



T-Squared Technology, Inc
903 Sneath Lane, Suite 125, San Bruno, Ca 94066, U S A
Tel: 650-871-0569 Fax: 650-873-6496
info: www.tsqtech.com e-mail: info@tsqtech.com

Chromatotron[®]

8924

Operation Manual

Copyright © Harrison

WARRANTY

The Chromatotron is warranted for a period of one year from date of delivery to be free from defects in workmanship and materials. All defects appearing more than one year from date of delivery shall be deemed to be due to normal wear and tear. Since the equipment and information given may be applied under conditions beyond our control, the user must determine the safety of procedures described and materials supplied under the user's conditions.

The warranty does not cover breakage of glass parts, corrosion from any cause or damage to painted or anodized surfaces.

REGISTERED TRADEMARKS

Chromatotron, Harrison

Teflon, E. I. Dupont.

Mineralight, UVP.

Ajax, Colgate Palmolive.

Florisil, Floridin Company

CAUTION

- * The Chromatotron is to be used only by those trained in the use of laboratory equipment and solvents.
- * Plug into a 3 pin (grounded) electrical outlet only.
- * Do not leave the Chromatotron unattended when solvent is flowing.
- * Unplug the Chromatotron if for any reason the rotor does not move freely.
- * The Chromatotron must be used with nitrogen or other inert gas.
- * The Chromatotron should be used in a hood with a good air flow to keep solvent vapor away from the operator.
- * Keep organic solvents away from the motor and electrical system.
- * After unplugging the Chromatotron, allow the rotor to come to rest without applying any braking force. External braking may loosen the rotor and damage the lid.
- * Do not use cracked rotors.
- * Organic solvents must be allowed to evaporate **completely** from sorbent layers before further drying by heating. Rotors that have been used with solvents (especially methanol) must not be placed in an oven.
- * Silicone oils and greases should not be used on or near the Chromatotron. The rotors may be contaminated irreversibly.
- * Silica gel and other finely powdered materials should be handled in a hood.
- * Wear suitable eye protection.
- * Use a filter to protect the pump from abrasive impurities in samples.
- * Pumps requires solvent for lubrication. Do not leave pumps running without solvent for more than a few min.
- * Do not pump toxic samples without suitable precautions to allow for accidental ejection under pressure.

CONTENTS

	Page
THE CHROMATOTRON AND HOW IT WORKS	
Introduction.	1
SETTING UP AND USING THE CHROMATOTRON	
Installation.	4
The Main Vessel.	4
The Teflon Lid.	4
The Solvent Inlet.	5
Solvent Pumps.	6
Nitrogen Flow.	8
Changing Rotors.	9
Sorbent Layer Thickness.	9
Prepurification of the Sample.	10
Solvent Choice.	10
Solvent Addition.	11
Introducing and Eluting the Sample	
Introducing the Sample.	12
Introducing Less Soluble Samples.	13
Maximum and Minimum Flow Rates.	14
Rapid Chromatography.	14
Interrupting Solvent Flow.	15
Heavy Loading.	15
Light Loading.	15
Detection of UV Absorbing Compounds on the Rotor.	15
Detection of Colorless UV Transparent Compounds.	16
Fraction Collection.	16
Clean-up and Regeneration of the Sorbent Layer.	17
Multiple Development.	18
Recycle.	18
Connecting Chromatotrons in Series.	19
COATING ROTORS WITH SORBENTS.	
Introduction.	20
Sorbents, Binders and Phosphors.	20
Recipes.	21
Coating Rotors Using Recipes 1-5, Gypsum-Bound Layers	
Introduction.	24
Cleaning and setting up the Rotor for Coating.	25
Mixing and Pouring the Recipe.	25
Drying the Sorbent Layer.	26
Scraping Sorbent Layers.	28
Storage of Coated Rotors.	29
Coating Rotors Using Recipe 6, Glue - Bound Layers.	29
Partition Chromatography, Recipe 4.	31
Correcting Band Slope.	32

	Page
MAINTENANCE AND REPAIRS	
Electrical Connections.	33
Solvent Pumps.	33
Adjusting the Main Vessel.	34
The Teflon Lid - Main Vessel Seal.	34
Replacing the Rotor Support Collar.	34
The Felt Seal.	34
Wear and Tear.	34
TROUBLESHOOTING.	35
CHROMATOTRON PARTS LIST.	40
APPENDIX	
The Test Mixture.	41
INDEX.	42

THE CHROMATOTRON AND HOW IT WORKS

INTRODUCTION

The Chromatotron is a preparative, centrifugally accelerated, radial, thin-layer chromatograph. The apparently simple construction hides a wealth of novel design details that ensure good resolution and ease of operation. The parts of the Chromatotron are shown on pages 2 and 3.

Chromatography is performed in a thin layer of sorbent **A** on a rotor **B**. The motor **F** drives the rotor at a constant speed by a shaft passing through a hole in the center of the main vessel **D**.

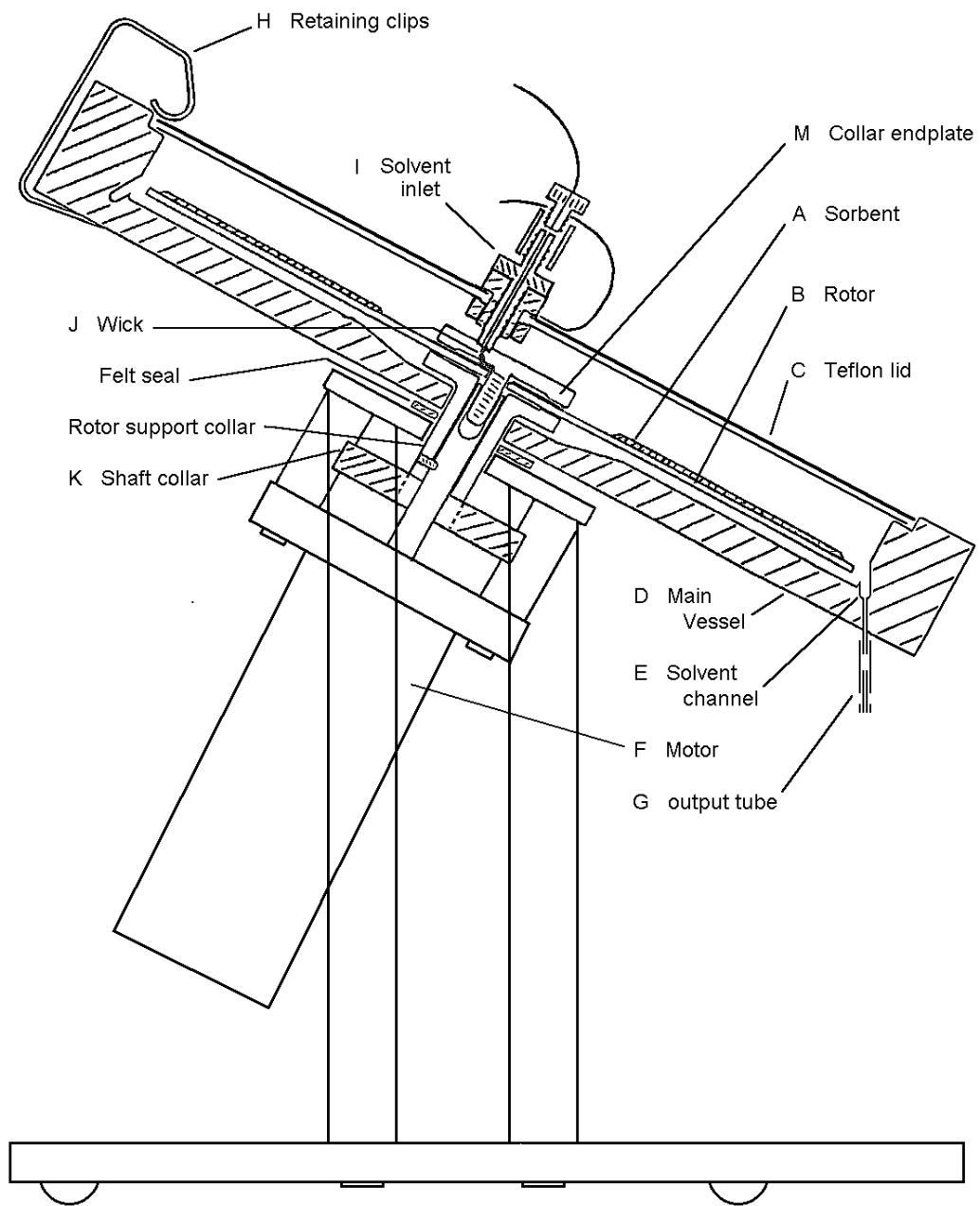
Solutions of samples to be separated are delivered to the rotor via the inlet **I** and wick **J**. Elution by solvent forms concentric bands of separated substances which leave the edge of the rotor together with solvent. Channel **E** collects the eluate and brings it to the output tube **G**.

Teflon lid **C** is transparent to UV light, allowing detection of UV absorbing bands. Eight retaining clips **H** hold the Teflon lid on the main vessel.

Chromatotrons can be connected in series by pumping the output of one to the input of the next. The output of a Chromatotron may be recycled to the input. Multiple development can be performed.

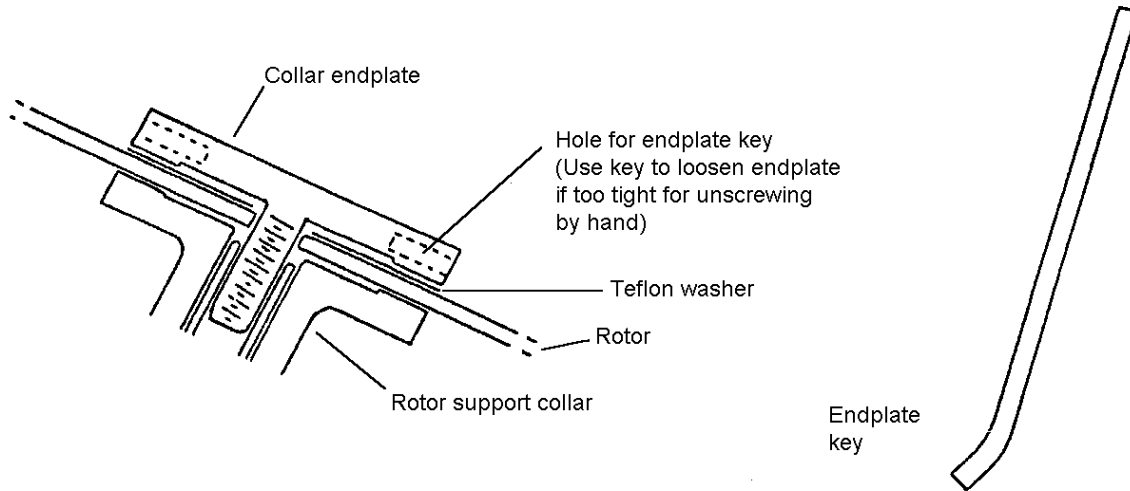
The Chromatotron was designed for preparative separations on silica gel and alumina. It is not useful for chromatography requiring cellulose or reversed phase sorbents.

