Parameters of Preparative Centrifugal Thin-Layer Chromatography

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Summary

The separation performance of preparative, centrifugal thin-layer chromatography was investigated for various flow rates and rotational speeds by means of a modified commercial instrument. High flow rates give rapid separations but require large amounts of solvent. Maximum separation (that is, greatest resolution R_s) is obtained at medium speeds and flow rates. The maximum sample size for a successful separation depends primarily on the type of substance. It varies from 10 to 1 000 mg per compound and per mm of layer thickness. Centrifugal TLC is superior to conventional "layer chromatography". Possible improvements of the instrument are pointed out.

Introduction

In 1955, Caronna [1, 2] published two articles in which he described a novel development technique for paper chromatography. He rotated a circular shaped paper about its centre in order to speed up the motion of the mobile phase through the effect of centrifugal force. With this so-called centrifugal chromatography a substantial saving of development time could be achieved.

The further development of this method has been summarized in a review article by Deyl, Rosmus and Pavlicek [3]. These authors were also the first to apply the centrifugal technique to thin-layer chromatography [4]. Its scale-up to a preparative technique eventually led to the construction of the "Chromatotron", a commercial instrument which has been available for some time.

Derguini, Balogh-Nair and Nakanishi [5] utilized this apparatus for separating isomeric esters. Hostettmann, Hostettmann-Kaldas and Sticher [6] separated xanthones, triterpenes and saponines. It has also been used for the isolation of furanocumarines [7].

The following experiments were made to study the role of some basic operational parameters of centrifugal thin-layer chromatography.

Experimental

Apparatus

Chromatotron model 7924 (HARRISON RESEARCH, Palo Alto, U. S. A.) (Fig. 1) for preparative radial TLC. The built-in motor generates a constant speed of 750 rpm. In the place of the standard machine we used a variable-speed motor and a revolution counter. Through this modification it was possible to vary speeds from 250 rpm up to 2000 rpm. The solvent was delivered by the wobble pump RP-G 150-OSSY (FMI, New York, U.S.A.).

The recording equipment consisted of a UV detector set at 254 nm (built by Physikalische Chemie, Saarbrücken, West Germany) and a pen recorder SERVOGOR 2, RE 520 (METRAWATT AG).

Preparation of Rotor Coatings

Kieselgel 60 PF_{254} gipshaltig (silica gel containing gypsum) and TLC-Kieselgel 60 GF_{254} (both from MERCK, D-6100 Darmstadt, FRG) served as coating materials. Layers
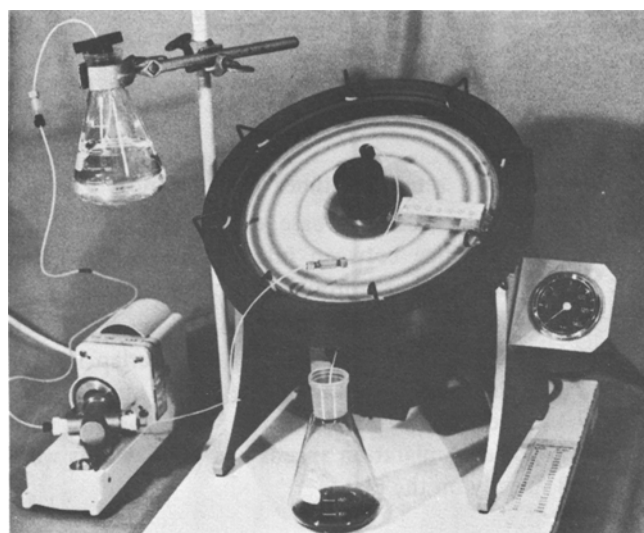

Fig. 1

View of Chromatotron with additional tachometer. Rotor plate with zones of three separated dyes is visible in centre. Fractions can be collected from short piece of tubing at bottom of chromatographic chamber. Pump is located on left. It transports solvent from supply vessel (upper left) to rotor. Speed gauge is attached to right side of chamber wall.

of 1, 2, and 4 mm thickness were prepared from Kieselgel 60 PF$_{254}$ gipshaltig according to the instructions of the Chromatotron manufacturer. Good 1 mm layers could also be made from a mixture of 40 g TLC-Kieselgel 60 GF$_{254}$, 90 ml water, and 1.2 ml Acronal 250 D(BASF AG,D-6700 Ludwigshafen, FRG) as binder. The Kieselgel content of one such layer amounted to 15 g. 2 mm layers occasionally showed fine cracks which had little influence on the separation performance, though. Attempts failed to prepare 4 mm layers from this material.

