INSTRUCTION MANUAL
model 8924

Chromatotron

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WARRANTY

Harrison Research warrants its products, except as listed below, 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, Harrison Research assumes no responsibility for the results of its use. 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.

The pump carries a 90 day limited warranty from Fluid Metering Inc.

REGISTERED TRADEMARKS

Teflon, E. I. Dupont.

Mineralight, UVP.

Ajax, Colgate Palmolive.

Florisil, Floridin Company.

Elmer's Glue-All, Carpenter's Wood Glue, Borden.

Adsorbosil, Alltech Associates - Applied Science Labs.

Polyseamseal, Ensign - Bickford Industries.

CAUTION

* The Chromatotron is to be used only by those trained in the use of laboratory equipment.

* Plug into a 3 pin (grounded) electrical outlet only.

* Do not leave the Chromatotron unattended when solvent is flowing.

* Switch off 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 and to cool the motor.

* Keep organic solvents away from the motor and electrical system.

* After switching off 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 absorbent layers before heating rotors 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.

* Do not pump toxic samples without suitable precautions to allow for ejection under pressure.

CONTENTS

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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 absorbent 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 pumped 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.

The 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, the output of one being pumped 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 absorbents.

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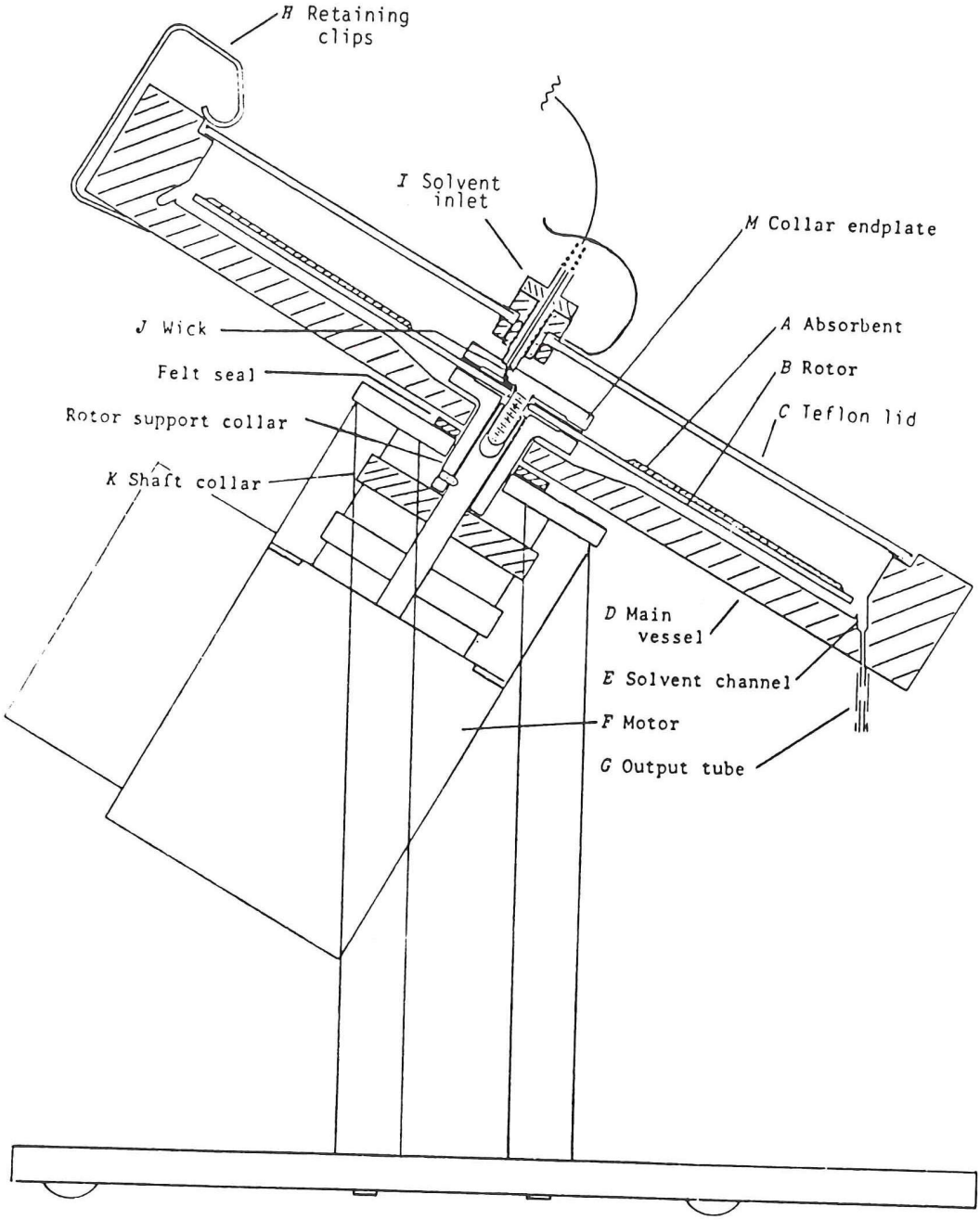
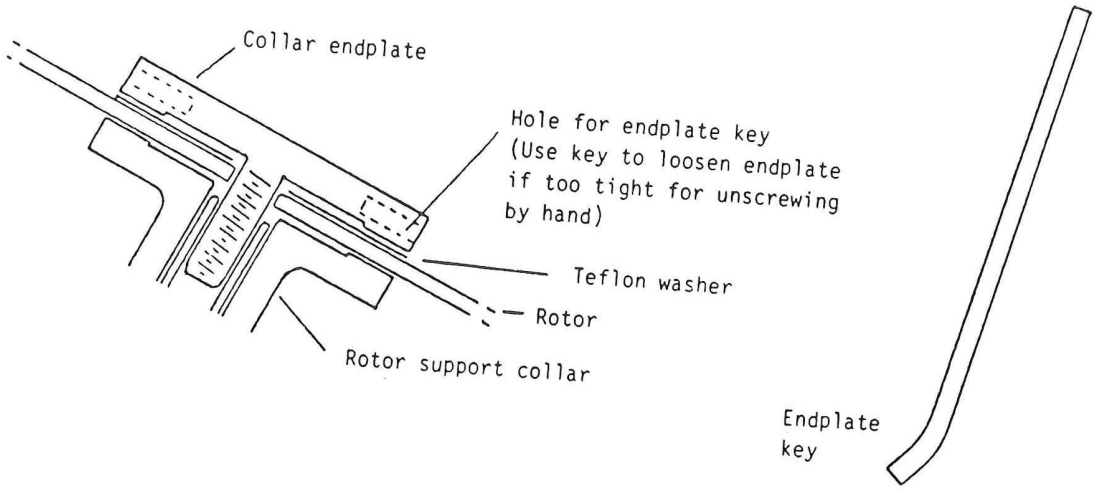
THE CHROMATOTRON