THE CHROMATOTRON®

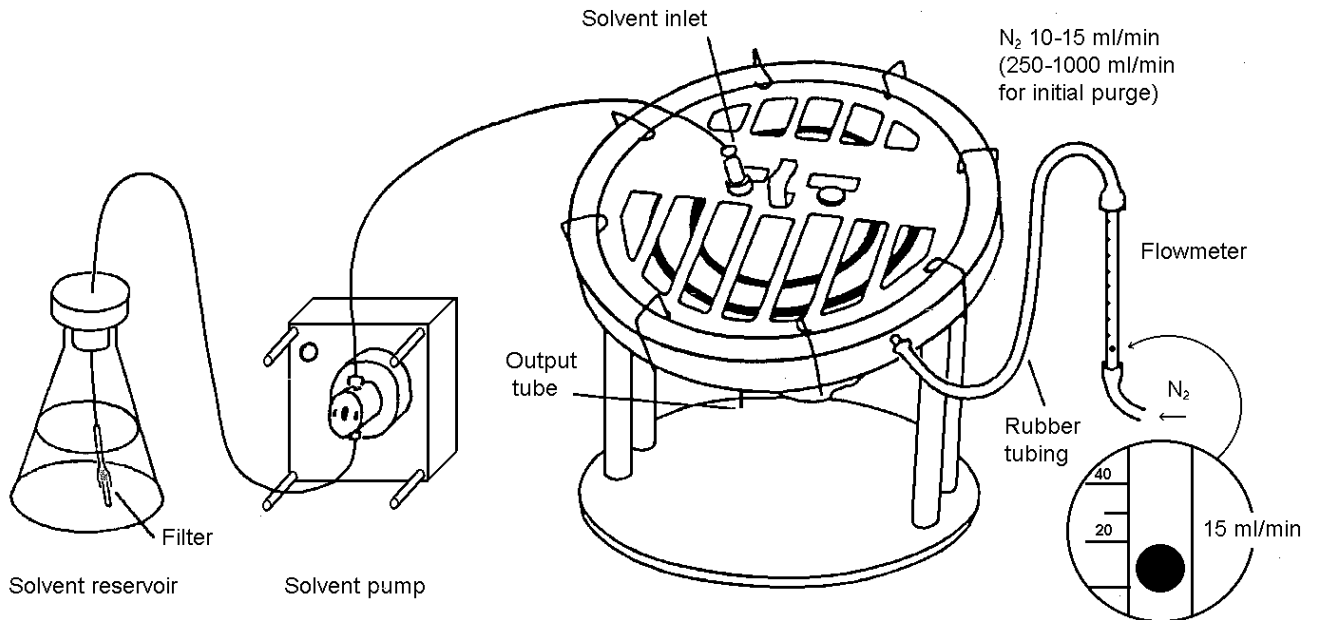


U.S. Patent no. 4139458

THE ROTOR SUPPORT SYSTEM



THE CHROMATOTRON AND PUMP SET-UP DIAGRAM



SETTING UP AND USING THE CHROMATOTRON

INSTALLATION

Set up the Chromatotron in a fume hood or similar area designed for the safe handling of organic solvents. Place the Chromatotron away from hotplates, steambaths or other sources of heat.

THE MAIN VESSEL

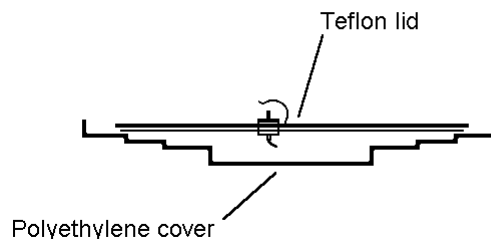
The main vessel is constructed from "acetal" polymer. After completion of chromatography, wash out solvents that soften or swell acetal (chloroform, dichloromethane, acetone, acetonitrile) with hexane, other hydrocarbons or ethyl acetate. The solvents ether, ethyl acetate, tetrahydrofuran, dioxane, methanol, hydrocarbons and carbon tetrachloride are relatively inert towards acetal. Acetic acid, trimethylamine and ammonia are acceptable as additives to chromatography solvents. The possibility of solvent damage to plastic parts can be reduced by leaving the nitrogen supply on when the Chromatotron is not in use.

Do not use mineral acids, formic or other acids stronger than acetic acid. Chloroform contains hydrochloric acid which will attack the main vessel, metal parts and your sample. Remove acid by adding alumina or other solid bases to the chloroform and check with wet pH paper. Consider using dichloromethane in place of chloroform.

Clean the solvent collection channel at intervals and clear the output tube with a straightened paper clip.

THE TEFLON LID

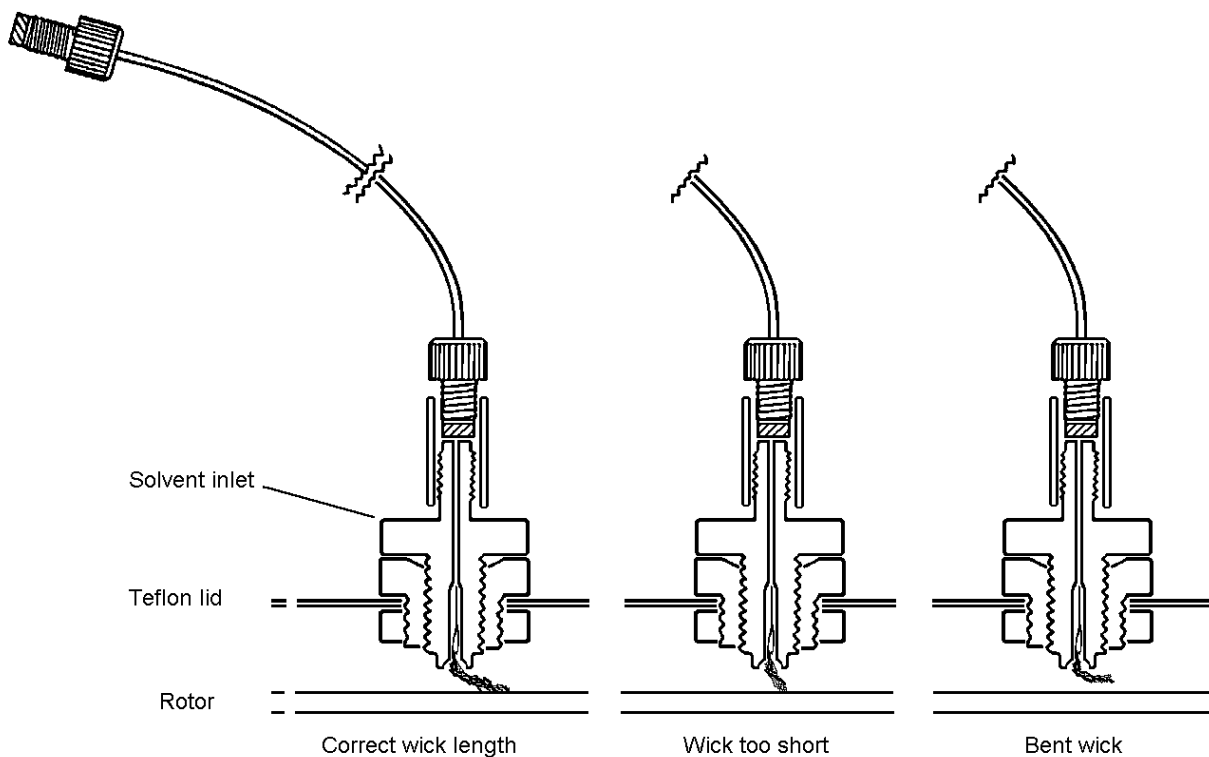
The polyethylene cover should be placed over the Teflon lid when the Chromatotron is not in use. When the Teflon lid is out of the Chromatotron, place it on the inverted polyethylene cover:



Maintain the Teflon lid clean. Large particles of dust on either side of the Teflon sheet, near the edge or in the corresponding recess in the main vessel, will cause solvent vapor leaks. A moist, soft paper towel or cloth will remove dust with a minimum of scratching.

THE SOLVENT INLET

Screw the inlet fully down into the holder in the Teflon lid.

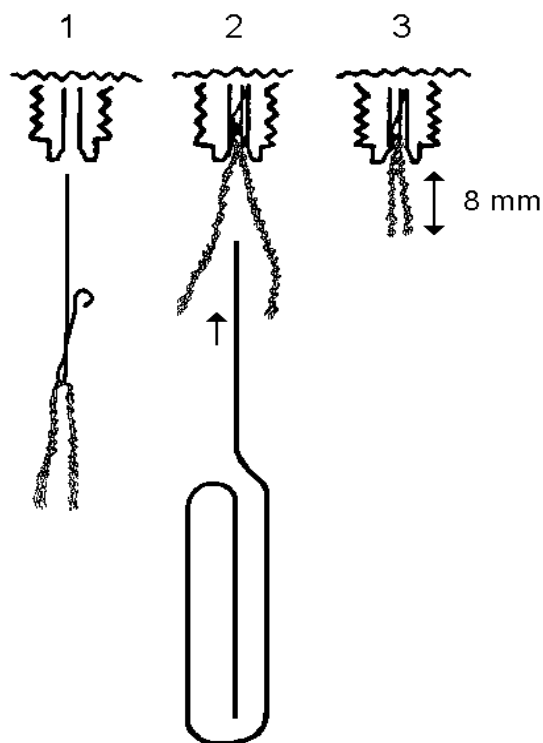


Maintain the wick in good condition. A short, worn or bent wick will pass solvent as a series of drops that give an uneven front on the rotor. Straighten the wick whenever the inlet or Teflon lid is removed from the Chromatotron.

Changing the wick

- 1 Pull out the wick and wire wick holder.
- 2 Replace the wick, insert into the inlet and push in with a straightened large size paper clip.
- 3 Trim the wick to about 8 mm.

Use the spare wick supplied or a similar thin fluffy polyester string. Sewing cotton or thread are not recommended.



SOLVENT PUMPS

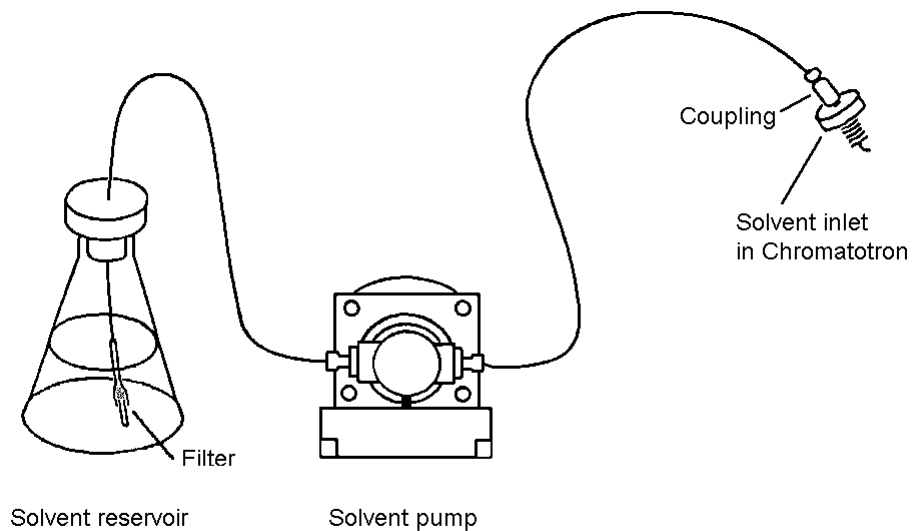
A pump is used not only for solvent addition but also for introduction of the sample solution and for recycle of eluted components back to the input.

The pump must have a low dead volume to prevent dilution of samples. Piston pumps and other pulsating types must also be asynchronous to avoid synchrony with the Chromatotron rotor. Peristaltic pumps are limited to the few solvents compatible with flexible tubing. The only suitable commercially available pump is:

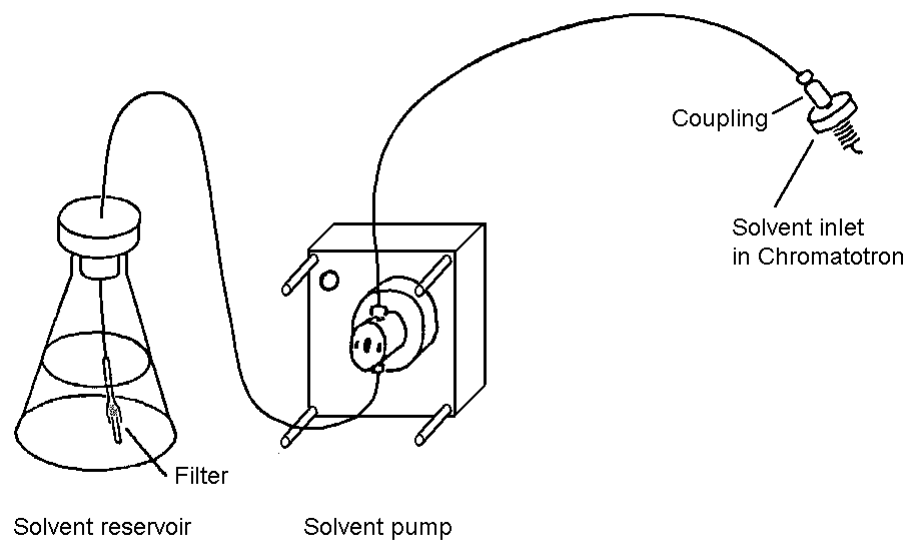
Model RHSYOOSTYLF from Fluid Metering Inc., www.fmipump.com
Pump connections kit, cat. no. H-53 is also required.