Test Solutions

Chromatographically purified Ceresrot 7B, Ceresgrün BB (BAYER AG, D-5090 Leverkusen, FRG) and Sudanrot G (BASF AG, D-6700 Ludwigshafen) were dissolved in toluene to give a concentration of 1.5 % each. Ceresrot 7B and Ceresgrün BB were also used as 2.4 % solutions in dichloromethane. Cycloheptanone- and cyclopentanone-2,4-dinitrophenyl hydrazones were dissolved in hexane-ethyl acetate (80 + 20) to give 0.15, 0.037, and 0.009 % solutions.

Operating Conditions

The 1 mm layer of TLC-Kieselgel 60 GF$_{254}$ with 1.5 % Acronal 250 D as binder was dried at 70 °C for 20 min and conditioned in air (relative humidity 35—40 %) for ten minutes prior to each test run.

The hydrazone mixtures were applied after saturation equilibrium had been reached in the chromatographic chamber. The dye mixtures were applied directly to the dry layer. Further conditions are given with the figures.

For (linear) TLC a layer thickness of 0.25 mm was used. Materials and solvents were the same as for centrifugal TLC. The separation efficiency was determined by calculating the resolution R$_s$. The latter was computed from the peak distances and peak widths according to the formula

$$R_s = \frac{2(t_{R2} - t_{R1})}{w_{b1} + w_{b2}}$$

where

t_{R2}, t_{R1} : total retention times of substances 1 and 2

w_{b2}, w_{b1} : peak widths at base of substances 1 and 2 (distance between intersections of base line and tangents through inflection points)

At R$_s$ = 1 (4σ separation) Gaussian peaks show an overlap of 3 %. At R$_s$ = 1.5 (6σ separation) the overlap is only 0.2 %, i. e. the separation can be regarded quantitative. When UV detection was not possible, for example, with toluene as solvent, migration speeds and zone widths were measured visually on the plate.

Results and Discussion

Coating Material and Layer Thickness

The comparison of the two silica gels and layers of different thickness yielded the following results (Table I):

a) Kieselgel 60 PF$_{254}$ gives somewhat inferior separations than TLC-Kieselgel 60 GF$_{254}$ with 1.5 % Acronal 250 D.

b) The separating efficiencies of the 1 mm and 2 mm layers are about equal; that of the 4 mm layer is comparable although a little worse. For thicker layers the solvent flow must be increased appropriately (see below).

c) With insufficient solvent flow the separating efficiency of the 4 mm layer deteriorates sharply. For this thickness the delivery of the company-supplied pump is too low by a factor of 2.

For the following experiments 1 mm layers of TLC-Kieselgel 60 GF$_{254}$ with 1.5 % Acronal 250 D were used.

Table I. Resolution R$_s$ for two sorbents and increasing layer thickness

Flow rate ml/min	TLC-Kieselgel 60 GF$_{254}$ with 1.5 % Acronal 250 D		Kieselgel 60 PF$_{254}$ containing gypsum		
	1 mm	2 mm	1 mm	2 mm	4 mm
4.7	2.8		2.1		
8.8		2.6		2.1	1.3
18.8					2.0

0.8 ml hydrazone test mixture (0.15 %) per mm layer thickness. Solvent: hexane-ethyl acetate (80 + 20). Speed: 750 rpm

Speed of Rotation and Flow Rate

Maximum flow rate

For each speed setting an upper limit for the flow rate exists. With higher influx the capacity of the layer is exceeded so that the solvent starts running across the surface.