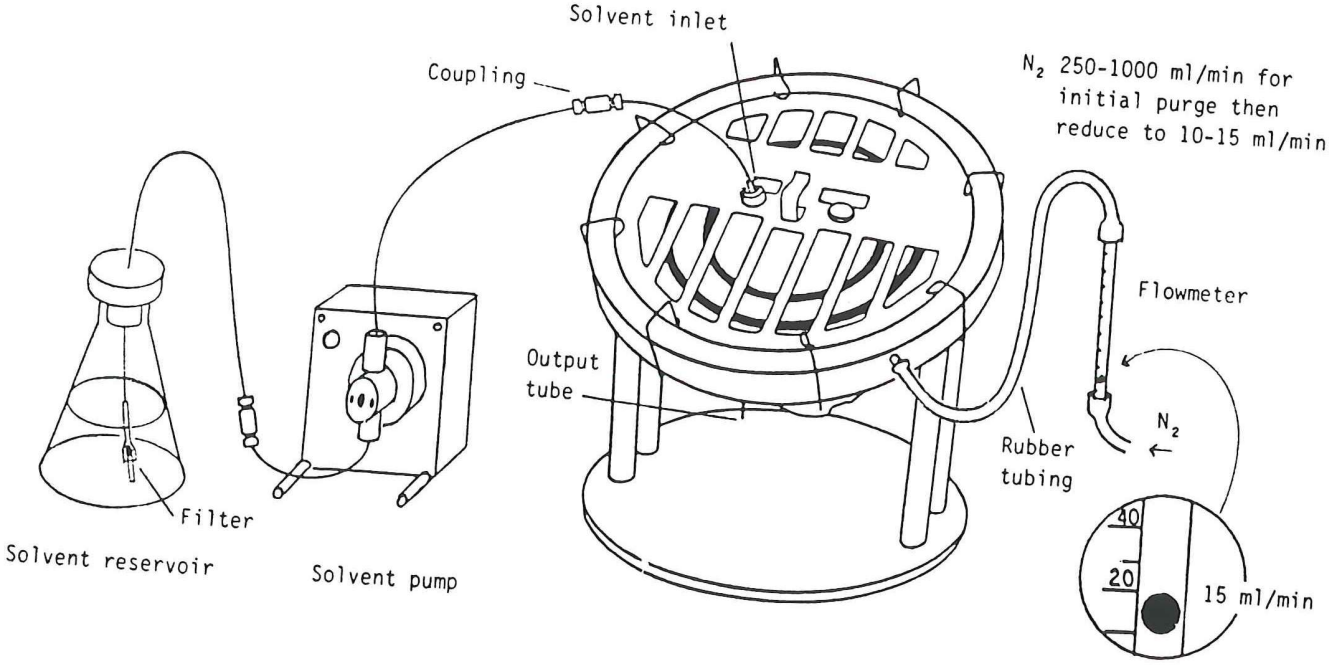


U.S. Patent no. 4139458
Patents pending in other countries.

THE ROTOR SUPPORT SYSTEM



THE CHROMATOTRON AND PUMP SET-UP DIAGRAM



Usual pump scale setting for 1 mm layers:

	60 Hz	50 Hz
2 mm	45-95	55-115
4 mm	140-190	170-230
	190-235	230-285

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. The air flow in such areas will give additional motor cooling. Operation in areas which are not well ventilated will allow heat rising from the motor to reduce the efficiency of the chromatography. A small fan should be used if no air flow is available. Hotplates, steambaths or other sources of heat should not be placed near the Chromatotron.

THE MAIN VESSEL

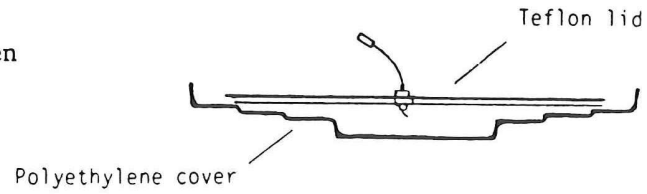
The main vessel is constructed from "acetal" polymer. When chromatography has been completed, solvents which soften or swell acetal (chloroform, dichloromethane, acetone, acetonitrile) should be eluted with hexane, other hydrocarbons or ethyl acetate. The solvents ether, ethyl acetate, tetrahydrofuran, dioxane, methanol, hydrocarbons and carbon tetrachloride are relatively inert towards acetal. Acetic acid, trimethylamine or ammonia may be added 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 provided should be placed over the lid when the Chromatotron is not in use. When the lid is out of the Chromatotron, place it on the inverted polyethylene cover:



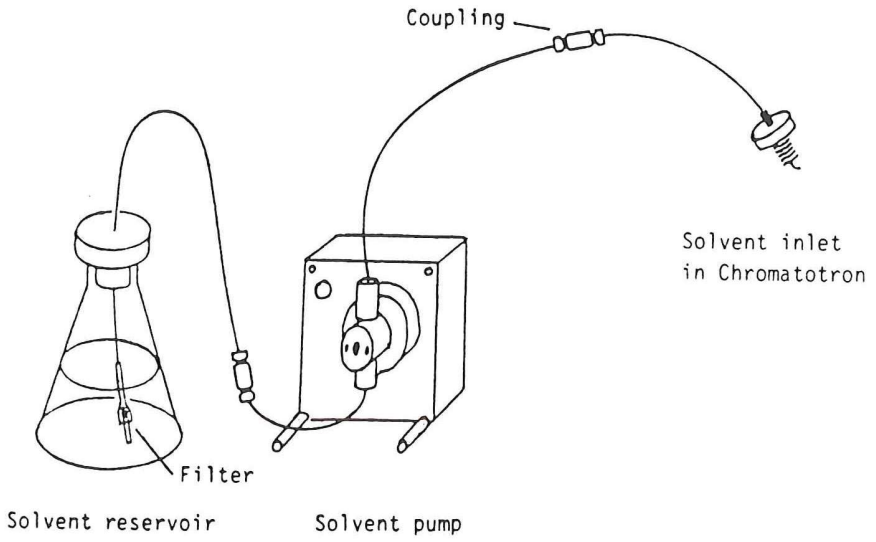
Maintain the teflon lid clean. Large particles of dust on either side of the Teflon sheet, near the edge or in the corresponding recess in the main vessel, will cause solvent vapor leaks. A moist, soft paper towel or cloth will remove dust with a minimum of scratching.

THE SOLVENT PUMP

The pump provides a variable solvent flow in the range 0 - 15 ml/min for solvent addition, for introduction of the sample solution and for recycle of eluted components.

Change flow rates (with the pump on or off) by turning the knurled adjustment ring on the pump head. The recommended flow rates and corresponding pump scale settings are given on the pump label and on page 10.

The Solvent Pump and Fluid Connections



Plastic tube end fittings should be screwed into the pump gently by hand only. If solvent leaks from the fittings check for blockages in the solvent inlet and tubing or damage to the flared tubing ends.

If the pump does not start, unplug the power cord, remove the cover (loosen thumbscrew at rear) and check that the motor turns freely. Also check the tension of the rubber drive belt.

Raise the solvent reservoir 20 cm if high ambient temperatures or very volatile solvents reduce flow rates by forming vapor locks (gas bubbles that remain in the pump). Alternatively, change the drive belt to decrease the pump stroke speed and use a higher flow rate setting at the pump head.

An inlet for introducing the sample directly to the rotor, by-passing the pump, is available. See CHROMATOTRON PARTS LIST, page 43.

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.

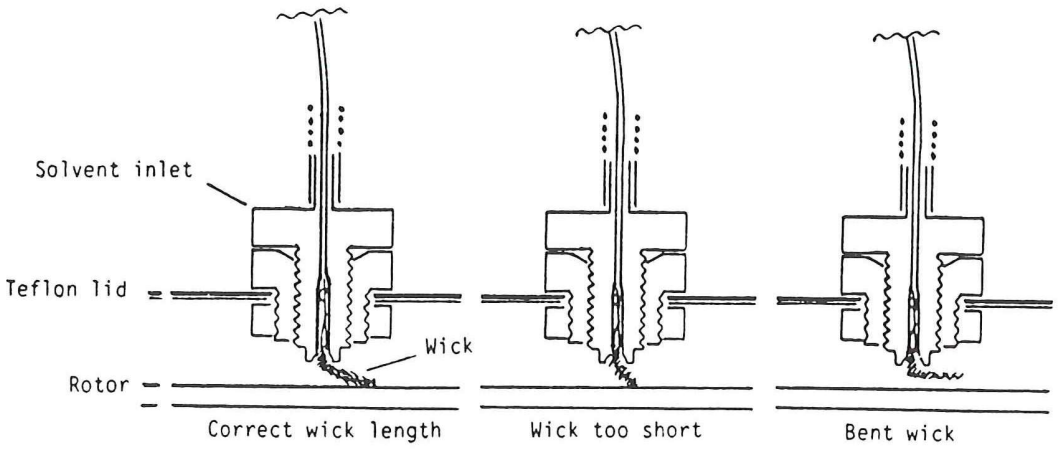
* Do not pump mineral acids. Wash out chloroform (which generates acid on exposure to air and light).