Fluid Connections for Pump Model RPG150-OSSY

This pump model is no longer available from Fluid Metering, Inc.



Fluid Connections for Pump Model RHSYOOSTYL



Screw tube end fittings into the pump gently by hand only!

Raise the solvent reservoir 20 cm if high ambient temperatures or volatile solvents reduce flow rates by forming vapor locks (bubbles in the pump).

Pumps are sensitive to abuse:

- * The cotton filter should be in place at all times when pumping sample solutions or solvent. Solid impurities must not enter the pump. Change the cotton at intervals. Synthetic material sold as "cosmetic puffs" is most suitable. Do not use glass wool; abrasive glass particles will be released into the pump.
- * Do not leave solutions of compounds in the pump. The piston may bind to the cylinder as the solvent evaporates.
- * Do not pump hot or supersaturated solutions. Solids may crystallize in the pump.
- * Do not pump mineral acids. Wash out chloroform (which generates acid on exposure to air and light).
- * Do not pump toxic materials without suitable precautions to allow for accidental ejection under pressure.

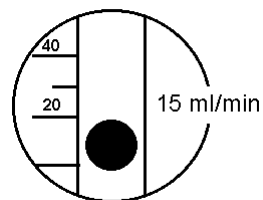
NITROGEN FLOW

A flow of inert gas is essential. Most compounds will partially oxidize when exposed to sorbents and air.

The flowmeter is calibrated for air and is reasonably accurate for nitrogen and argon. If a bubbler is used, place it on one side for high flow rates to allow the gas to pass over rather than through the liquid. Choose a liquid of low volatility, e.g. a phthalate ester. *Do not use silicone oil.* Silicones may irreversibly contaminate the rotors, preventing adhesion of the sorbent layer. Volatile liquids such as xylene are unsuitable if UV absorption is used for detection of compounds on the rotor. *Do not use sulfuric acid in wash bottles.*

Before using the Chromatotron, flush out air with a nitrogen flow of 250-1000 ml/min then reduce to about 15 ml/min (read at center of ball) and maintain this rate during the chromatography. The latter flow rate corresponds to about 1 - 2 bubbles/sec from a 6 mm i.d. tube in a bubbler. Excessively high rates will dislodge sorbent.

Flowmeter Scale

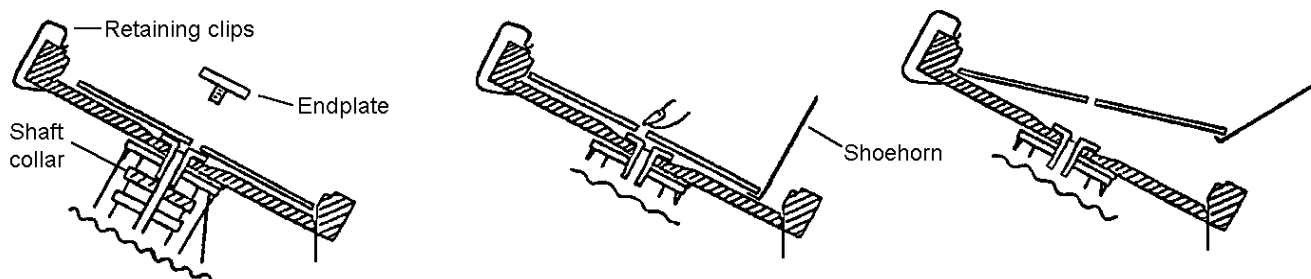


Solvent vapor will pour out from the nitrogen inlet if it is left open to the air.

CHANGING ROTORS

Lift the retaining clips with a finger or thumb and slide back about 1 cm. The Teflon lid can now be removed. Hold the shaft collar stationary when screwing the endplate in or out. Alternatively and more conveniently, turn the shaft collar and hold the endplate stationary.

- 1 Unscrew and remove endplate. →
- 2 Slide rotor up with finger and place shoehorn under lower edge. →
- 3 Remove rotor. ↓
- 6 Use endplate in center hole to slide rotor up into position. Screw in endplate by hand only. ←
- 5 Slide rotor up with finger to allow removal of shoehorn. ←
- 4 Lower new rotor into position. ←



A tight endplate can be levered loose with the endplate key. Insert into one of the holes at the side of the endplate. See diagram page 3.

If the slack between the screw threads on the endplate and the hole in the rotor causes slight eccentricity, loosen the endplate and screw up in a new position relative to the rotor. Repeat until concentric. Eccentricity of the sorbent layer, or the glass rotor, does not affect performance.

SORBENT LAYER THICKNESS

1 mm Layers. For separation of small samples, up to 100 mg per component, 250 mg total sample.

2 mm Layers. For larger sample loads, up to 300 mg per component, 750 mg total sample. Also for small samples of low solubility or which tend to tail. The resolving powers of 1 and 2 mm layers are not significantly different for light loadings.

4 mm Layers. For very large loads, up to about 1.5g. Gain experience with 1 and 2 mm layers before using these thick layers.

Layers up to a thickness of 8 mm can be prepared but are troublesome to use.

Scraper blades are available for sorbent layers of any thickness within the range 0.3 to 4 mm. See CHROMATOTRON PARTS LIST, page 40.

PREPURIFICATION OF THE SAMPLE

Samples that are free of "baseline" impurities need not be prepurified. All samples containing very polar impurities should be prepurified by slow filtration of a solution in a polar solvent, e.g. ethyl acetate, through a layer of sorbent (column chromatography grade) in a short wide column or a sintered glass funnel. Evaporation of the solvent gives the prepurified sample. This simple procedure removes very polar compounds that react irreversibly with sorbents.

Very polar impurities will form a line of darker impervious spots at the inner edge of the sorbent on the rotor. Ultimately, bands will streak and broaden. Remove the offending sorbent by rescraping the layer with a scraper blade displaced radially outwards from the normal position in the scraping tool. Removal of 2-3 mm of sorbent (in increments of 1 mm, to prevent chipping) from the inner edge will restore the utility of the layer.

Substances that are rendered insoluble by calcium ions are present in crude extracts of plant or animal matter. Add gypsum to the sorbent for prepurification of these samples.

SOLVENT CHOICE

The Chromatotron requires an R_f lower than for regular TLC. Preferably choose solvents giving an R_f in the range 0.2 - 0.4 using conventional analytical TLC. A higher R_f is acceptable for easy separations. If UV absorption is to be used for detection, see Detection of UV Absorbing Compounds on the Rotor, page 15, before choosing a solvent. The range of usable solvents extends from hexane to methanol. Glue-bound silica gel layers are not loosened even by aqueous solvents.

The equilibrium conditions in the Chromatotron, versus the non equilibrium conditions of standard TLC, will occasionally cause disparate results. Bands may separate in the Chromatotron but not on analytical TLC plates, or vice versa. This effect is most noticeable when using mixtures of solvents with very different polarities, e.g. chloroform - methanol. The TLC results can usually be reproduced in such cases by reducing the proportion of the polar solvent or by introducing the sample before the rotor is completely wetted with solvent (page 13).

Most compounds will "tail" when the solvent contains only low or medium polarity components such as hexane and dichloromethane. The addition of a small quantity of a polar solvent, e.g. 0.1% of methanol, will sharpen up the bands considerably.

Low solubility of the sample is troublesome with solvents based on mixtures of hexane and a polar solvent, e.g. hexane - ethyl acetate. Increase solubility by replacing part of the hexane with toluene or dichloromethane while decreasing the proportion of the polar solvent in order to maintain a reasonably low R_f. See also Introducing Less Soluble Samples, page 13.

Gradient elution is recommended for most separations. Step gradients, from the batchwise addition of solvent mixtures with increasing amounts of a polar solvent, work well. Equilibration through the vapor space in the Chromatotron partially smoothes out the gradient. Increase the polarity much more rapidly than is usual for column chromatography, using only 3 or 4 steps. A long smooth polarity change will give a very large number of dilute fractions rather than an improved separation.

Sudden large increases in solvent density, e.g. a change from hexane to dichloromethane, may cause radial streaking of bands. Use a short gradient to smooth out the change.

The complete elution process should not take more than about 30 min if solvents giving a reasonable R_f have been chosen.

SOLVENT ADDITION

For solvents of normal viscosity the usual flow rates are:

1 mm sorbent layers: 2 - 4 ml/min
2 mm sorbent layers: 6 - 8 ml/min
4 mm sorbent layers: 8 - 10 ml/min

Before the sample solution is introduced, the sorbent layer should be completely wetted with solvent and at least a further 5 min allowed for equilibration. If the sample is introduced without allowing a short period for equilibration, then sharp bands will be formed on the rotor but evaporation in the solvent collection channel will cause slight tailing during fraction collection. The solvent flow can be reduced or turned off during the 5 min equilibration.

The dark band at the edge of the rotor consists of sorbent completely wetted with solvent. Other parts of the sorbent are only partially wetted.

Observe the solvent front during the initial solvent introduction. It should be circular and concentric for at least the first 5 cm of travel. The front may become slightly eccentric later due to the large temperature rise that occurs as the solvent meets the sorbent. A front that appears to wobble during the first few cm is an indication of a worn or bent wick, an inhomogeneous sorbent, vapor leaks around the Teflon lid or an excessive flow of nitrogen. See page 35, TROUBLESHOOTING.

Vapor locks (bubbles that remain in the pump) may reduce the flow rate when ambient temperatures are high. Raise the solvent reservoir about 20 cm or change to less volatile solvents to correct this problem. See page 38, TROUBLESHOOTING.

After 10-20 min of operation, condensed solvent droplets will appear on the Teflon lid. Warm with the hand to clear slight fogging, tap the Teflon to coalesce larger droplets. *Do not increase the nitrogen flow to remove condensed solvent drops!*

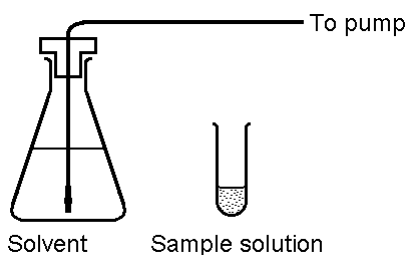
Polar solvents do not flow smoothly through Teflon tubing. Remove the output tube if solvent backs up or flows intermittently.

INTRODUCING AND ELUTING THE SAMPLE

Dissolve the sample, e.g. 1-2 mg of the test mixture (Appendix, page 42), in a small volume (0.5 - 2 ml) of the eluting solvent. Suitable solvents for the test mixture are heptane - isopropyl acetate, or hexane - ethyl acetate, both 8:2, or toluene. If this is the first run, choose toluene. The test mixture is very soluble in this solvent and good resolution is easily obtained. Toluene is UV absorbing and thereby blocks detection of UV absorbing samples.

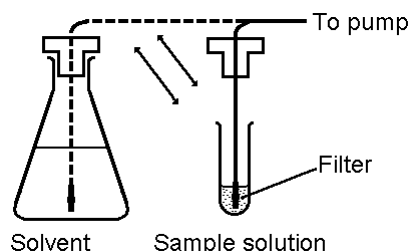
INTRODUCING THE SAMPLE:

1 Pump solvent to the Chromatotron until the sorbent is completely wetted and allow a further 5 min or more for equilibration.



2 Take up the sample solution through the filter and pump input tube. The sample must be completely in solution. Filter or centrifuge as required.