Fig. 2 shows that the maximum flow rate is also dependent on the solvent. Higher viscosity results in a slower motion and thus necessitates a lower pumping speed.
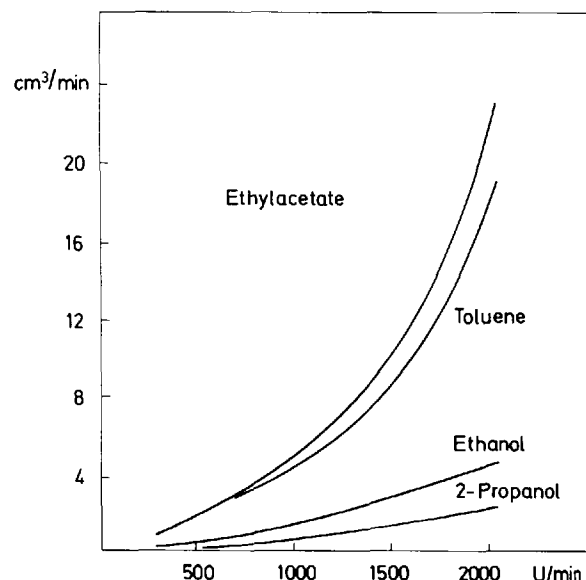

Fig. 2

Maximum flow rate for different rotational speeds and solvents. Coating: 1 mm layer, TLC-Kieselgel 60 GF$_{254}$ with 1.5 % Acronal 250 D.

Zone width

The distribution of the solvent within the layer is not even. High saturation persists at the beginning and the end of the length of run with an area of lower concentration in between. The extension of those areas depends on the speed of rotation and has a considerable influence on the peak widths.

With proper settings for rotational speed and flow rate the zone widths increase steadily to approach a maximum (Fig. 3). Slower rotation (Fig. 4) results in a definite deterioration which can be related to the changed solvent distribution within the layer.

The collection system, too, contributes to zone broadening. The extent of this contribution depends on the speed of rotation. At speeds above 1500 rpm the draining of the solvent from the chromatographic chamber is seriously

inhibited. The dead volume increases and a spray of fine droplets is dispersed through the apparatus.

Various flow rates and speeds were tried to find the best settings for separations (Fig. 5). It must be noted that the results are not solely dependent on the separating layer but also include the contribution from the collection system. The curves show maxima at medium speeds. The best separation is reached at 750 rpm and a flow rate of 3.5 ml.

Solvent consumption and separation time

The solvent consumption rises strongly with increasing flow rate. The speed of rotation has a smaller effect. Higher speeds result in a slight decrease of solvent consumption (Fig. 6). The total time for a chromatogram and the solvent consumption are inversely proportional: rapid separations require a great amount of solvent and vice versa.

Choice of speed and flow rate

If the best possible separation is desired a flow rate of 3—6 ml/min per mm of layer thickness is selected. Then the Chromatotron is started with a speed of 1000 rpm until equilibrium is reached, that is, the solvent flow through the apparatus and the saturation of the atmosphere are constant. After this the minimum speed is sought by lowering the rotation down to the point where the solvent just starts running across the layer. This minimum speed is raised by about 100—200 rpm and the separation is carried out.
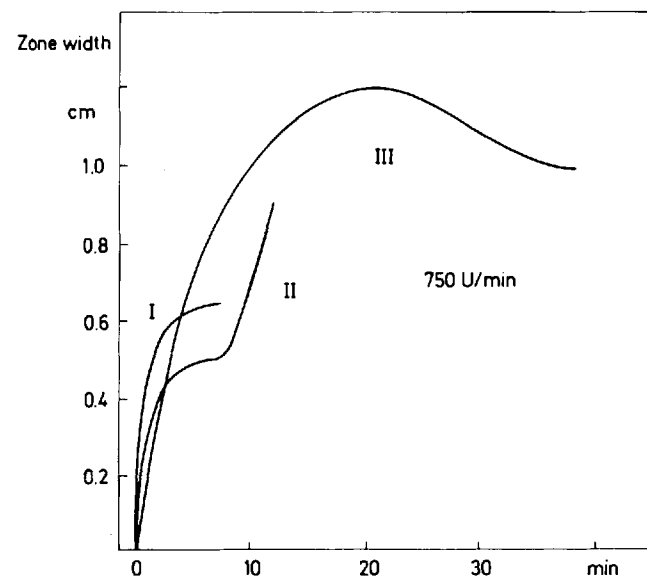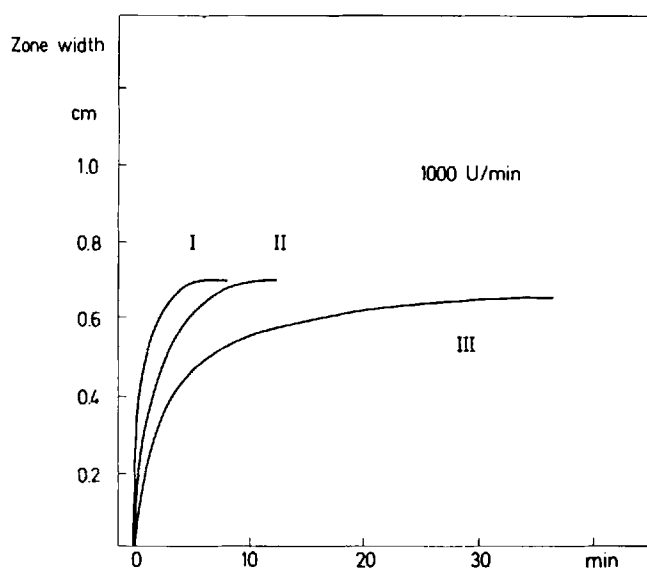