* Do not pump toxic materials without suitable precautions to allow for accidental ejection under pressure.

THE SOLVENT INLET

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

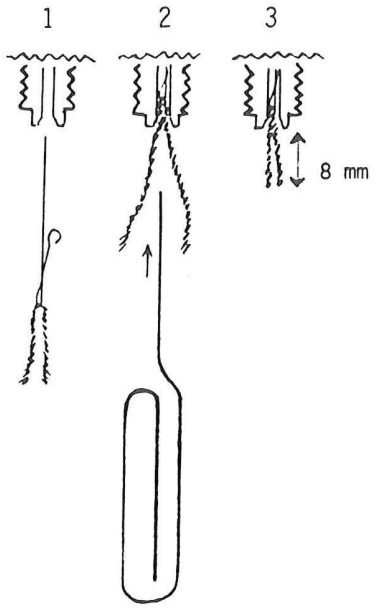
The Solvent Inlet



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 8 mm.

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



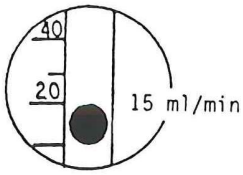
NITROGEN FLOW

A flow of inert gas is essential. Even relatively stable compounds can partially oxidize when exposed to absorbents and air. Connect a nitrogen or other inert gas source to the inlet tube on the side of the main vessel. The flowmeter supplied or a bubbler is required. The gas cylinder must have both reducing and needle valves to avoid sudden blasts of gas.

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 absorbent layer. Volatile liquids such as xylene are unsuitable if UV absorption is to be used for detection of compounds on the rotor. **Do not use sulfuric acid** or other corrosive materials 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 particles of absorbent.

Flowmeter Scale

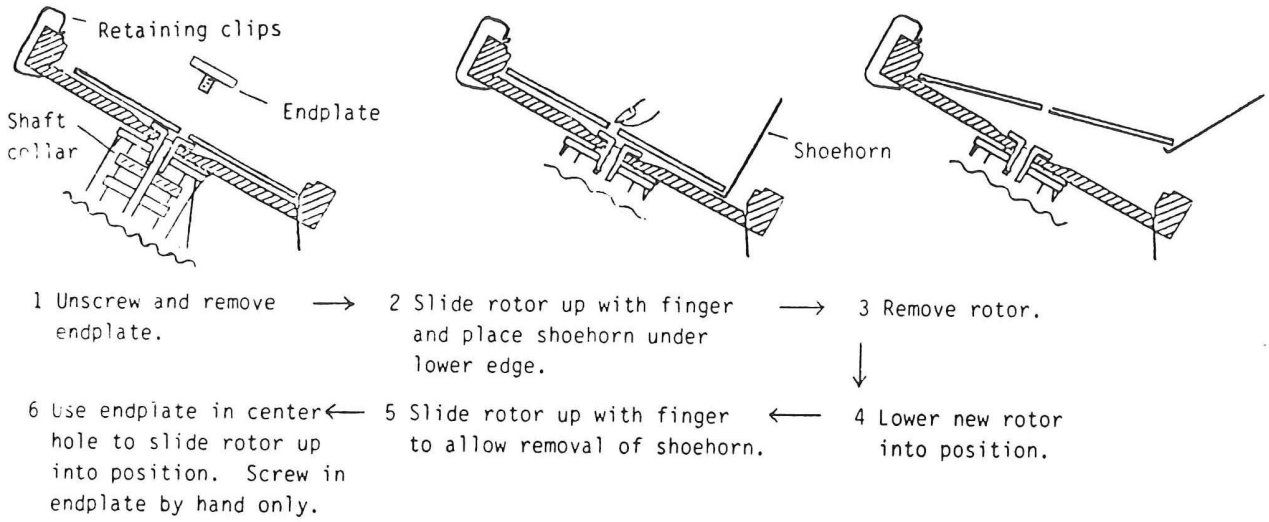


Nitrogen exits from the main vessel via the felt seal around the rotor support collar.

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.



A tight endplate can be levered loose with the endplate key inserted 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 absorbent layer, or the glass rotor, does not affect performance.

ABSORBENT 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 1.5 g. Gain experience with 1 and 2 mm layers before using these thick layers.

Scraper blades are available for absorbent layers of any thickness within the range 0.4 to 4 mm, see CHROMATOTRON PARTS LIST, page 43.

PREPURIFICATION OF THE SAMPLE

Samples which 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 absorbent (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 which react irreversibly with absorbents.

Very polar impurities will form a line of darker impervious spots at the inner edge of the absorbent on the rotor. Ultimately, bands will streak and broaden. The offending absorbent can be removed by rescraping the layer using a scraper blade displaced radially outwards from the normal position in the scraping tool. Removal of 2-3 mm of absorbent (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 absorbent for prepurification of these samples.

SOLVENT CHOICE

The Chromatotron requires an Rf lower than for regular TLC. **Preferably choose solvents giving an Rf in the range 0.2 - 0.4** using conventional analytical TLC. A higher Rf is acceptable for easy separations. If UV absorption is to be used for detection, see Detection of UV Absorbing Compounds on the Rotor, page 14, before choosing a solvent. The range of usable solvents extends from hexane to methanol. Glue-bound silica gel layers are not loosened even by 100% water.

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 12).

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. Increased solubility can be obtained 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 Rf. See also Introducing Less Soluble Samples, page 12.

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 Rf have been chosen.

SOLVENT ADDITION

For solvents of normal viscosity the recommended flow rates are:

		Pump Scale Setting	
		60 Hz	50 Hz
1 mm absorbent layers:	2 - 4 ml/min	45 - 95	55 - 115
2 mm absorbent layers:	6 - 8 ml/min	140 - 190	170 - 230
4 mm absorbent layers:	8 - 10 ml/min	190 - 235	230 - 285

The above settings assume that zero on the pump scale corresponds to zero flow rate. Check by adjusting the pump to zero flow (bubbles in the tubing do not flow forwards or backwards) and if required loosen the pump scale (unscrew small knurled knob) and set to 000.

Before the sample solution is introduced, the absorbent 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 absorbent completely wetted with solvent. Other parts of the absorbent contain nitrogen and solvent vapor as well as solvent.

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 which occurs as the solvent meets the absorbent. A front that appears to wobble during the first few cm is an indication of a worn or bent wick, an inhomogeneous absorbent, vapor leaks around the Teflon lid or an excessive flow of nitrogen. See page 35, TROUBLESHOOTING.

Vapor locks (bubbles which remain in the pump) may reduce the flow rate when ambient temperatures are high. Raise the solvent reservoir about 20 cm to correct this problem. See page 40, 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.

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

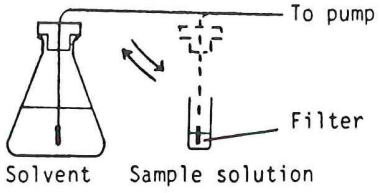
Introducing the Sample. Dissolve the sample, e.g. 1-2 mg of the test mixture (Appendix 3, page 46), in a small volume (0.5 - 2 ml) of the eluting solvent. Suitable solvents for the test mixture are heptane - isopropyl acetate, or hexane - ethyl acetate, both 8:2, or toluene. If this is the first run, 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.

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

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

* Wash in with several squirts of solvent.

* 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 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.

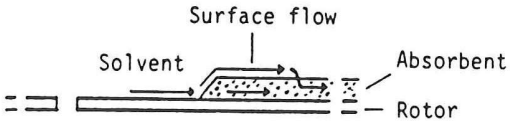
It is possible to introduce the sample after only a small part of the rotor has been wetted with solvent. Under these non equilibrium conditions relative Rf values and the separations obtained may be abnormal. Evaporation in the solvent collection channel will cause slight but usually acceptable tailing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

An inlet which allows introduction of the sample solution directly to the rotor, by-passing the pump, is available. See CHROMATOTRON PARTS LIST.