3 Flush the last traces of the sample into the filter and tube using several squirts of solvent.



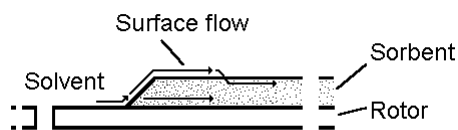
4 Return the filter and input tube to the solvent reservoir.

The volume of solvent used to add the sample is not as critical as for regular prep TLC although the concentration should not exceed about 10%. The high viscosity of more concentrated solutions will distort bands on the rotor. Sample solution volumes of 1-2 ml (per mm of layer thickness) are usual but volumes as large as 4-8 ml (i.e. up to about 30 ml for a 4 mm layer) will give reasonably narrow bands. If a band is broad it will sharpen as the development proceeds. There is no reason to have initial bands of less than about 3 mm width since resolution will not be improved.

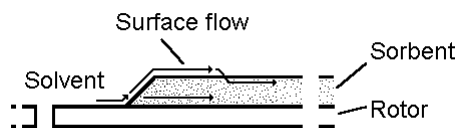
It is possible to introduce the sample after wetting only a small part of the rotor with solvent. Under these non equilibrium conditions relative R_f values and the separations obtained may be abnormal. Evaporation in the solvent collection channel will cause slight but usually acceptable tailing of fractions.

Introducing Less Soluble Samples (1 and 2 mm layers only). If samples will not dissolve in a reasonable volume of solvent, wet the rotor completely with a more polar solvent, introduce the sample in this solvent, allow a few minutes for solvent to drain from the rotor and then dry out the sorbent with an increased nitrogen flow (1-1.5 L/min). Elution can then be performed with the desired solvent. Use sufficient of the polar solvent to form a broad band; a narrow band may crystallize on drying out. The solvent evaporation is very slow since the rotor becomes cold; 45-75 min will be needed. Do not take off the Teflon lid to speed up evaporation; water will condense on the cold sorbent.

Samples of low solubility can also be handled by the standard method used with column chromatography, that is introduction as a solution in a small volume of a polar solvent after wetting the sorbent with the less polar elution solvent. Success depends on minimizing the amount of polar solvent that compromises chromatography until diluted.



Maximum and Minimum Flow Rates. Good resolution will be obtained on silica gel (1 mm) with flow rates of 2-5 ml/min. At a flow rate of about 5-7 ml/min, depending on the type of silica, the solvent will flow over the surface of the sorbent at the inner edge causing streaking and loss of resolution for bands in this area. With lighting from directly above, surface flow is visible as a shiny band (pulsating if a pump is used for solvent supply). Dichloromethane and other high density, low viscosity solvents can be used at higher flow rates without surface flow.

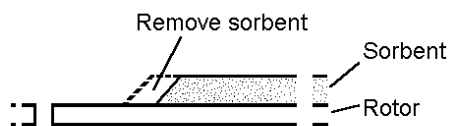


The maximum useful rate of solvent flow varies with the density and viscosity of the solvent but the above rates are approximately correct for the low viscosity solvents commonly used for chromatography. With more viscous solvents, use the test mixture (Appendix, page 41) first. A sharp band even with no resolution, indicates proper solvent flow.

At high flow rates a dark band appears at the inner edge of the sorbent where solvent completely fills the pores.

There is no minimum solvent flow rate, however, low rates do not improve resolution and will exaggerate defects such as the sloping of bands through 2 and 4 mm layers.

Rapid Chromatography. For rapid chromatography increase the flow rate after the bands have moved away from the central area where surface flow occurs. Alternatively, increase the diameter of the sorbent-free area at the center of the rotor by mounting in the coating arbor and turning with a slow speed stirrer motor while scraping with a spatula. Removal of 2 cm or more of the layer will allow much higher solvent flow rates without surface flow. For example, the test mixture (page 41) separates within 5 min using silica gel and toluene at double the normal flow rate.



Interrupting Solvent Flow. Solvent flow may be shut off for a considerable time during elution with no effect on the resolution ultimately obtained. If the flow is to be off for more than 20 min, increase the nitrogen flow to evaporate the solvent from the sorbent. The bands of compounds on the dry rotor will not spread by diffusion. The new solvent flow will sharpen the bands.

Heavy Loading. For heavy loadings, dissolve the mixture in several ml of solvent. Concentrated solutions (>10%) produce eccentric bands (due to the increased viscosity). Slight eccentricity is normal for heavy loading.

Light Loading. Very small quantities of UV absorbing mixtures may not be detectable when spread out as circular bands on a rotor. More concentrated bands are obtained if the solution of the mixture is applied directly to the dry sorbent on the rotor as a single spot or a series of spots in a short arc. To observe the bands in UV light, turn off the solvent flow and stop the rotor. Choose a solvent which gives a low R_f so that equilibrium conditions will be attained before the compounds are eluted. Resolution of mixtures applied as single spots or short arcs is better than that from the complete circles of the normal procedure.

Use thin sorbent layers to maintain higher concentrations of small samples. See the scraper blades in the CHROMATOTRON PARTS LIST.

DETECTION OF UV ABSORBING COMPOUNDS ON THE ROTOR

A UV lamp (e.g. a Mineralight, short wave, UVP Inc.) held over the Teflon lid of the Chromatotron will reveal bands of UV absorbing compounds. Bands will be visible directly under the lamp and also for some distance around the rotor due to the delay between absorption of UV and emission of visible light from the phosphor. Detection at short (254 nm) wavelengths is the most generally useful.

A band of apparently even density will have most material near the center and very little at the leading and trailing edges. Two compounds that are 95% separated may therefore appear as a single broad band.

For detection at the output tube, spot the eluate on a TLC plate held under a UV lamp. The lack of pressure at the output and the presence of gas bubbles, prevents direct connection to a UV monitor.

Some hand lamps do not produce sufficient UV light, especially after long use. This problem is easily solved by removing the light filter (which absorbs most of the UV light). Shading the equipment from room lights will also give an improvement. A further enhancement can be obtained by turning off the solvent flow and stopping the rotor. If detection difficulties are due to a small sample size, see Light Loading, above.

Solvents must not absorb UV light. Some suitable UV transparent solvents are hexane, heptane, dichloromethane, ether, tetrahydrofuran, acetonitrile, methanol, ethanol and isopropanol. Ethyl acetate absorbs UV light but is usable in most cases. Use dichloromethane if the choice is between dichloromethane and chloroform. Benzene is a common impurity in some grades of hexane and ethanol and will interfere with detection. The ease of detection of a sample in a particular solvent can be checked by ordinary TLC. Observe the still-wet TLC plate in UV light.

If acetone (UV absorbing) is used as a clean-up solvent, it must be removed completely from the pump, tubing and sorbent. Preferably, air-dry the rotor. A solvent wash can be used to remove acetone but surprisingly large volumes are required to remove the last traces from the sorbent. A UV absorbing test substance with a high R_f, such as phenanthrene or other aromatic hydrocarbon, provides a convenient means of checking that all is well before introducing more valuable compounds.

DETECTION OF COLORLESS UV TRANSPARENT COMPOUNDS

Compounds which cannot be detected by UV absorption should be analyzed by conventional TLC after collection. Gradient elution covering a wide polarity range will ensure that all compounds have been eluted.

Compounds may be detected at the output tube by spotting on a clean ground glass stopper. Evaporation of the solvent leaves an observable residue.

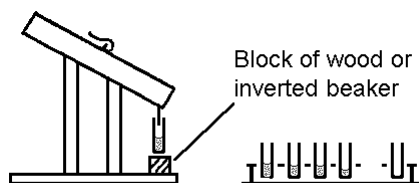
Some compounds lacking chromophores can be detected on sorbents with long wavelength (365 nm) phosphors (H.K. Desai, E.R. Trumbull and S.W. Pelletier, *J. Chromatography* 1986 **366** 439). Bands on a rotor can be visualized, inconveniently, by evaporating the solvent and applying iodine (H.K. Desai, B.S. Joshi and S.W. Pelletier, *J. Chromatography* 1985 **322** 223). Most of the methods used for detection of UV transparent compounds on regular TLC plates should be applicable in the Chromatotron. These include UV light with berberine (L. Mamlok, *J. Chromatographic Science* 1981 **19** 53) in the sorbent and (for polar compounds) hexane (K. Suyama and S. Adachi, *J. Chromatographic Science* 1987 **25** 130).

Chromophores can be designed into the intermediates of a reaction sequence by including protecting groups with an aromatic system e.g. benzoic esters or trityl ethers.

FRACTION COLLECTION

The narrow bands produced by the Chromatotron require that fractions be small. Collection in disposable test tubes or culture tubes allows a decision on how to cut or mix fractions to be made later after analytical TLC. Collection in a small number of Erlenmeyer or round-bottom flasks inevitably remixes parts of close fractions.

The output tube should be within the test tube to prevent loss by splashing as the bubbles in the eluate burst.



A small amount of a colored compound with a high R_f , e.g. azobenzene or the test mixture (page 42), added with the mixture to be separated, will mark the "solvent front"; collection of fractions can then be delayed until this has been eluted. **When collecting UV absorbing compounds, continue to collect several fractions after the UV absorbing band has apparently passed from the rotor.** The last part of the band is not easily seen and significant amounts of compounds can be missed. The trailing edge of a band can be detected by spotting the eluate on a TLC plate and observing the residue in UV light after the solvent has evaporated.

Since the separations are completed in a very short time, automatic fraction collection has few advantages over hand collection. If a fraction collector is used, choose drop counting or time mode. Small fractions will partially remix in collectors using a siphon tube. The output from the Chromatotron will not pass through long lengths of narrow tubing. Use tubing with a bore of about 1/16" (1.5 mm) to connect to a fraction collector and keep the gradient to a minimum. The base of the Chromatotron can be removed to allow positioning over a fraction collector.

CLEAN-UP AND REGENERATION OF THE SORBENT LAYER

After each separation or when sorbent layers have become contaminated with polar compounds, a clean-up with a polar solvent such as acetone is required. Use at least 40 ml per mm of layer thickness. At the start of the clean-up, unscrew the inlet about a quarter turn to move the wick and solvent flow closer to the center of the rotor where traces of samples may remain.

The clean-up solvent can be removed from the sorbent by drying in the open air, allowing sufficient time (at least 12 hr for 1 mm, 24 hr for 2 mm and 48 hr for 4 mm layers) for evaporation of the solvent and of water that condenses on the cold sorbent. A metal "heat sink" under the rotor will encourage the evaporation. Drying with slight heating (the lamp and bucket on page 27 is effective) may also be used (and will be essential in humid climates) after the solvent has **completely** evaporated. In case some solvent is still present, **do not use an oven.** *Take care to remove all traces of acetone from the solvent reservoir and tubing if UV absorption is to be used later for detection.*

Clean-up solvent can also be removed by a solvent of intermediate polarity such as dichloromethane (at least 40 ml per mm layer thickness) followed by an equal volume of the solvent to be used in the separation. The direct transition from acetone to a solvent of low polarity such as hexane - ethyl acetate (9:1) requires much larger volumes of solvent (at least 125 ml). High Rfs, broad bands and poor resolution will result from the use of insufficient new solvent for removal of the clean-up solvent.

For a more rapid removal of clean-up solvent from 1 or 2 mm layers, first evaporate with nitrogen (1 - 1.5 L/min) then follow with the new solvent. An overnight nitrogen flow of about 250 ml/min will also remove solvent.

Methanol may be used to remove very polar impurities from sorbent layers. Removal of the methanol by other solvents is a slow process, preferably dry the rotor in the open air. For most separations a silica gel layer cleaned once with methanol will give sharper bands, however, repeated use of 100% methanol will slowly remove the gypsum binder and change the nature of the sorbent. Relative Rf values of the components of a mixture will also change.

When chromatography and clean-up have been completed and no further separations are contemplated, wash out chloroform, dichloromethane, acetone and acetonitrile with hexane or ethyl acetate or leave a slow stream of nitrogen passing into the Chromatotron to reduce the possibility of solvent damage to plastic parts.