Fig. 3 and Fig. 4

Change of zone widths during chromatography. I Ceresrot 7B, II Ceresgrün BB, III Sudanrot G. Coating: 1 mm TLC-Kieselgel 60 GF_{254} with 1.5 % Acronal 250 D, solvent: toluene, 3.5 ml min.
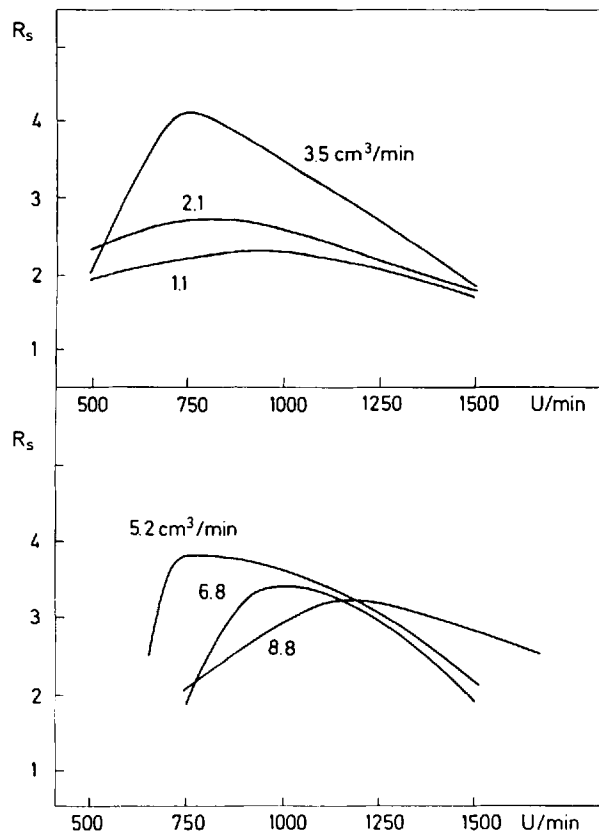

Fig. 5

Resolution R_s as function of rotational speed and flow rate. Hydrazone test mixture (0.15 %). Coating: 1 mm layer, TLC-Kieselgel 60 GF_{254} with 1.5 % Acronal 250 D. Solvent: hexane-ethyl acetate (80 + 20).
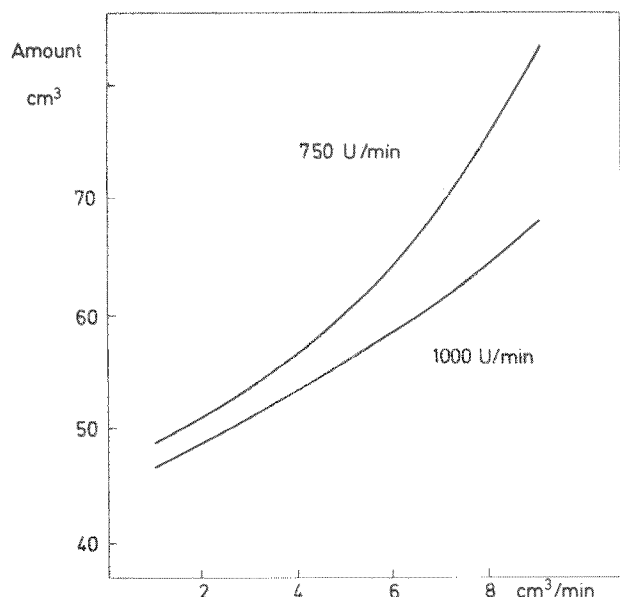
Fig. 6
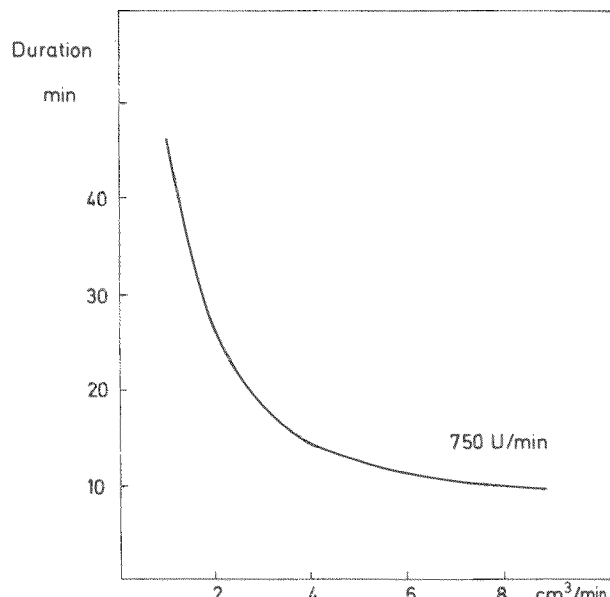Solvent consumption at different flow rates and rotational speeds. Conditions see Fig. 5.