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 absorbent 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 absorbent.

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 while wetting the absorbent and eluting with less polar solvent. Success depends on minimizing the amount of polar solvent which 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 absorbent 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 pulsating shiny band.



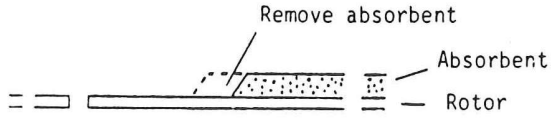
Dichloromethane and other high density, low viscosity solvents can be used at higher flow rates without surface flow.

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 3, page 46) 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 absorbent 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 use the more porous silica gel HF (recipe 3, page 21) and increase the flow rate after the bands have moved away from the central area where surface flow occurs. Alternatively, use regular absorbents and increase the diameter of the absorbent-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 46) can be separated 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 absorbent. The bands of compounds on the dry rotor will not spread by diffusion, even during 24 hr. The new solvent flow will sharpen the bands.

Heavy Loading. For heavy loadings the mixture should be dissolved 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 absorbent 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 Rf 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.

Thin absorbent layers (e.g. 0.5 mm) can also be used to maintain higher concentrations. 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 most generally useful.

A band of apparently even density will in fact have most material near the center and very little at the leading and trailing edges. Two compounds which 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 has a limited life. 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, page 13.

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 absorbent. Preferably air-dry the rotor. Washing with solvent can be used to remove acetone but surprisingly large volumes are required to remove the last traces from the absorbent. A test substance with a high Rf, 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 in fact 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.

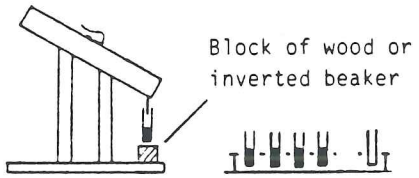
Some compounds lacking chromophores can be detected on absorbents containing long wavelength (365 nm) phosphors (H.K. Desai, E.R. Trumbull and S.W. Pelletier, J. Chromatography 1986 **366** 439). Bands on a rotor can be visualized, inconveniently, by evaporating the solvent and applying iodine (H.K. 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 absorbent 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 and sharp separations 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 Rf, e.g. azobenzene or the test mixture (page 46), 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 compound 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" 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 ABSORBENT LAYER

After each separation or when absorbent 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. Start the addition with the rotor turning backwards for 1-2 turns (turn the shaft collar by hand) to allow solvent to wash the central area, then switch on the motor.

The clean-up solvent can be removed from the absorbent by drying in the open air, allowing sufficient time (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 absorbent. Oven drying may also be used **after the solvent has completely evaporated.** Take care to remove all traces of acetone from the pump 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 absorbent 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 may change relative Rf values of components of mixtures and will slowly remove the gypsum binder.

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.

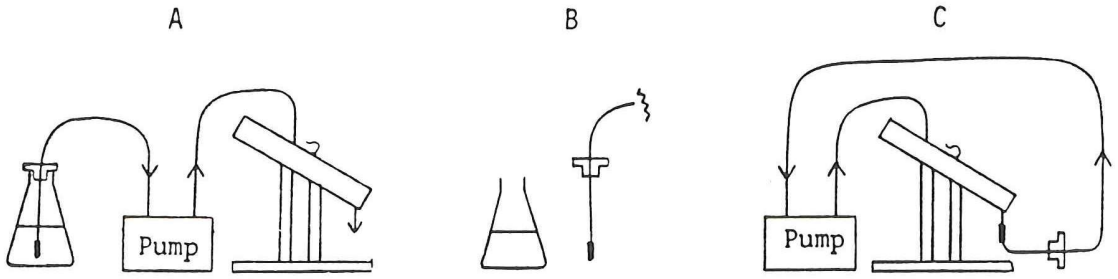
MULTIPLE DEVELOPMENT

Broad or irregular bands on 1 and 2 mm layers may be sharpened 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 absorbent. 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 absorbent.

RECYCLE

Recycle is a simple strategy for increasing the resolving power of the Chromatotron. For a trial run use the test mixture (Appendix 3, page 46) 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 absorbent 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 C (with Teflon tip removed). The pump will recycle solvent from the Chromatotron for about 1 min then both solvent and vapor will recycle.



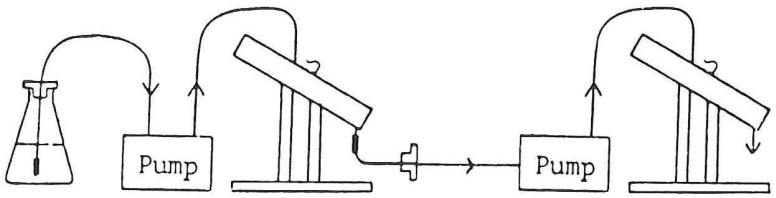
If the 10 sec delay at stage B is omitted, solvent may back up slightly in the collection channel causing some remixing of bands. **The solvent flow rate must be held constant for some time before recycle commences to prevent surges of solvent flow.**

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 absorbent. 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 17). If the Rf 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 from 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.



Series connection of Chromatotrons is useful when the lack of a chromophore in the sample prevents the application of recycle.

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COATING ROTORS WITH ABSORBENTS

INTRODUCTION

Absorbent layers on rotors are produced by casting absorbent-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 ($CaSO_4.1/2H_2O$, 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. Manufacturers often produce silica gel - gypsum with a defective (probably partly hydrated) binder. See Appendix 1, page 45, for regeneration by heating.

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

Rotors are made of regular glass, not a heat resistant type. Do not submit them to unnecessary thermal shocks.

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

ABSORBENTS, BINDERS AND PHOSPHORS

	Catalog/item no.
Silica gel 60 PF-254 with Calcium sulfate, E. Merck	7749
Silica gel 60 HF-254+366, E. Merck	7741
Adsorbosil-Plus P (silica gel), Alltech Associates - Applied Science Labs	16031
Silica gel, Aldrich Chemical Company	28,858-6
Florisil, Aldrich Chemical Company	28,871-3
Aluminum oxide 60 PF-254, E. Merck	1103
Aluminum oxide 60 PF-254+366, E. Merck	1104
Calcium sulfate hemihydrate ($CaSO_4.1/2H_2O$) TLC reagent, J.T.Baker	1463
Zinc silicate phosphor, Sigma Chemical Company	z4500
Polyethylene glycol 8000, Aldrich Chemical Company	20,245-2

E. Merck products are available through EM Science, VWR Scientific and through regular laboratory suppliers. Do not confuse "Silica gel PF-254 with calcium sulfate", item no. 7749, with other types of "Silica gel PF-254". TLC grade calcium sulfate must be used. Other grades contain impurities such as the dihydrate which influence the setting rate, the adhesion to the rotor and the cracking tendency.

RECIPES

The temperature and relative amounts of the ingredients given below are varied according to the nature of the absorbent and the thickness of the layer. These details have been optimized empirically to reduce the tendency of bands of compounds to slope through the thickness of 2 and 4 mm layers. It may be necessary to adjust the proportion of water to allow for batch to batch absorbent variations.

Where a recipe requires cooling of the absorbent, this should be done in the container to be used for mixing. Cool for at least 2 hr.

The layers from recipes 1 - 6 (with gypsum binder) must be allowed to set for at least 1 hr (overnight OK) before drying.

Amounts of absorbents and binders are given in grams, amounts of water in ml and temperatures in °C. Full details of the mixing/coating procedures are given on pages 23 - 31.

Mixing Jar Sizes

Layer thickness:	1 mm	2 mm	4 mm
Mixing jar size:	12-16 oz (350-500 ml)	16-20 oz (500-600 ml)	24-32 oz (750-1000 ml)

For easy mixing the jar should be less than half full with the dry absorbent.

Recipe 1, Silica Gel 60 PF-254

The preferred absorbent for most separations.