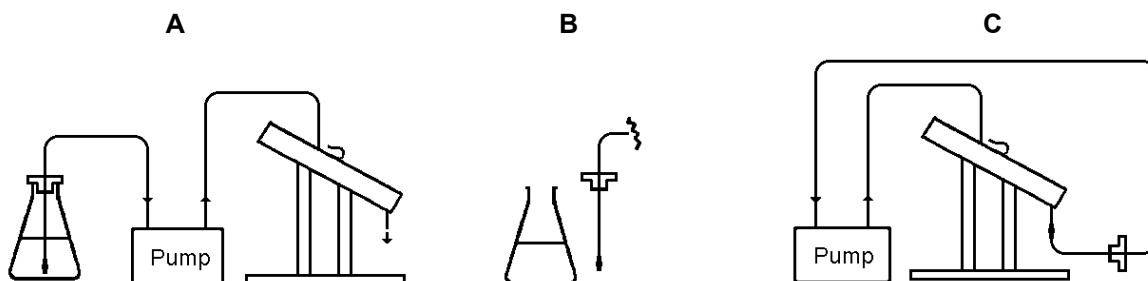
MULTIPLE DEVELOPMENT

Sharpen broad or irregular bands on 1 and 2 mm layers by multiple development. Turn off the solvent flow and wait a few minutes for the flow at the output to decrease to a low rate. Increase the nitrogen flow to 1- 1.5 L/min for 45-60 minutes or more to evaporate the remaining solvent from the sorbent. The evaporation is a slow process since the rotor becomes very cold. Decrease the nitrogen flow and restart the solvent addition. Bands will become considerably sharper. Multiple development is less effective when Rf values are low. *Do not remove the Teflon lid to evaporate solvent; water will condense on the cold sorbent.*

RECYCLE

Recycle is a simple strategy for increasing the resolving power of the Chromatotron. For a trial run use the test mixture (Appendix, page 41) with hexane - ethyl acetate (6:4). This solvent mixture, containing a large proportion of the polar component, gives incomplete separation in one pass and complete separation on recycle.

For recycle, proceed as in normal elution **A** until the bands to be recycled are about 1 cm from the edge of the sorbent then remove the solvent input tube from the solvent reservoir, **B**. After waiting about 10 sec to reduce the amount of solvent in the pump and Chromatotron, connect the filter (with cotton) to the output tube (with detachable part removed), **C**. The pump will recycle solvent from the Chromatotron for about 1 min then both solvent and vapor will recycle.



If the 10 sec delay at stage **B** is omitted, solvent may back up slightly in the collection channel causing some remixing of bands. *The solvent flow rate must be held constant for some time before recycle commences to prevent surges of solvent flow.*

When the bands of interest have been returned to the rotor it is preferable to return to mode **A** since in mode **C** evaporation will slowly reduce the amount of solvent available for recycle.

Bands will be discouragingly diffuse when newly recycled back to the sorbent. However, on further development they will sharpen up considerably. After one, two or three stages of recycle, depending on the loading and the tailing tendency of the particular compounds, the bands will become so broad that further recycle will cause the head of one to catch up with and overlap the tail of another. At this stage the band or bands should be eluted or if recycle is to continue they must be sharpened by multiple development (page 18). If the R_f of the bands is 0.5 or more, multiple development is very effective for band sharpening, allowing an unlimited number of recycles to be performed.

CONNECTING CHROMATOTRONS IN SERIES

Resolving power and capacity can be increased by connecting two (or more) Chromatotrons in series. Pump the output of one to the input of the next. Back-up of solvent in the first Chromatotron can be prevented by setting the intermediate pump at a higher flow rate so that both solvent and some vapor are pumped.

COATING ROTORS WITH SORBENTS

INTRODUCTION

Sorbent layers on rotors are produced by casting sorbent-binder mixtures followed by scraping down to 1 mm, 2 mm or 4 mm thickness with a rotating scraping tool. More binder is required than in conventional analytical TLC.

Calcium sulfate hemihydrate (plaster of Paris, dried gypsum) is the most frequently used binder. Setting is very sensitive to the pH, the temperature, and to the presence of other solids or solutes.

Satisfactory binders for C18-silica and other hydrophobic sorbents are not available.

Rotors are made of regular glass, not a heat resistant type. Do not submit them to unnecessary thermal shocks. Rotors with wet layers are likely to crack at the center if placed in an oven at temperatures above 50°C.

Recipe 1 (page 21) forms silica gel layers that satisfy 95% of the needs of most chemists. Other recipes are useful in special cases.

SORBENTS, BINDERS AND PHOSPHORS

Sigma-Aldrich item no.

Silica gel, TLC standard grade (no binder) with fluorescent indicator	288586
Fluorescence indicator green 254 nm (zinc silicate)	02554
Polyethylene glycol 8000	202452

Aluminum oxide SAI	Scientific Adsorbents Inc.	Cat. no. 04344-1
Aluminum oxide GF-254		E.Merck, Item 1092

Sigma-Aldrich: sigma-aldrich.com
Scientific Adsorbents Inc: saiadsorbents.com

Calcium sulfate hemihydrate (plaster of Paris, dried gypsum) is available from various chemical suppliers. Plaster of Paris from hardware stores is usually of better quality.

E. Merck products are available through VWR Scientific and other laboratory suppliers.

RECIPES

Check the quality of the calcium sulfate hemihydrate binder: Stir a 5g sample and 5 ml of water with a spatula in a 25 ml beaker then mix continuously by swirling. The mix should thicken after about 7-14 min to the point where it will not move when tilted. Thickening outside of this time interval indicates the presence of calcium sulfate dihydrate impurity, that will make rotor coating difficult. If sorbent layers crack, try another source of plaster of Paris or regenerate at about 160° for 8 hr.

Cooling of the water in the recipe reduces air pressure build-up during the shaking of the container. A thick, creamy but pourable mixture is required.

Amounts of sorbents and binders are given in grams, amounts of water in ml and temperatures in °C.

Suggested Mixing Jar Sizes

Layer thickness	1 mm	2 mm	4 mm	8 mm
Mixing jar size	250-400 ml	300-400 ml	400-600 ml	750-1100 ml

For easy mixing, the jar should be not more than 70% full with the dry sorbent and binder.

Recipe 1, Silica Gel TLC Standard Grade - Gypsum

Silica gel TLC standard grade (contains 254 nm fluorescent indicator) is the most useful sorbent.

	1 mm	2 mm	4 mm
Silica gel, TLC standard grade	33	45	75
Calcium sulfate hemihydrate	13	18	30
Water at 0-10°	82	112	187

Allow the layers to set for at least 1 hr (overnight OK) before drying. See page 24 for the mixing and pouring procedure.

Recipe 1B, Silica Gel TLC Standard Grade - Gypsum, 8 mm Layers

	8 mm
Silica gel, TLC standard grade	137
Calcium sulfate hemihydrate	55
Water at 0-10°	343

Allow the layers to set for at least 1 hr (overnight OK) before drying.
See page 24 for the mixing and pouring procedure.

The 8 mm layers are not recommended. Drying of the layers requires several days. The drying out of solvents from the layers after use is also a long process. A 4 mm layer used twice will give the same results with less trouble.

When coating with the 8 mm recipe, use 1" (2.5 cm) masking tape around the rotor and also around the central metal disk of the coating arbor. A long, strong spatula is required for the initial stirring.

Solvent flow rates for 8 mm layers should be the same as for 4 mm layers. Higher flow rates may cause splashing.

Recipe 2, Acidified Silica Gel-Gypsum

Acidification of silica gel layers will prevent tailing of acidic samples. Only very weak acids such as ammonium sulfate (giving a pH about 4.5) can be added to the recipe. Oxalic and citric acids inhibit setting of the binder.

Use recipe 1 with ammonium sulfate (1 mm - 0.45g,
2 mm - 0.65g, 4 mm - 1.2g) dissolved in the water.

Allow 4hr for setting.

Ammonium sulfate is not eluted by acetone but is removed by methanol.

If a stronger acid is required, prepare layers from recipes 1 or 6 and add acetic acid to the eluting solvent.

Recipe 3, Silica Gel - Silver Nitrate - Gypsum

For the separation of olefins, cf. L.J. Morris, Chem. and Ind. (1962) 1238.

Use recipe 1 with silver nitrate (1 mm - 2g, 2 mm - 3g, 4 mm - 5g) dissolved in distilled water.

Allow 4hr for setting. The finished layers are rather soft and will slowly darken if stored in light.

Hexane - ethyl acetate should be used for elution and ethyl acetate for clean-up. Silver nitrate is eluted by solvents containing methanol, tetrahydrofuran or acetone. UV detection is not effective in the presence of silver nitrate.

Recipe 4, Silica Gel - Polyethylene Glycol - Gypsum

For partition chromatography of non polar samples, cf. J.H. Dhont et al., J. Chromatography, (1971) **60** 265. See page 31 for a discussion.

Use recipe 1 or 2 with polyethylene glycol 8000 or 6000 (1 mm - 6.5g, 2 mm - 9g, 4 mm - 15g) dissolved in the water.

Allow 4 hr for setting. Layers are rather soft.

For elution use only hexane, ether, and acetone; polyethylene glycol is soluble in most other solvents. Only non polar hydrocarbon solvents give good resolution.

Recipe 5, Aluminum Oxide - Gypsum

Type SAI aluminum oxide is preferred. Aluminum oxide GF must be regenerated (heat at 160° for 3 hr) to avoid unpredictable results.

	1 mm	2 mm	4 mm		1 mm	2 mm
Aluminum oxide Type SAI	55	90	160	Type GF	55	90
Calcium sulfate hemihydrate	13	22	38		10	16
Fluorescent indicator	0.5	1	1.5		-	-
Water at 0-10°	55	90	160		62	100

See page 24 for the mixing and pouring procedure. The mixture, particularly for 1mm layers, does not flow freely over the rotor. Spin the coating arbor and rotor by hand to encourage flow to the edge. If necessary, pick up and tilt the rotor. Finally bump the rotor and arbor. Setting requires at least 4 hr.

Recipe 6, Silica Gel - Glue

Glue-bound layers are stable to very polar (including aqueous) solvents.

1 mm

Silica gel TLC standard grade	30
Glue	2.5
Water	78

See page 29 for the coating procedure and a discussion.

Recipes for Florisil and Cellulose

Florisil layers can be made using glue as in recipe 6. The layers are very alkaline and bands "tail".

Cellulose layers are difficult to prepare and do not perform well.

COATING ROTORS USING RECIPES 1-5, GYPSUM-BOUND LAYERS

Introduction

The beginner should first prepare a 1 mm layer of silica gel from recipe 1 (page 21) for which the procedure is least critical. Typically, the processing time for the first rotor coated is excessive and a less than perfect layer is produced. However, the technique is easily mastered and the second rotor prepared is usually a success.

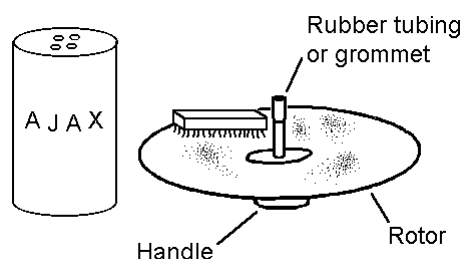
Before starting the coating procedure obtain a clear cake cover, cardboard box or plastic bowl to cover the rotor while the layer is setting. Have a strong spatula ready for stirring.

All rotors, new or used, must be thoroughly cleaned within 1/2 hr of coating. Do not rely on glass washing services. Follow the cleaning instructions carefully! Detachment of sorbent layers will be a serious nuisance if rotors are not scrupulously clean.

Cleaning and Setting Up the Rotor For Coating

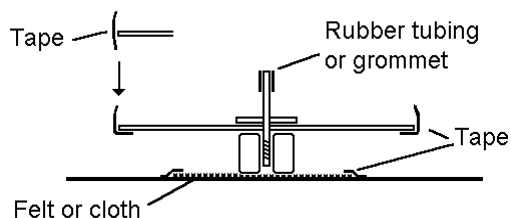
Mount the rotor ground side up in the coating arbor and tighten gently by turning the edge of the rotor while holding the plastic handle. The rate of accidental rotor breakage will be high if the arbor and handle are not used during cleaning.

