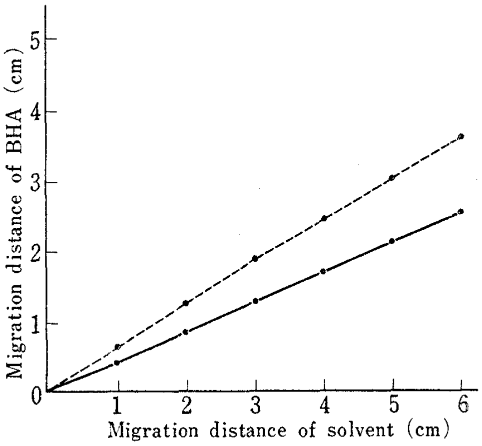

Fig. 4-b. Mobility of BHA showing the Effect of Prior Equilibration

plate: silica gel
solvent system: benzene-acetone (9:1)
------: unequilibrated chromatogram
——: chromatogram equilibrated over solvent for 15 min

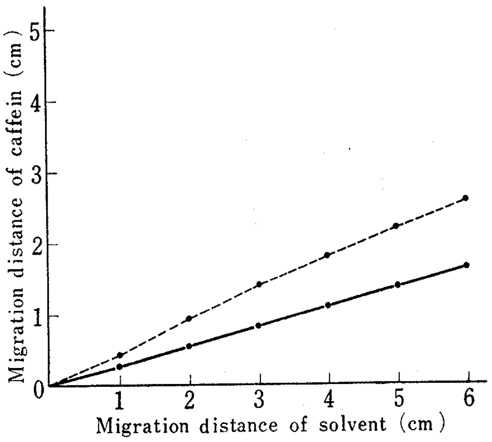

Fig. 4-c. Mobility of Caffeine showing the Effect of Prior Equilibration

plate: silica gel
solvent system: ethyl acetate-methanol-acetic acid (8:1:1)
------: unequilibrated chromatogram
——: chromatogram equilibrated over solvent for 15 min

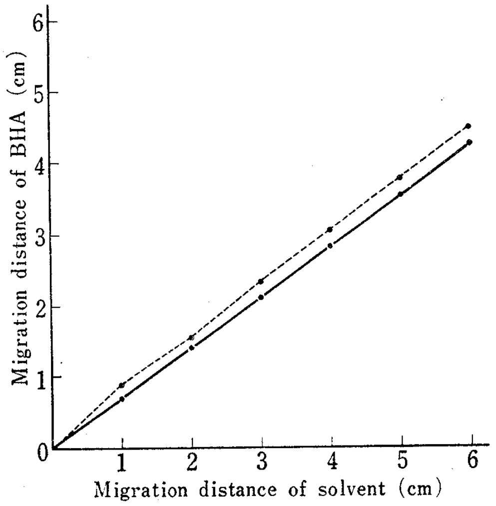

Fig. 4-d. Mobility of BHA showing the Effect of Prior Equilibration

plate: polyamide
solvent system: methanol
------: unequilibrated chromatogram
——: chromatogram equilibrated over solvent for 15 min

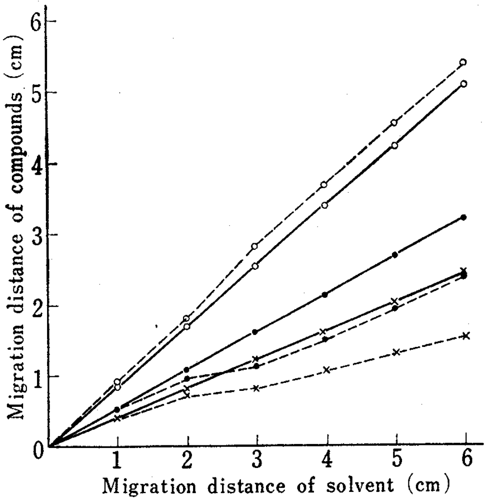

Fig. 4-e. Mobility of Dehydroacetic Acid, Sorbic Acid, and Benzoic Acid showing the Effect of Prior Equilibration

plate: polyamide
solvent system: benzene-acetic acid (40:1)
○: dehydroacetic acid ●: sorbic acid
×: benzoic acid
------: unequilibrated chromatogram
——: chromatogram equilibrated over solvent for 15 min