Fig. 7
Total run time for different flow rates. Conditions see Fig. 5.

If one is primarily interested in a speedy separation and poorer resolution and higher solvent consumption are acceptable a high flow rate is selected (Fig. 7). Again the speed should be 100–200 rpm above the minimum value. An especially low solvent consumption can be achieved with very low flow rates. The price is an inferior separation and an enormous increase of analysis time. At low flow rates the minimum speed is no longer defined by solvent overflow at the starting site but rather by the point where the ejection of liquid at the rim ceases completely. Under these conditions separations are no longer possible.

Sample Size

Fig. 8 shows how the separation of the hydrazone test mixture is affected by the total mass of the sample. It should be noted that the maximum is independent of the concentration of the test solutions. The maximum amount that can be separated on 1 mm layers have been compiled in Table II along with their Rf-distances in normal TLC. Although such a correlation can only be approximate it is certainly useful for practical purposes since in most cases TLC tests will be made anyway prior to the preparative run in order to select the most suitable solvent and coating material. The results for the dye mixtures show that 1300 mg can be separated with sufficiently large Rf distances. The substantially lower values for the hydrazones stem from their tendency for tailing.

Comparison With Linear Preparative TLC

Centrifugal TLC is a circular form of preparative TLC. A comparison with the normally used linear preparative TLC [8, 9] is therefore of special interest. The latter utilizes large size layers with a width up to 1 m [9, 10] whose dimensions and weight (one set of five such layers weighs about 20 kg) makes their handling very cumbersome. They
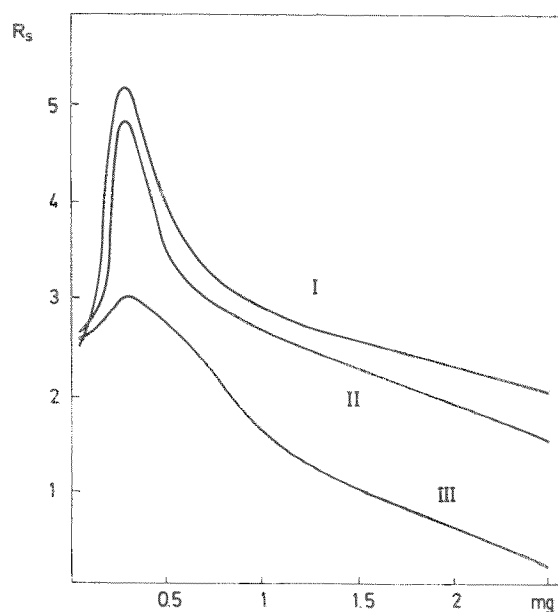

Fig. 8
Resolution R_s for various amounts of hydrazone test mixture.
I : Concentration of sample solution c_I = 0.15 % per component
II : concentration of sample solution c_{II} = 1/4 of c_I
III : concentration of sample solution c_{III} = 1/16 of
Layer and solvent as given in Fig. 5. Flow rate: 5.2 ml/min
Speed 750 rpm.