Thoroughly scrub the upper surface with water and Ajax or similar abrasive household cleanser powder, using a wad of paper towels, nylon scouring pad or a small stiff brush. Wash well and check for effective cleaning by allowing the water to drain off for about 15 sec. If separate drops of water form on the rotor then the cleaning is not complete and should be repeated.



Dry with paper towels only. Use at least two, wiping first the cleaned upper surface then the other parts of the rotor and arbor. A final wipe of the dry rotor with a dry towel will remove lint. Do not dry with acetone or by heating in an oven.

Attach 3/4" (2 cm) masking tape (preferred) or other adhesive tape (cellulose tape may be used but will leave adhesive on the glass) to the edge of the rotor as shown. Press the tape on firmly to the edge and underside of the rotor. Attach extra pieces of tape as required. For easy removal of the tape later, form a tab by bending over the tape end.

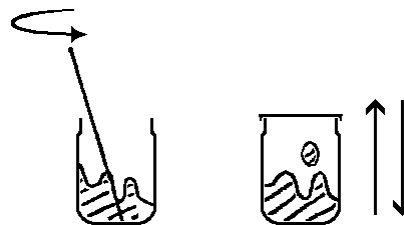


Place on a rigid **level** surface covered by a piece of thin felt, cloth or a single layer of a paper towel, taped down. The surface used must be free from vibration caused by nearby equipment. Use grommets or a short length of rubber tubing on the rod of the arbor as a finger grip.

Mixing and Pouring the Recipe

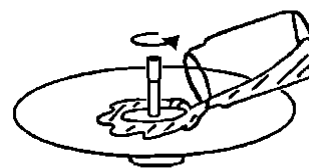
Weigh out the sorbent and binder into a preserve or pickle jar or other wide-mouth container with a screw-on cap giving a liquid-tight seal. Erlenmeyer (conical) flasks are inconvenient. For easy mixing the jar should be not more than 70% full of the dry sorbent and binder. Usual jar size: 1 mm layers, 250-400 ml, 2 mm, 300-400 ml, 4 mm, 400-600 ml.

Measure out the water, cool, in a refrigerator and add to the jar. The following stages must be completed within 3 min since the mixture will begin to set within 3-10 min.



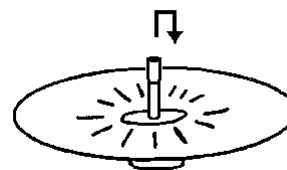
Stir the mixture with a spatula until most dry pockets at the bottom and sides of the jar have been wetted (about 10 sec). Remove the spatula (ignore the mixture that is attached) then cover and shake *very vigorously* end to end for 10-20 sec.

Turn the rotor slowly (1/2 - 1 revolution/sec) by the central rod and pour the mixture in a continuous stream in overlapping circles close to the central metal disk. Keep the jar close to the rotor to minimize the formation of separate drops of the mixture. Slight agitation will encourage thick mixtures to flow. Touch the mouth of the jar to the metal disk of the arbor to remove the last hanging drop.

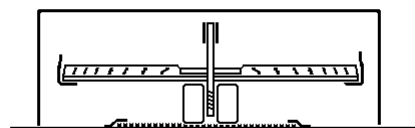


If sufficient water has been used, 95% of the mixture will flow readily out of the jar.

Grip the center rod of the arbor and raise the rotor about 1 cm then lower, bumping the handle below the rotor against the felt (or cloth) covered surface. Do not turn during the bumping. Repeat about 3 times. Bumping liquefies the mixture releasing air bubbles, allowing gravity to spread the mixture and smooth out inhomogeneous parts.



Cover the rotor with a clear cake cover or cardboard box (protection from drafts) and allow the layer to set for at least 1 hr (recipe 1) or 4 hr (recipes 2-5). Overnight setting is convenient.



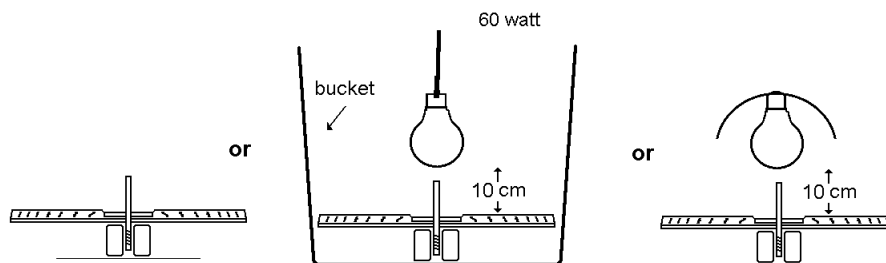
Remove the masking tape after the setting is complete. The rotor can remain in the coating arbor for air drying of the layer.

Drying the Sorbent Layer

The traditional "activation" of regular TLC plates by heating at 100° is only a reversible drying. The sorbent will quickly re-equilibrate with atmospheric moisture unless the plates are stored in a dry atmosphere. For Chromatotron rotors, air drying alone will give satisfactory "activity" in dry climates. In humid climates oven drying at 40-50° will be required.

Air Drying. *It is a common mistake to assume that a layer is dry when the surface appears to be dry. Air dry to constant weight $\pm 0.5g$ (weigh the complete rotor-arbor assembly). Rotors coated with recipe 1 or 1B can be dried in the open with no heating and no special precautions.*

Slight heating with a lamp as shown will provide a more rapid drying, especially useful for the thicker sorbent layers.



With a lamp heater, constant weight is usually reached in about 12 hr for a 1mm layer, within about 36 hr for a 4 mm layer

Some types of silica gel (e.g. Merck silica gel PF, cat. no. 7749), that were previously suggested for use with the Chromatotron, are sensitive to drafts. Non-circular bands were formed on layers dried in a unidirectional air flow.

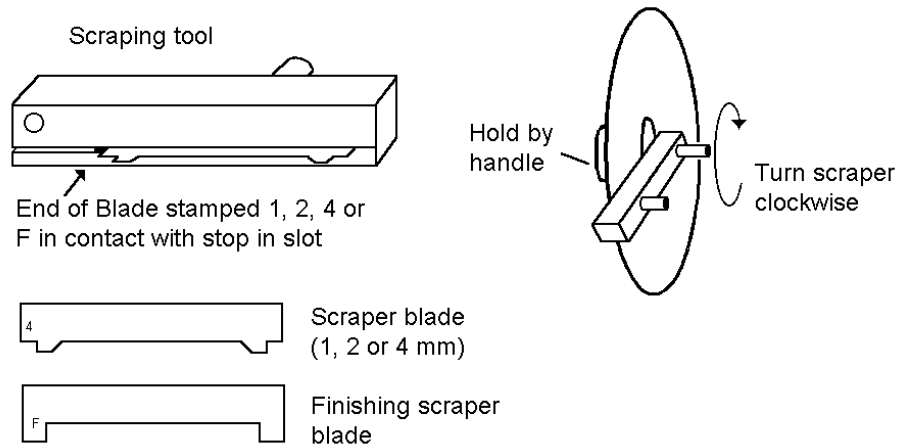
Oven Drying. Scrape off a circle of sorbent around the metal disk of the arbor with a spatula or piece of wire, to allow removal of the rotor from the arbor. Unscrew by turning the edge of the rotor while holding the handle of the arbor. *The glass will crack if the rotor is not removed from the arbor before heating.* Oven dry at 40-50°. *Rotors may crack if a wet layer is placed in an oven at higher temperatures. Also, layers become fragile at temperatures above 70°.* Any oven that is not sealed will be suitable. *Dry to constant weight $\pm 0.5g$, about 6 hr for 1 mm layers, 10 hr for 2 mm and 20 hr for 4 mm.* The progress of the drying can be checked by touching the underside of the rotor with the fingers while in the oven. The glass will remain cool until most of the water has evaporated.

Although air drying of sorbent layers is most convenient, oven drying gives better adhesion to the glass rotor and is preferable if large volumes of methanol are to be used for elution.

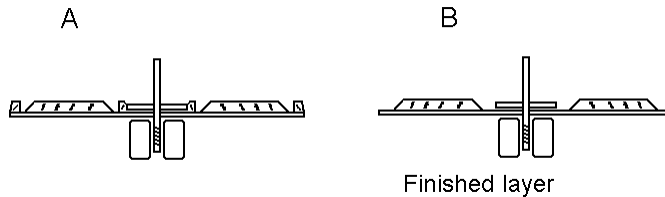
Rotors that have been used with solvents (especially polar solvents such as methanol) should not be placed in an oven. Use the lamp heater above, after all solvent has evaporated.

SCRAPING SORBENT LAYERS

Allow oven dried rotors to cool completely before scraping. Hot rotors may be slightly distorted. Replace the rotor in the coating arbor, removing if necessary any loose sorbent between the rotor and the arbor. Hold the rotor vertical and place the scraping tool (with 1, 2 or 4 mm blade) on the shaft as shown. Turn the scraper clockwise. To avoid chipping the sorbent, apply only slight pressure. This is a noisy operation !



Continue scraping until stage A is reached with 2 channels scraped completely down to the glass. If the scraping tool can be turned only with difficulty, hold the outer end instead of the handle. If the scraper bounces over the sorbent, forming radial ridges, again hold the end of the tool. Alternatively, scrape in reverse for a few turns.



Change to the finishing scraper to remove unwanted sorbent at the edge and center (stage A - B).

Remove the rotor from the coating arbor and use the end of a scraper blade to chisel off any sorbent remaining at the center. Rub off sorbent at the edge or on the underside of the rotor.

Blow off loose sorbent dust from the surface into a fume hood. Blowing by mouth or with compressed air/nitrogen from a pinched rubber tube is satisfactory. Most electric hand-held blowers are too weak. Any dust remaining on the rotor will appear on the Teflon lid or in the solvent collection channel.

The finished sorbent layer may have a few circular ruts and several bubble pits. These do not affect performance. Scuff marks and the loss of small pieces of the sorbent at the edge, have a negligible effect. Loose pieces of sorbent should be removed since they may detach later.

Scraper blades are easily sanded down by rubbing on a sheet of emery paper. A 4 mm blade, for example, can be adjusted to 3.9 mm to allow scraping of a layer that is not quite the intended 4 mm thickness. Adjusted blades can also be used to clean off a thin layer of sorbent from an old or damaged layer.

STORAGE OF COATED ROTORS

Rotors stored in the open will pick up volatiles from the lab air but most of these wash out in the first few ml of solvent. Before mounting rotors in the Chromatotron, blow off dust that will otherwise gather around the outlet hole in the collection channel.

Old rotors develop slight pH differences between the upper and lower layers of sorbent. Only the separation of acidic or basic samples is affected.

In moist climates, store rotors in a cabinet with drying agents or a slow stream of dry air/nitrogen or re-dry with the lamp or lamp-bucket assembly before use.

Label rotors at the edge with a pencil:



COATING ROTORS USING RECIPE 6, GLUE-BOUND LAYERS

A variety of common aqueous latex glues are satisfactory adhesives for silica gel (recipe 6, page 24). The most useful are **clear** "siliconized" (or "with silicone") acrylic latex sealants for kitchen and bathtub caulking sold in squeeze tubes by hardware stores.

Weigh out the sorbent into a 250-300 ml jar or flask, add all but 10 ml of the water and stir with a spatula until completely wetted. Suspend the glue in 10 ml of water, by magnetic stirring or with a spatula, add to the wet sorbent and stir. The mixture may thicken initially but will thin out within a few minutes. Cover with aluminum foil (without removing the spatula) and allow the mixture to stand for at least 1/2 hr. If many bubbles are trapped in the mix, stir gently and bump the jar to allow bubbles to rise and break. If the mix is too thick for easy stirring, add water in 2 ml increments.