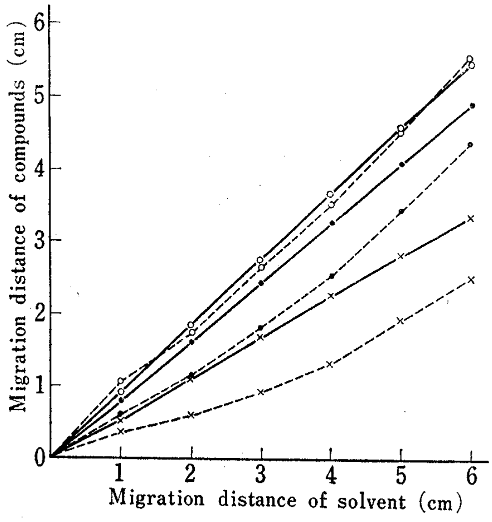

Fig. 4-f. Mobility of Naphthol Yellow S, Tartrazine, and Sunset Yellow FCF showing the Effect of Prior Equilibration

plate: polymide
solvent system: butanol-pyridine-conc. NH_4-OH-H_2O
○: Tartrazine ●: Sunset Yellow FCF
×: Naphthol Yellow S
------: unequilibrated chromatogram
——: chromatogram equilibrated over solvent for 15 min

immersion. In the development of sorbic acid on polyamide plates, it took longer time to obtain a constant *Rf* value than in the case of Butter Yellow on silica gel plates, because the solvent system E is less volatile than solvent system A.

Therefore, a condition for obtaining reproducible *Rf* value of a compound was not related directly to the temperature at which the compound was developed, but was related to whether equilibration of the chromatographic media with the solvent vapor was completely established before commencing the actual development or not. It is natural that the time required for such equilibration should be decided by the kinds of solvents used in the solvent system.

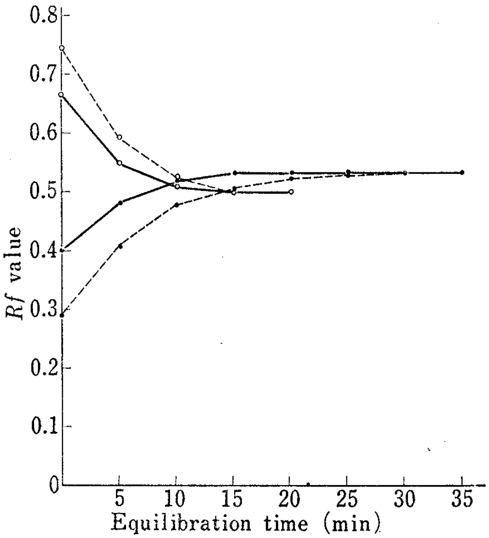

Fig. 5. Effect of Prior Equilibration on Developing Temperature

plate: silica gel and polyamide
solvent system: ethyl acetate for Butter Yellow, and benzene–acetic acid (40:1) for sorbic acid
○: Butter Yellow ●: sorbic acid
------: at 10±1°, ——: at 20±1°

In order to speed up the equilibration at a low temperature, following steps may be recommended: The solvent system is previously warmed at 30—40° so that the constitution of the solvent system is not changed, and then the plates are suspended without immersing in the solvent using the proposed chamber until the temperature of the solvent and the vapor in the chamber reach the developing temperature, and developed without opening the glass-cover of the chamber according to the developing manner (b).

Conclusion

The chromatographic chamber equipped with a suspender by which plates could be immersed into solvent systems at a desired time was deviced. By using this chamber, nine compounds were developed on ready-made silica gel or polyamide plates with several solvent systems, consisting of one or more kinds of a solvent. When the compounds were developed after the chromatographic media had completely been equilibrated with the solvent vapor by suspending the plates without immersion, all the compounds always showed constant *Rf* values without regard to the migration distance of the solvent or to the temperature during the development. The time required to obtain equilibration naturally, depended on the kind of solvents used, capacity of the chamber, and temperature at which the development was carried out.

An interesting result was obtained on comparison between the patterns of the chromatograms when the compounds were developed according to the conventional manner and after vapor equilibration of the chromatographic media, the *Rf* values obtained in the former manner were higher or lower according to the solvent systems used than those obtained by the latter manner.

As the plates can be suspended with this apparatus, the bottoms of the chamber need not be flat, and moreover the lower part of the plates can be immersed into the solvent to a desired distance even if much solvent be placed in the chamber.

This chromatographic chamber is highly useful in obtaining reproducible *Rf* values in partition and adsorption chromatography.