	1 mm	2 mm	4 mm
Silica gel 60 PF-254 with calcium sulfate	45	65 (0-5°)	115 (0-5°)
Water	90 (0-5°)	130 (0-5°)	200 (0-5°)

See page 23 for the mixing and pouring procedure.

Recipe 2, Adsorbosil-Plus P - Gypsum

This recipe illustrates the use of analytical grade TLC silica gel with added binder. Other brands of silica gel may be used although the recipe will require a different proportion of water.

	1 mm	2 mm	4 mm
Adsorbosil-Plus P	30	43	77
Calcium sulfate hemihydrate	8	13	23
Water	75 (0-5°)	110 (0-5°)	190 (0-5°)

See page 23 for the mixing and pouring procedure.

Recipe 3, Silica Gel HF - Gypsum

A more porous absorbent for rapid chromatography at high flow rates. Bands are broad at low flow rates.

	1 mm
Silica gel HF	36 (0-5°)
Calcium sulfate hemihydrate	9 (0-5°)
Water	100 (0-5°)

See page 23 for the mixing and pouring procedure.

Recipe 4 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, 4mm - 1.2g) dissolved in the water.

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

If a stronger acid is required, prepare layers from recipes 1, 2, 3 or 7 and add acetic acid to the eluting solvent.

Recipe 5, 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 the water.

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 6, Silica Gel - Polyethylene Glycol - Gypsum

For partition chromatography, cf. J.H. Dhont et al., J. Chromatography, (1971) **60** 265. For non-polar samples, partition can be used in place of reversed phase chromatography. See page 31 for a discussion.

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

For elution use only hexane, ether, and acetone; polyethylene glycol is soluble in most other solvents.

Recipe 7, Silica Gel - Glue

Glue-bound layers are stable to very polar (including aqueous) solvents. Silica gel from the Aldrich Chemical Company forms layers that have the least tendency to crack during drying. Silica gel HF is also usable.

	1 mm
Silica gel (Aldrich)	30
Glue	2.5
Water	78

See page 29 for the coating procedure and a discussion.

Recipe 8, Florisil - Glue

	1 mm
Florisil (Aldrich)	33
Glue	1.5
Water	69

Use the coating procedure described on page 29 for silica gel - glue. Florisil layers are alkaline.

Recipe 9, Aluminum Oxide PF (254 or 254+366)

	1 mm
Aluminum oxide PF	60
Water	70

See page 31 for a procedure based on the method of H.K. Desai, E.R. Trumbull and S.W. Pelletier, J. Chromatography (1986) **366** 439. Aluminum oxide layers are slightly alkaline.

COATING ROTORS USING RECIPES 1-6, GYPSUM-BOUND LAYERS

Introduction

The beginner should first prepare a 1 mm layer of silica gel 60 PF-254 (recipe 1, page 20) 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.

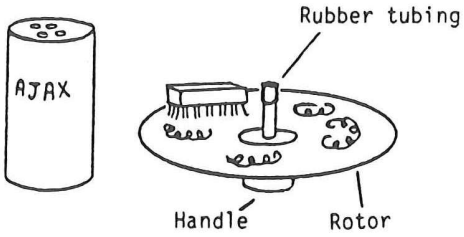
Before starting the coating procedure obtain a cardboard box or plastic bowl to cover the rotor while setting.

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 absorbent layers will be a serious nuisance if rotors are not scrupulously clean.

Cleaning and Setting Up the Rotor For Coating

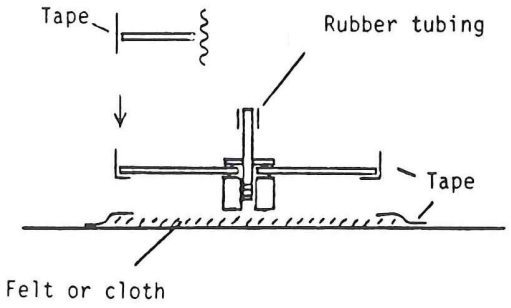
Mount the rotor (ground side up if one side is ground, either side up if the rotor is of the unground type) in the coating arbor and tighten gently by turning the edge of the rotor while holding the plastic handle. The rate of accidental rotor breakage will be high if the arbor and handle are not used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

Thoroughly scrub the upper surface with water and Ajax or similar abrasive household cleanser powder, using a small, stiff scrubbing brush or wad of paper towels. 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. Place on a rigid level surface covered by a piece of felt or cloth, taped down. The surface used must be free from vibration caused by nearby mechanical equipment. A short length of rubber tubing is required on the rod of the arbor as a finger grip.

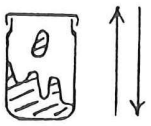


Mixing and Pouring the Recipe

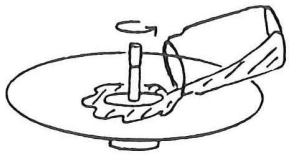
Weigh out the absorbent (and binder if required) into a preserve or pickle jar or other wide-mouth container with a cap giving a liquid-tight seal. Erlenmeyer (conical) flasks may be used but are inconvenient. For easy mixing the jar should be less than half full of the absorbent and binder. Usual jar size: 1 mm layers, 12 - 16 oz (350 - 500 ml); 2 mm layers, 16 - 20 oz (500 - 600 ml); 4 mm layers, 24 - 32 oz (750 - 1000 ml).

Measure out the water, cool to the specified temperature and add to the jar. The following stages must be completed within 5 min since the mixture will begin to set within 5-10 min.

Swirl the mixture until most of the absorbent has been wetted (releasing air which will otherwise create a slight pressure) then cover and shake vigorously for 15-30 sec. During the shaking, check that air pockets have not formed. If necessary break up air pockets with a spatula.

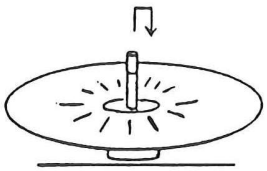


Turn the rotor slowly (1/2 - 1 revolution/sec) by the central rod and pour the mixture in a continuous stream in overlapping circles close to the central metal disk. Keep the jar close to the rotor to minimize the formation of separate drops of the mixture. Slight agitation will encourage thick mixtures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

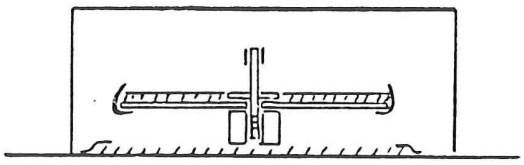


If sufficient water has been used, 95% of the mixture will flow readily out of the jar.

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 5 times. Bumping liquefies the mixture releasing air bubbles, allowing gravity to spread the mixture and smooth out inhomogeneous parts.



Cover the rotor with a bowl or cardboard box (protection from drafts) and **allow the layer to set for at least 1 hr** (overnight OK).



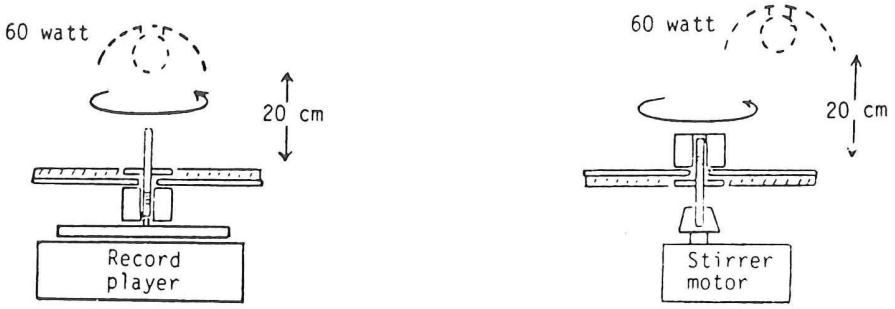
Remove the masking tape after the setting is complete. The rotor can remain in the coating arbor for air drying of the layer.

The white spiral that appears on the absorbent surface during setting is of no significance.