A tape edge cannot be used around the rotor when pouring glue-bound layers. The tape detaches during the many hours that the mixture remains fluid. With experience and some luck sorbent layers can be poured without the tape edge. A more consistent solution is shown in the diagram on the next page.

Give the mixture a final stir and pour in the usual way. Bump the rotor and arbor until the mixture flows to the edge at all points. If the mixture is too thick to flow, pick up the rotor and tilt.

Allow the layer to dry to constant weight in the open (do not cover with a box). The drying time is about 48 hr in dry climates, The lamp heater can be used after about 12 hr when the layer has partially set. After air drying, remove the rotor from the arbor and place in an oven and heat at 50° for about 1 hr.

Wash the scraped rotor in the Chromatotron with at least 120 ml of acetone to remove large amounts of unpolymerized glue.

Normal Phase Chromatography. Silica - glue layers perform well in normal phase operation, e.g. the test mixture (page 41) in toluene. Bands of compounds are usually narrow and concentric. The layers do not detach from the glass even with aqueous solvents.

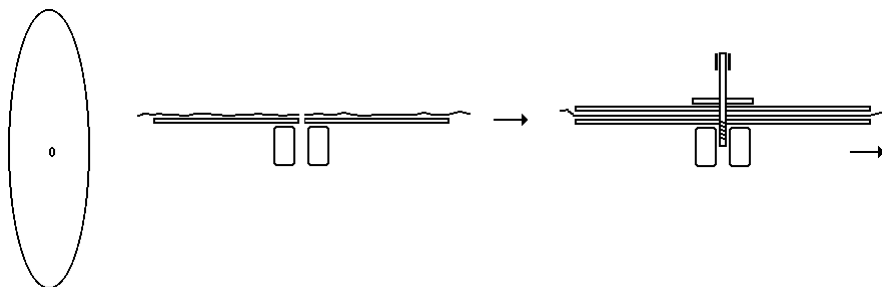
Reversed Phase Chromatography. A very limited form of reversed phase chromatography is possible on silica - glue layers. The test mixture will separate in reverse order with acetone - water (1:1). Apply the test mixture as a filtered saturated solution (about 2 ml) in acetone - water (6:4).

Aqueous solvents skate over the surface when applied to a dry sorbent. Start with 100% organic solvent then change to the aqueous solvent. As the percentage of water in the solvent is increased, a point is reached where solvent begins to splash around and the utility of the Chromatotron is lost.

Only non polar samples separate well with this sorbent under RP conditions and resolution does not approach that obtained by regular TLC on silanized layers.

Rotor Set-Up for Slow Setting Layers

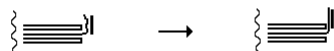
Masking tape in contact with a wet layer for more than about 20 min will detach from the rotor and allow the mixture to leak out. The rotor set-up below prevents leakage.



1 Clean and dry a rotor. Remove from the coating arbor and dry the central area.

2 Place another rotor on the coating arbor handle, cover with a square sheet of aluminum foil. Press the central area with the fingers to mark the center hole then pierce with the coating arbor.

3 Place the clean rotor on top, insert the coating arbor and screw in. Full tightening requires touching both rotors (outer 5 mm only!) to allow turning the rotors while holding the handle. Cut the aluminum foil to a rough circle extending about 2 cm beyond the rotor edge.



4 Push the aluminum foil up and apply masking tape in short (10 cm) pieces. The tape is for stiffening only, not for sealing.

5 Press the foil and tape together forming a neater edge.

PARTITION CHROMATOGRAPHY, RECIPE 4

Partition of compounds between a moving solvent and a liquid stationary phase retained by silica gel provides a separation method quite different from adsorption chromatography. Partition is particularly successful for the separation of mixtures of compounds that differ in the ratio of polar to non polar groups, such as homologs, and for separating unrelated compounds which by chance have the same R_f by adsorption chromatography. Only hydrocarbon solvents give good resolution

Recipe 4, page 23, for partition layers containing polyethylene glycol is based on the data of J.H. Dhont, J.C. De Beauveser and G.G. Kuijpers, J. Chromatography 1971 **60** 265.

Polar solvents are also suitable as stationary phases. For example, chromatography by partition between acetonitrile and hexane may be performed as follows. Run in acetonitrile, previously equilibrated with hexane, onto a 1 mm silica gel layer in the Chromatotron until the solvent front is about halfway across. Disconnect the solvent delivery system and remove all acetonitrile from the tubing, inlet and pump. After allowing the acetonitrile stationary phase to equilibrate on the rotor for at least 30 min, run in hexane, previously equilibrated with acetonitrile, and apply the sample, also in hexane.

The two bands from the test mixture (page 41) will be sharper and more completely resolved by partition than by adsorption chromatography.

CORRECTING BAND SLOPE

When 2 and 4 mm layers are prepared from recipes with certain silica gels (e.g. Merck silica gel PF, cat. no. 7749) or with an excess of water then bands of compounds will slope as shown.

Bands will broaden and separated bands may remerge. Product will appear at the output tube before the observed band on the surface has reached the edge of the rotor. *The slope is more pronounced at low flow rates and with high density solvents.*



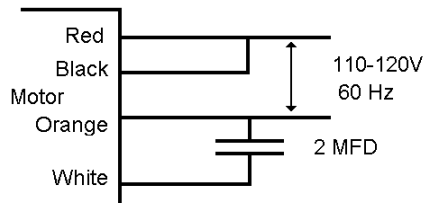
To reduce band slope:

- Avoid chloroform (high density solvent).
- Increase flow rates.
- Reduce the amount of water in the recipe.
- Cool the water and sorbent.

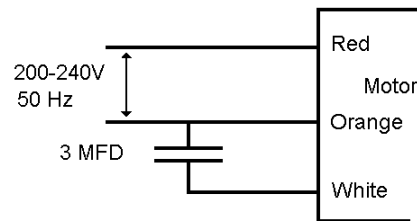
MAINTENANCE AND REPAIRS

ELECTRICAL CONNECTIONS

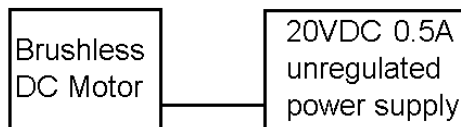
ELINCO motor 115V Model



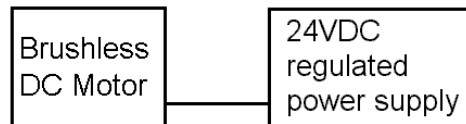
ELINCO motor 220V Model



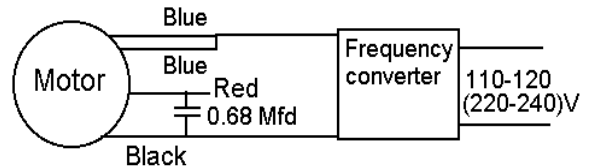
20V DC Model



24V DC Model



Models using frequency converter



SOLVENT PUMPS

See page 38, TROUBLESHOOTING, and the literature provided with the pump. The following routine maintenance is suggested for model RHSYOOSTYLF pumps at one year intervals or whenever the pump head or motor become stiff.

Remove the pulley and apply an automotive type oil to the one visible motor bearing. Wipe off excess oil and replace the pulley and drive belt.

ADJUSTING THE MAIN VESSEL

If the rotor support collar touches the main vessel, loosen the four screws holding the vessel to the metal frame of the Chromatotron, adjust the vessel and retighten the screws. The rotor support collar can also be raised slightly.

THE TEFLON LID - MAIN VESSEL SEAL

The Teflon lid must seal well to the main vessel. If the lid is held up by damage to the recess in the main vessel, use fine sandpaper to sand it down.

REPLACING THE ROTOR SUPPORT COLLAR

If the rotor support collar (diagram page 2) is removed, note that on replacement, the two set screws must be aligned with the flats on the motor shaft. If the rotor support collar and the shaft collar are not fully locked in position, they will move when the endplate is screwed in, causing the shaft to press against and break the glass rotors near the center hole.

THE FELT SEAL

The rotor support collar passes through a felt seal (diagram page 2) below the main vessel. Deposits of organic materials on the felt may cause it to adhere to the rotor support collar, producing a rubbing sound. No maintenance is required unless the adhesion causes a slow start up of the motor.

The deposits can be removed by holding the Chromatotron with the plane of the main vessel vertical while applying solvent to the upper edge of the felt, allowing it to pass through and run off the lower edge. Use a range of solvents but finish with a hydrocarbon. Keep solvents away from the motor bearings. Unplug the Chromatotron before starting this operation.

WEAR AND TEAR

The original prototype Chromatotron was retired, still in working order, after 10 years of daily use. The Chromatotron motor is rated for continuous duty and is expected to have a long life. Pumps are more subject to wear and damage from abuse.

TROUBLESHOOTING

PROBLEM	CAUSE	CURE
Irregular or eccentric solvent front and bands. Most evident with UV illumination.	1 Sorbent mixture prepared and poured too slowly, partly set during pouring.	
	2 Sorbent layer not protected from drafts during setting.	Cover with cardboard box if the sorbent has gypsum binder.
	3 Uneven drying of Merck silica gel PF.	Use the lamp/bucket dryer page 27 or turn rotors during drying. Use silica gel standard grade.
	4 Sorbent layer not completely dried.	Dry to constant weight. In humid climates use an oven or a lamp (page 27).
	5 Inlet wick is too short, bent up or of incorrect material causing solvent to flow down wick as separate drops.	Change or adjust wick (pages 5-6).
	6 Surface used for pouring and setting of sorbent suffers from vibration.	Test with beaker of water. Vibration forms visible waves.
	7 Home made glass rotors are not flat.	
	8 Solvent mixture has components of extreme polarity range, e.g. hexane-acetone. Solvent separates into two phases visible as dark spots at inner edge of sorbent.	Use less extreme range, e.g. hexane-ethyl acetate.
	9 Vapor leaks due to dust at the edge of the Teflon part (either side) of the lid.	Clean lid and recess with a moist paper towel.
	10 Vapor leaking out of disconnected nitrogen inlet.	
Broad bands. See also radial streaking (below).	1 Overloaded.	
	2 Clean-up solvent not removed completely.	
	3 Sample solution has undissolved material in suspension.	Filter or centrifuge sample solution.
	4 Bands slope through 2 or 4 mm layers, especially with dense solvents. Separated bands may remerge.	Common problem with Merck silica gel PF. See Correcting Band Slope, page 32. Use Standard grade silica gel
	5 Heat from nearby steambaths, hotplates, etc. disturbing equilibrium conditions.	Move the Chromatotron to a hood. Cool with a small fan. Use less volatile solvents.