Table II. Correlation between maximum load per component and respective peak locations and peak separations (ΔRf_{lin}) normal TLC

Location of TLC zones	ΔRf_{lin}	Hydrazones	Dyes
medium and upper half	1	10 mg	130 mg
	2–3		1300 mg
lower half	1	15 mg	
	2–3		900 mg

* Figures are mg compound per mm layer thickness

can be used only once while the handy rotor layers of centrifugal TLC may be used repeatedly.

The sample solution is usually applied in form of a band. Drying is needed after each application so that a relatively long time is required. Furthermore an uneven distribution of the substances in the starting band may take place (concentration on the surface through evaporation, formation of multiple zones). Some substances may crystallize and so cause strong tailing of their zones and an uneven flow of the solvent. Others may even undergo chemical reactions during the drying process [11]. Other techniques use one or several grooves which are scratched into the layer and loaded with the solid [12] or dissolved sample [13]. They, too, look clumsy when compared with centrifugal TLC where the sample solution is supplied continuously and all of the above drawbacks are avoided.

The development time of preparative TLC is relatively long especially since multiple development is advisable [9, 10]. With centrifugal TLC multiple development is also possible but not necessary. Further time saving results from the faster flow of the solvent through the centrifugal force so that the additional time for eluting the zones hardly matters. Another positive aspect is the smaller zone width of the circular technique [14, 15] (Fig. 9) and the greater capacity. In linear preparative TLC about 50 mg of sample can be applied per cm of the starting band, while with centrifugal TLC under comparable conditions about 150 mg/cm can be separated.

Another considerable advantage of the centrifugal technique is the possibility of eluting the zones. Such elution techniques are also known for linear TLC [16–18] but compared with centrifugal TLC they are very tedious, troublesome and time consuming. Scratching-off and further processing the individual components is equally unattractive.

Comments on the Chromatotron

Centrifugal TLC has proved to be superior to conventional preparative TLC in several important points. It is also a valuable alternative to column chromatography (CC) because of its greater separation power. Compared to HPLC it offers the advantage of greater simplicity and lower cost.
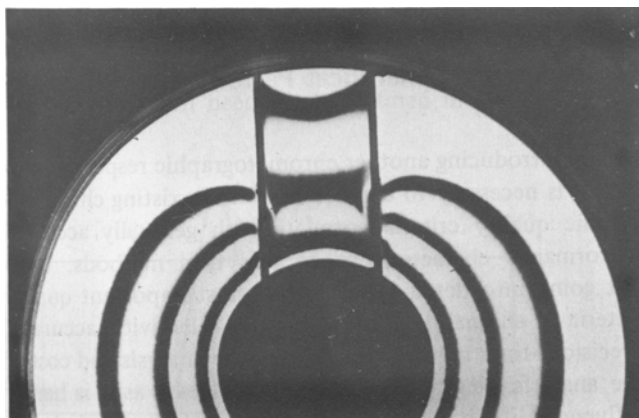

Fig. 9

Comparison of linear and radial development. Zone widths are significantly smaller for radial development.

There are also some disadvantages, though. Strongly polar or polymeric substances must be removed before the sample can be applied to the Chromatotron; otherwise clogging of the starting site occurs which must be of even more concern than with the linear technique. Another problem is detection: the installation of detectors is difficult and limits the usefulness of the method.

The most serious problem however is contamination of the plastic collector dish with unwanted sample components. These stem from several sources. Sample application is not entirely free of splashing. Also some crystallization may occur at the starting site which is, however, of minor importance.

The main reason for contamination is splashing during the collection of the droplets thrown off. A small part is thus not caught in the drain but whirled away as an aerosol.

The degree of contamination becomes greater with increasing sample size. Thicker layers with higher flow rates aggravate the problem. The situation is less severe with the commercial pump which works at lower but inadequate flow rates. Splashing is thus diminished but the separation cannot be carried out under optimum conditions (see Table I).

The most detrimental effect, however, is diffusion of the collected substances into the plastic (Delrin^R) collection dish. Its purification takes a lot of solvent and time. An easy-to-clean material like stainless steel would certainly be of great advantage.

The contamination of the collection system renders the isolation of pure fractions problematic, especially when large samples are to be separated quickly. Often the zones which had already been separated in the layer are partially remixed in the collection system. With smaller samples, however, this effect becomes negligible and no tailing due to the collection system is usually observed.

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