Drying the Absorbent Layer

The traditional "activation" of regular TLC plates by heating above 100° is in fact only a reversible drying. The absorbent 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 very humid climates oven drying at 60-70° will be required.

Air Drying. Air dry to **constant weight** (weigh the complete rotor-arbor assembly) while turning (to average out the effect of drafts on the absorbent) with a slow speed stirrer motor or a record player at the lowest speed. Drying is usually complete in 20-30 hr for a 1 mm layer. In moist climates or if faster drying is desired, heat slightly with a 60 watt lamp 20 cm above the rotor.



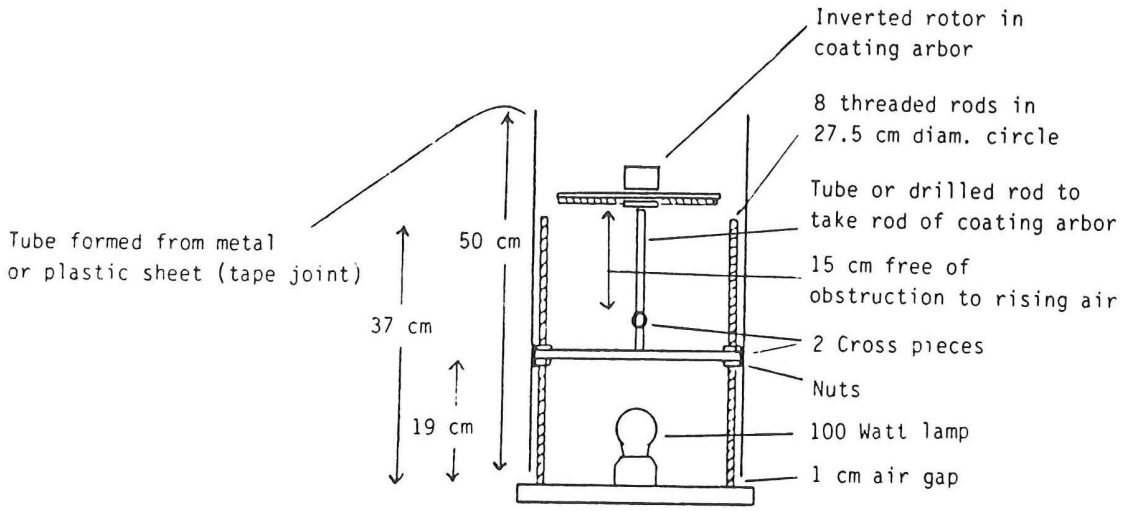
If no equipment is available for turning, place the rotor away from areas with unidirectional drafts (fume hoods etc.) and sources of heat or cold. The downdrafts of cold air that form below windows in a heated room will prevent even drying of the absorbent.

Oven Drying. Scrape off a circle of absorbent 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 60-70°, preferably turning the rotor about 1/3 turn at 5 min intervals for the first 20 min. Any oven that is not sealed will be suitable. Dry to constant weight, about 3 hr for 1 mm layers, 5 hr for 2 mm and 12 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. Dry for a further 1 hr after the rotor is too hot to hold.

Although air drying of absorbent 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. A motor for turning rotors within an oven has been described (R.F. Vieth and H.R. Sloan, J. Chromatography, 1986 **357** 311).

The gypsum binder will partly revert to calcium sulfate hemihydrate at oven temperatures above 70°. The absorbent layers will then be soft and easily damaged.

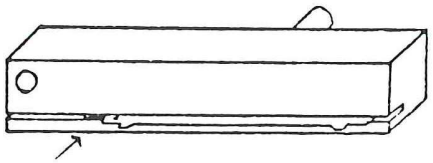
The rotor dryer shown below requires no rotation for even drying.



Rotors that have been air or oven dried with rotation will form bands of separating compounds which are regular and concentric to ±1 mm. Drying in the open air without rotation increases the possible irregularity and eccentricity to about ±3 mm. If the rotors are not covered during the setting, irregularities will be even larger.

SCRAPING ABSORBENT 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 absorbent 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 absorbent, apply only slight pressure. Continue scraping until stage A (diagram, next page) is reached with 2 channels scraped completely down to the glass. This is a noisy operation!

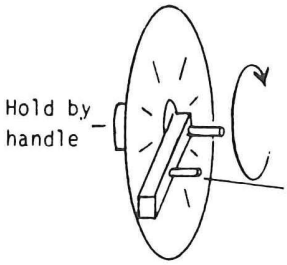


Scraping tool

End of blade stamped 1, 2, 4 or F in contact with stop in slot

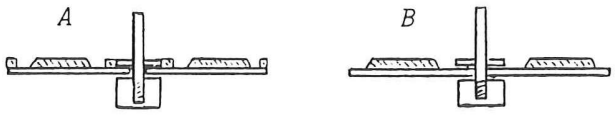
Scraper blade (1, 2 or 4 mm)

Finishing scraper blade



If the scraping tool can be turned only with difficulty, hold the outer end instead of the handle. If the scraper bounces over the absorbent, 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 absorbent at the edge and center (stage A → B).



Scraped layer Finished layer

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

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

The absorbent scrapings are of little value. Reuse requires crushing and sieving of the residues as well as regeneration of the binder. If economy in the use of materials is important then scale down the quantities specified in the recipes.

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 which will otherwise gather around the outlet hole in the collection channel.

Old rotors develop slight pH differences between the upper surface and lower layers of absorbent. 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 in an oven at 60-70° before use.

COATING ROTORS USING RECIPE 7, GLUE-BOUND LAYERS

A variety of common aqueous latex glues are satisfactory adhesives for silica gel (recipe 7, page 22) and Florisil (recipe 8 page 23). These include Elmer's Glue-All, Carpenter's Wood Glue (Elmer's) and clear Polyseamseal (an adhesive/sealant sold in caulking tubes by hardware stores). Polyseamseal is recommended, Elmer's Glue-All forms a non-wetting surface on the rotor, disturbing the smooth flow of very polar solvents.

Weigh out the absorbent into an 8-10 oz (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 absorbent and stir. The mixture thickens initially but thins 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 (silica gel) or 1 hr (Florisil), with occasional stirring and bumping of 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 absorbent 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 without rotation (do not cover with a box). The drying time is about 48 hr in dry climates, 20 hr with an optional 60 watt lamp 20 cm above the center of the rotor. After air drying, remove the rotor from the arbor and place in an oven preheated to 70° then slowly raise the temperature to about 100° and maintain at this value for 1 hr. Cool slowly to avoid cracking the glass.

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 46) in toluene. Bands of compounds are usually narrow and concentric. The layers do not detach from the glass even with 100% water.

Reversed Phase Chromatography. A limited form of reversed phase chromatography is possible on silica - Polyseamseal 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). Only non polar samples separate well with this absorbent under RP conditions.

Increased amounts of the glue give improved capacity and resolution in RP mode but the layers are difficult to scrape and have a low porosity (requiring low flow rates) if more than 5g is used.

Aqueous solvents skate over the surface when applied to a dry absorbent. 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.

Elmer's Glue-All forms a non-wetting film on glass rotors. Use oxidizing acid cleaning solutions before recoating.

Rotor Set-Up for Slow Setting Layers

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

2 Place another rotor on the 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 to stiffen the foil. The tape is for stiffening only, not for sealing.

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

COATING ROTORS USING RECIPE 9, ALUMINUM OXIDE PF LAYERS

Masking tape in contact with aluminum oxide PF - water may detach from the edge of rotors. Use the tapeless rotor set-up shown on the previous page.

Following the procedure of H.K. Desai, E.R. Trumbull and S.W. Pelletier, J. Chromatography, 1986 **366** 439, weigh out 60g of aluminum oxide PF in an 8-16 oz (250-500 ml) jar, add 70 ml of water, shake vigorously for 2 min then allow to stand for 15 min. Give the mixture a final swirl, pour and bump in the usual way.