PROBLEM	CAUSE	CURE
Bands separate then merge or broaden, especially with dense solvents on 2 or 4 mm layers.	Bands slope through the layer.	Common problem with Merck silica gel PF. See Correcting Band Slope (page 32). Use standard grade silica gel.
Broad initial band.	<ol style="list-style-type: none"> 1 Sample solution diluted by solvent in tubing and pump head. 2 Flow rate too high. Solvent flows over surface of sorbent (bands may disappear down into the sorbent). 	Allow alternating segments of solvent and air to follow the sample through the pump.
Radial streaking of bands (same appearance as broad bands when rotor is in motion).	<ol style="list-style-type: none"> 1 Inner edge of the sorbent has become impervious by reaction with polar compounds. Impervious part may be visible as a line of dark spots. 2 Sample solution contains undissolved material in suspension. 3 Sudden increase in solvent density, e.g. from hexane to dichloromethane. Denser solvent streaks through the lighter solvent. 	<p>Prepurify samples (page 10). Remove impervious part of the sorbent (page 10).</p> <p>Filter or centrifuge sample solution.</p> <p>Avoid solvent changes to dichloro-methane/chloroform or use a short solvent gradient.</p>
Chromatotron does not separate sample although separation is possible on regular TLC plates.	<ol style="list-style-type: none"> 1 Rf is too high. The Rf is sometimes higher in the Chromatotron than on regular TLC plates. Common problem with chloroform - methanol. 2 Equilibrium conditions in the Chromatotron versus non equilibrium conditions of regular TLC. 3 Sorbent layer not fully dried before scraping or air drying to remove cleanup solvent has left moisture in the layer. 	<p>Use less polar solvent mixture.</p> <p>See pages 10 and 13 for operation under non equilibrium conditions.</p> <p>Dry to constant weight.</p>
Dark band at outer edge of sorbent.	Not a problem. Solvent completely fills the sorbent pores at the outer edge of the layer.	
Dark band at inner edge of sorbent.	At high flow rates the solvent completely fills the sorbent pores at the inner edge. Not a problem but an indication that flow rate is approaching maximum where solvent flows over surface.	
Lumpy sorbent mixture during pouring. Irregular surface after setting. Short concentric cracks may form. Samples will give non-circular bands.	<ol style="list-style-type: none"> 1 Insufficient water used in recipe. 2 Ineffective mixing before pouring. 3 Rapid setting catalyzed by traces of hydrated gypsum in the mixing jar. 4 Mixing and pouring of recipe performed too slowly. Mixture has partly set before pouring. 	<p>Add extra 2-5% of water.</p> <p>Shake more vigorously. Break up air pockets with a spatula.</p>

PROBLEM	CAUSE	CURE
Sorbent layer slides off or pieces flake off from outer edge. Layers crack during drying. Mixed recipe may set before pouring is complete.	1 Sorbent-gypsum mixture was manufactured with partly hydrated binder. Common problem with Merck silica gel PF.	Use standard grade silica gel. Check quality of binder (page 21).
	2 Ineffective cleaning of rotor before coating.	An abrasive household cleaner must be used. Clean with oxidizing acid cleaning mixtures. Place two rotors in contact with the cleaning mixture between. Use oven drying of the sorbent layer at 50° in place of air drying. Oven dried layers are more firmly bound.
	3 Cleaned rotor was contaminated before coating.	Use glue bound layers (page 29).
	4 Mixing jar contains hydrated gypsum from previous pourings.	Dry cleaned rotors with paper towels only. Do not dry in an oven or with acetone. Coat rotors within 1/2 hr of cleaning.
	5 Ambient temperature too high.	Do not coat rotors when ambient temperatures are high. Adhesion is significantly weaker when the setting takes place above 30°C (86°F).
Very soft powdery layers. Scraper leaves part of layer unscraped.	Oven temperature too high.	Use 50° max.
	1 Coating performed on sloping bench.	
	2 Metal disk of the coating arbor is not parallel to the rotor due to sorbent between them.	Increase quantities in recipe.
Short arcs gouged in the sorbent during scraping.	3 Insufficient sorbent - water mixture. The layer is too thin before scraping.	Performance is not affected significantly by this defect.
	Fibers or other foreign material in the sorbent.	Performance is not affected in any way.
Circles gouged in the sorbent during scraping.	Scraper blade has rough edge.	
UV absorbing compounds not detectable.	1 Acetone or other UV absorbing solvent is present in the sample, sorbent, pump or solvent.	Evaporate clean-up solvents completely.
	2 Benzene is present as an impurity in hexane solvent.	Use benzene free grades of hexane. Use heptane.
	3 UV absorption of chloroform is masking a weak chromophore.	Use dichloromethane in place of chloroform.

PROBLEM	CAUSE	CURE
	4 Weak UV lamp.	Remove the UV filter from the lamp. Add extra zinc silicate phosphor (0.5-2%) (page 20).
	5 Manufacturer has added insufficient phosphor to the sorbent.	Add 0.5-2% of zinc silicate (page 20).
	6 Sample size too small or Chromophore is weak.	Stop solvent flow and switch off Chromatotron. Observe bands on stationary rotor. Apply sample as one spot on rotor (Light loading, page 15). Use 1/2 mm layers to maintain higher concentrations of small samples.
Condensation on lid.	This is normal, not a problem. Absence of condensation after 30 min operation may indicate vapor leaks.	A slightly fogged lid can be cleared by warming with the hand. Larger drops will coalesce if the Teflon is tapped.
Sample solution splashes onto the lid.	Flow rate too high during sample introduction.	Do not exceed 7 ml/min during sample introduction.
Solvent backs up in the collection channel.	1 Outlet blocked. 2 Polar solvents do not flow smoothly through Teflon tubing.	Clear outlet with straightened small size paper clip. Remove output tube.
Fluffy particles in solvent collection channel. May block output hole.	Dust from rotors stored in the open.	Blow off dust before mounting rotors.
Funnel stopcock develops slow leak.	Loose stopcock	Remove and squeeze stopcock slightly end to end in a vise.
Solvent flow ceases or is reduced to a low rate.	1 Stainless steel filter mesh in funnel stopcock assembly is clogged. 2 RH pump drive belt is loose.	Remove stopcock assembly from funnel and backwash. Loosen the two screws holding the motor and adjust motor position to tighten the belt.
	3 Cotton filter clogged. Bubbles will form in the tubing.	Replace cotton. Do not use glass wool.
	4 Cotton packed too tightly in the filter.	
	5 Vapor lock in the pump. Common problem with very volatile solvents at high ambient temperatures.	Raise the solvent reservoir about 20 cm to increase inlet pressure. Use less volatile solvents. Move to a cooler location.
	6 Blockage in tubing or inlet	Disconnect tubing and inlet. Check each part separately.

PROBLEM	CAUSE	CURE
Solvent leaks from pump or tube fittings.	1 Increased pressure due to blockage on output side of pump. 2 Flared ends of tubing damaged.	Disconnect tubing and inlet. Check for blockages. Do not tighten with tools! Hand tighten only. Thread tape is not effective.
Pump does not start.		Unplug pump, remove cover (thumbscrew at rear of RH pump). Take off drive belt, turn pulley by hand to determine if problem is in pump head or motor. Check belt tension.
Solvent inlet becomes tight in lid.	1 Inlet has swelled through absorption of solvents. 2 Deposits on threads.	Allow inlet and lid to dry out for 24 hr. Clean with solvents or scrape off deposits. Rethread with 5/16-18 tap and die.
Particles of sorbent on Teflon lid.	1 Sorbent dust not blown off after scraping rotor. 2 Excessive nitrogen flow.	Blow off sorbent after scraping. Wipe lid with moist paper towel.
Chromatotron oscillates from side to side.	1 Rotor unbalanced due to uneven evaporation of solvent. 2 Unbalanced home made glass rotor.	Evaporate solvent from rotor with nitrogen before switching off. Remove rotor and allow solvent to evaporate.
Rotors crack at center.	Rotor support collar and shaft collar loose.	See Replacing the Rotor Support Collar, page 34.
Endplate becomes etched. Flaky patches in main vessel.	Corrosion by strong acids, usually HCl impurity in chloroform.	Add solid sodium bicarbonate or alumina to chloroform. Test with wet pH paper. Use dichloromethane in place of chloroform.
Chromatotron produces a rubbing sound that continues after switching off, until the instant the rotor stops.	Deposits on felt seal causing adhesion to the rotor support collar.	No maintenance required unless adhesion prevents start up of motor. See the Felt Seal, page 34.
Motor does not start.	Rotor support collar in contact with main vessel.	See Adjusting the Main Vessel, page 34.
Elinco motor has insufficient torque. Slow start up. May run at half speed.	Low supply voltage.	Use motor capacitor 1 MFD larger than specified or increase supply voltage with variable transformer.
Shoehorn or endplate key lost.		Use a small hex key (Allen wrench).

CHROMATOTRON PARTS LIST

Part Number	Description
8924-03	Instruction manual for model 8924 Chromatotron
H-06	Glass rotor
H-070	Scraping tool
H-071	Scraper blade, 1 mm
H-072	Scraper blade, 2 mm
H-074	Scraper blade, 4 mm
H-07-X	Scraper blade, X mm. .Give required X in range 0.3 to 4.0
H-079	Finishing scraper blade
H-16	Coating arbor
H-19	Collar endplate (with Teflon washer)
H-24	Teflon washer for collar endplate
H-34	Key for endplate
H-36	Output tube
H-40	Shoe horn
H-48	Lid retaining clips, set of 8
H-53	Pump connections kit for RH, RPG and QG pumps. Filter/stopper/tubing/inlet
H-53-3	Coupling
H-54	Solvent filter
H-55	Solvent pump model RH. Includes pump connections kit H-53
H-65	Wick and 2 wire wick holders
H-67	Solvent inlet for use with pump
H-68	Solvent inlet for direct introduction of sample (diagram below)
H-90	Gilmont flowmeter, 10-2100 ml/min, for nitrogen
H-92	Teflon lid with solvent inlet for use with pump
H-93	Replacement Teflon sheet for Teflon lid
H-99	Polyethylene cover
H-106	24V power supply

THE TEST MIXTURE

A mixture of high R_f colored compounds is useful for testing sorbent layers. "The test mixture" refers to a mixture of the 2,4-dinitrophenylhydrazones of cyclopentanone and cycloheptanone. The same derivatives of open-chain ketones are also suitable. Azobenzene derivatives and the commercial dye mixtures used with regular TLC plates are not recommended since they are not completely stable and contain low R_f impurities that remain on the sorbent.

INDEX

- adsorbent See sorbent
- air drying of layers 27
- aluminum oxide layers 23

- band slope 32
- bands
 - broad 35, 36
 - eccentric 35
 - irregular 35
 - streaking 36
- binders 20

- changing
 - rotors 9
 - wick 5, 6
- chromatography
 - partition 31
 - rapid 14
 - reversed phase 30
- Chromatotron
 - diagram 2
 - installation 4
 - introduction 1
 - parts list 40
 - set-up diagram 3
- coating rotors
 - cellulose layers 24
 - introduction 20
 - glue-bound layers 29
 - gypsum-bound layers 24
- cleaning rotors 25
- clean-up of sorbent layers 17
- collection of fractions 16
- connection in series 19

- detection
 - small samples 15
 - UV absorbing samples 15
 - UV transparent samples 16
- development, multiple 18

- electrical connections 33

- felt seal 34
- fraction collection 16
- fraction collectors 17

- glue-bound layers 29
- gradient elution 11

- inlet 5
- insoluble samples 11, 13

- layer thickness 9
- layers
 - flaking 37
 - loose 37
 - soft 37
- less soluble samples 11, 13

- main vessel 4
 - adjusting 34
- maintenance and repairs 33
- maximum flow rate 14
- mixing jar 21, 25
- multiple development 18

- nitrogen flow 8

- oven drying 27

- partition chromatography 31
- parts list 40
- phosphors 20
- polyethylene glycol 20, 23
- prepurification of samples 10
- pumps 6-8, 33, 38, 39

- rapid chromatography 14
- recipes
 - acidified silica gel - gypsum 22
 - aluminum oxide 23
 - mixing and pouring 25
 - silica gel - glue 24
 - silica gel - polyethylene glycol - gypsum 23
 - silica gel - silver nitrate - gypsum 23
- recycle 18
- Rf 10
- rotor support collar 34
- rotors
 - changing 9

- cleaning 25
- coating 20-32
 - glue-bound layers 29
 - gypsum-bound layers 24
 - partition layers 31
- set-up for slow setting layers 31
- storage 29

- sample
 - introducing and eluting 11, 12, 13
 - loading 15
 - low solubility 11, 13
 - prepurification 10
 - volume 13
- scraping sorbent layers 28
- series connection 19
- silica - gel glue layers 24, 29
- silica gel - gypsum layers 21 - 23
- solvent
 - addition 11
 - choice 10
 - condensation 12, 38
 - flow rates 11, 14
 - maximum 14
 - inlet 5
 - interrupting flow 15
 - leaks 38, 39
 - pumps 6-8, 33, 38, 39
 - surface flow 14
- solvent inlet 5
- sorbent
 - clean-up 17
 - layer thickness 9
 - loose layers 37
- sorbents
 - (list) 20
 - coating rotors 20
 - drying layers 26
 - scraping layers 28
- storage of coated rotors 29
- surface flow 14

- tailing 10
- Teflon lid 4, 34
- test mixture 41
- Troubleshooting 35

- vapor locks 12, 38

- wick 5, 6