Allow the layer to air dry to constant weight (24-48 hr, less with heat from a 60 watt lamp 20 cm above the center of the layer). Oven heating at 70°, after air drying and removal from the arbor, may be used if desired.

Aluminum oxide PF is slightly alkaline; labile esters suffer hydrolysis during chromatography. Allow the scraped layer, wet with ethyl acetate, to stand in the Chromatotron (with nitrogen flow reduced) overnight to reduce the basicity.

The layers have a low porosity requiring slightly reduced flow rates to prevent surface flow of solvent.

PARTITION CHROMATOGRAPHY, RECIPE 6

Partition of compounds between a solvent and a liquid stationary phase absorbed on silica gel provides a separation method quite different from absorption chromatography. Partition is particularly successful for the separation of mixtures of compounds which differ in the ratio of polar to non polar groups, such as homologs, and for separating unrelated compounds which by chance have the same Rf by absorption chromatography.

Recipe 6 , page 22, 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. A suitable solvent series is hexane - ether - acetone. Other solvents may extract the stationary phase.

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 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.

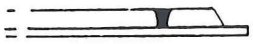
The two bands from the test mixture (page 46) will be sharper and more completely resolved by partition than by absorption chromatography.

OPTIMIZING RECIPES

The recipes for 1 mm silica gel layers are uncritical, the relative amounts of the ingredients or the temperature can be changed considerably without adverse effects. For 4 mm and to a lesser extent 2 mm layers, the situation is quite different. Changes in the recipes for these thicker layers and batch to batch absorbent variations may cause bands of compounds to slope as shown below. The observed bands will broaden and separated compounds may merge again near the rotor edge.

For best results check each newly prepared layer at normal and reduced (e.g. 1/2) solvent flow rates with the test mixture (page 46) or acetone 2:4- dinitrophenylhydrazone (or other colored compound) and acetone solvent. Use the list below to find a partial cure for band slope and for improvements to the recipe. A good layer will have bands from 3 to 6 mm wide near the edge of the rotor.

Correcting Band Slope

If bands are sharper at normal flow rates then bands slope backwards:	If bands are not sensitive to flow rates then bands are optimum and do not slope:	If bands are sharper at low flow rates then bands slope forwards:
		
Partial cure:	Increase flow rate. Use less dense solvents, avoid CH_2Cl_2, $CHCl_3$	Decrease flow rate.
Recipe changes to reduce band slope. Use one or more as required:	1 Reduce temp. of H_2O. 2 Reduce temp. of absorbent. 3 Reduce proportion of H_2O. (e.g. by 5-10%) 4 Reduce setting time to 25 min. and dry in an oven preheated to 60-70°. 5 Reduce setting time to 25 min and dry on an aluminum sheet (baking sheet) preheated in an oven at 60-70°.	Increase temp of H_2O. Increase temp. of absorbent. Increase proportion of H_2O.

Reducing the proportion of water (change no. 3) may reduce the porosity of the layer requiring lower solvent flow rates.

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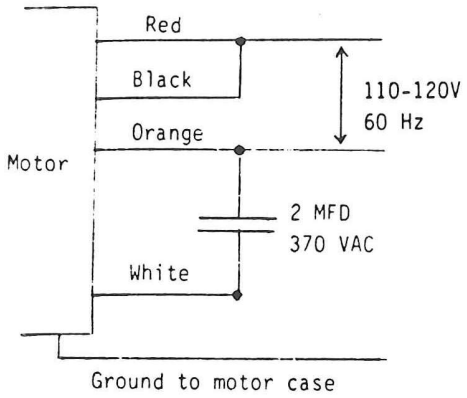
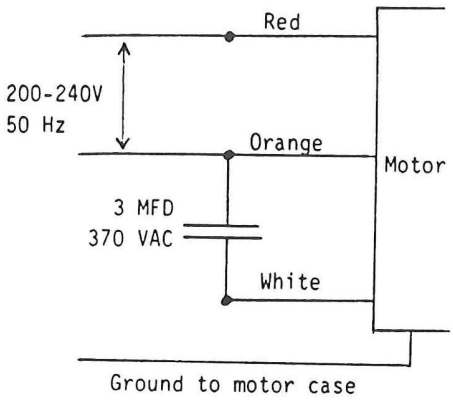
ELECTRICAL CONNECTIONS

115V Model



220V Model



For operation at 100V use 3 MFD capacitor

THE SOLVENT PUMP

See page 40, TROUBLESHOOTING, and the literature provided with the pump.

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 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 emery cloth to sand it down.

REPLACING THE ROTOR SUPPORT COLLAR

If the rotor support collar is removed, note that on replacement, the two set screws must be aligned with the flats on the motor shaft. The rotor support collar should be in contact with the shaft collar (diagram page 2). 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 rotors near the center hole.

THE FELT SEAL

The rotor support collar passes through a felt seal (diagram page 2) immediately 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 20 ml of acetone followed by 20 ml of dichloromethane. 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. The pump is more subject to wear and is predicted to be the first major component requiring replacement.

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TROUBLESHOOTING

PROBLEM	CAUSE	CURE
Irregular or eccentric solvent front and bands. Most evident with UV illumination.	1 Absorbent mixture prepared and poured too slowly, partly set before completion of pouring.	
	2 Absorbent layer not covered during setting, affected by drafts.	Cover with cardboard box.
	3 Uneven drying of absorbent.	Turn rotor during drying.
	4 Surface used for pouring and setting of absorbent suffers from vibration.	Test surface with beaker of water. Vibration forms visible waves.
	5 Inlet wick short or bent up.	Change or adjust wick (page 6-7).
	6 Incorrect material used for wick. Solvent flows down wick as a series of drops.	See Changing the wick page 7.
	7 Solvent mixture has components of extreme polarity, e.g. hexane-acetone. Solvent takes up moisture from the absorbent and separates into two phases. Darker spots of the polar separated phase will be visible at inner edge of the absorbent.	Use less polar solvents, e.g. hexane-ethyl acetate. Use dry acetone and well dried rotors.
	8 Vapor leaks due to dust at the edge of the Teflon part (either side) of the lid or damage to the corresponding recess in the main vessel.	Clean lid and recess with a moist paper towel. Carefully sand down damaged recess.
	9 Vapor pouring out of disconnected nitrogen inlet.	
	10 Home made glass rotors are not flat.	

PROBLEM	CAUSE	CURE
Broad bands. See also radial streaking, below.	1 Overloaded.	
	2 Clean-up solvent not removed completely.	
	3 Sample solution contains undissolved material in suspension.	Filter or centrifuge sample solution.
	4 Bands slope through thickness of layer (2 mm and 4 mm only). Separated bands may remerge later.	See Optimizing Recipes, page 32.
	5 Heat from motor disturbing equilibrium conditions.	Use less volatile solvents. Move the Chromatotron to a hood. or area with lower ambient temp. Use a fan to cool the motor.
Broad initial band.	Sample diluted by solvent in pump head.	Set pump drive belt to lower stroke rate and increase scale setting. Allow alternating segments of solvent and air to follow the sample through the pump.
Broad initial band. Bands may disappear.	Solvent flowing over surface of absorbent. Flow rate too high.	
Radial streaking of bands. Same appearance as broad bands when rotor is in motion.	1 Inner edge of the absorbent has become impervious by reaction with polar compounds. Impervious part may be visible as a series of dark spots.	Prepurify samples (page 9). Remove impervious part of the absorbent (page 9).
	2 Sample solution contains undissolved material in suspension.	Filter or centrifuge sample solution.
	3 Sudden increase in solvent density, e.g. from hexane to dichloromethane. Denser solvent streaks through the other solvent.	Avoid solvent changes to dichloromethane/chloroform or use a short solvent gradient.
Bands on 2 or 4 mm layers separate then remerge.	Bands slope through the thickness of the layer, i.e. the bands move at different rates on the top and bottom of the layer.	See Optimizing Recipes, page 32.

PROBLEM	CAUSE	CURE
Chromatotron does not separate mixture although separation is possible with regular TLC plates.	1 Rf too high. The Rf is sometimes higher in the Chromatotron than on regular TLC plates. Common problem with chloroform - methanol mixtures.	Use less polar solvent mixture.
	2 Equilibrium conditions in the Chromatotron versus non equilibrium conditions of regular TLC.	See page 9 for operation under non equilibrium conditions.
	3 Absorbent layer not fully dried before scraping. Rf too high.	Dry to constant weight.
Dark band at outer edge of absorbent.	Normal, not a problem. Solvent completely fills the absorbent pores at the outer edge.	
Dark band at inner edge of absorbent.	At high flow rates solvent completely fills the absorbent pores at inner edge. Not a problem but indicates flow rate is approaching maximum where solvent flows over surface.	
Lumpy absorbent mixture during pouring. Irregular surface after setting. Short concentric cracks may form. Samples will give non-circular bands.	1 Insufficient water used in recipe.	Add extra 5-10 % of water.
	2 Ineffective shaking before pouring.	Shake more vigorously. Check for air pockets, break up with a spatula.
	3 Rapid setting caused by traces of fully hydrated gypsum in the mixing jar.	
	4 Mixing and pouring of absorbent delayed too long. Mixture has partly set before pouring.	
Absorbent layer slides off or pieces flake off from outer edge.	1 Silica gel-gypsum has been manufactured with partly hydrated binder.	See appendix 1, page 45, for regeneration by heating.
	2 Ineffective cleaning of rotor before coating.	An abrasive household cleaner such as Ajax must be used.

PROBLEM	CAUSE	CURE
		Clean with oxidizing acid cleaning mixtures. Place two rotors in contact with the mixture between.
		Use oven drying of the absorbent at 70° in place of air drying. Oven dried layers are more firmly bound.
		Use glue-bound layers (page 29).
	3 Cleaned rotor has been contaminated before coating.	Dry cleaned rotors with paper towels only. Do not dry in an oven or with acetone. Coat rotor with absorbent within 1/2 hr of cleaning.
	4 Mixing jar contaminated with fully hydrated gypsum from previous pourings.	
	5 Ambient temperature too high.	Do not coat rotors when ambient temperature is high. Adhesion is significantly weaker when the setting takes place above 30°C (86°F).
	6 Absorbent layer dried in a strong draft.	Do not dry with a fan unless in an oven.
Cracked or very soft layers. Loose powdery surface.	1 Deficiency of binder.	
	2 Oven temperature too high.	Use 70°.
Scraper leaves part of layer unscraped.	1 Bench surface used for pouring is not level.	
	2 Metal disk of the coating arbor is not parallel to the rotor due to the presence of absorbent between them.	
	3 Insufficient absorbent - water mixture. The layer is too thin before scraping.	

PROBLEM	CAUSE	CURE
Short arcs gouged in absorbent during scraping.	Fibers or other foreign material in the absorbent.	Performance is not affected significantly by this defect.
Circles gouged in absorbent during scraping.	Scraper blade has rough edge.	Performance is not affected in any way.
UV absorbing compounds not detectable.	1 Acetone or other UV absorbing solvent present in the absorbent, solvent or sample.	Evaporate clean-up solvents from the absorbent 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 weak chromophore.	Use dichloromethane in place of chloroform.
	4 UV lamp too weak.	Remove filter from the lamp. Add extra phosphor (1-2% of the absorbent). See page 19 for zinc silicate phosphor.
	4 Sample size too small or chromophore weak.	Stop solvent flow and switch off Chromatotron. Observe bands on stationary rotor. Apply sample as one spot on the rotor (See Light Loading, page 13). Use 1/2 mm layers. See Chromatotron Parts List for corresponding blades.
Condensation on lid.	This is normal, not a problem. Absence of solvent 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 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.	Clear outlet with straightened paper clip.
	2 Polar solvents do not flow smoothly through Teflon tubing.	Remove output tube.

PROBLEM	CAUSE	CURE
Fluffy particles in solvent collection channel. May block output hole.	Dust from rotors stored in the open.	Blow off dust before mounting rotors.
Solvent flow ceases or is reduced to low rate. Pump may produce unusual noises.	1 Motor-pump drive belt too loose. Belt may become loose after pump warms up or after several months of use.	Loosen the two screws holding the motor and adjust motor position to tighten belt. At 150 strokes/min belt tension is less critical. Replace belt with an ordinary rubber band (3 mm).
	2 Drive belt too tight. Belt vibrates.	Adjust motor position.
	3 Cotton filter clogged.	Replace cotton. Do not use glass wool.
	4 Cotton packed too tightly in filter.	
	5 Vapor lock in pump.	Raise the solvent reservoir higher. Change belt drive in pump to decrease pump stroke rate. Increase setting on flow rate scale.
	6 Blockage in tubing or inlet.	Disconnect tubing and inlet. Check each part separately.
Solvent leaks from pump or tube fittings.	1 Blockage in inlet or tubing on output side of pump causing increased pressure.	Disconnect tubing and inlet. Check for blockages. Do not tighten fittings with tools! Hand tighten only.
	2 Flared ends of tubing damaged.	
Pump does not start.		Unplug pump, remove cover (loosen thumbscrew at rear) and check that motor turns freely. Check drive belt tension.
Solvent inlet becomes tight.	1 Inlet has swelled through absorption of solvent.	Allow inlet to dry out for 24 hr.

PROBLEM	CAUSE	CURE
	2 Deposits on threads.	Clean with solvents or scrape off deposits. Rethread with 5/16"-18 tap and die.
Particles of absorbent on Teflon lid.	Absorbent dust not completely removed from rotor after scraping.	Blow off loose absorbent after scraping. Wipe lid with moist paper towel or wash with stream of water.
Chromatotron oscillates from side to side.	1 Rotor unbalanced due to evaporation of solvent from part of rotor.	Evaporate solvent from rotor with nitrogen before switching off. Remove rotor and allow solvent to evaporate.
	2 Unbalanced home-made rotor.	
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 alumina to chloroform. Test with wet pH paper.
Chromatotron produces a rubbing sound which 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.
Rotor produces tapping sound on each revolution.	Damaged threads preventing alignment of endplate with rotor.	Sand threads of endplate with emery cloth. Rethread motor shaft with 1/4"-20 tap.
Motor does not start.	1 Rotor jammed.	
	2 Rotor support collar in contact with main vessel.	See Adjusting The Main vessel, page 33.
	3 Loose electrical connections in the junction box on the motor.	
	4 Defective motor capacitor.	

PROBLEM	CAUSE	CURE
Motor has insufficient torque. Start up is slow. May run at half speed.	Low supply voltage.	Use motor capacitor 1 MFD larger than specified. Increase supply voltage to specified value with variable transformer.
Shoe horn lost.		Use endplate key or a small hex key.
Endplate key lost.		Use a small hex key.

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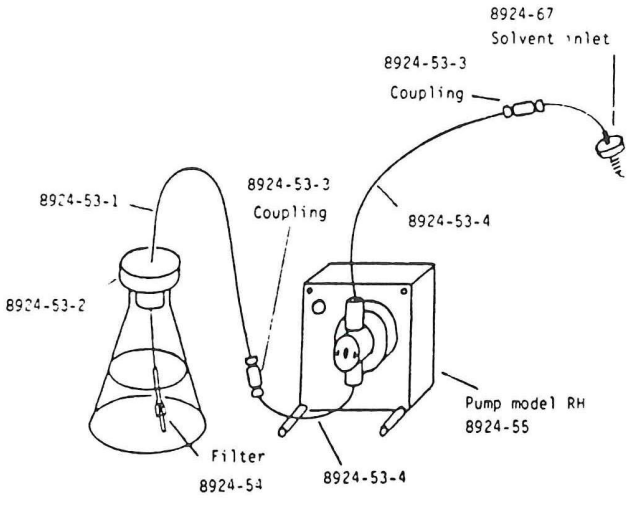
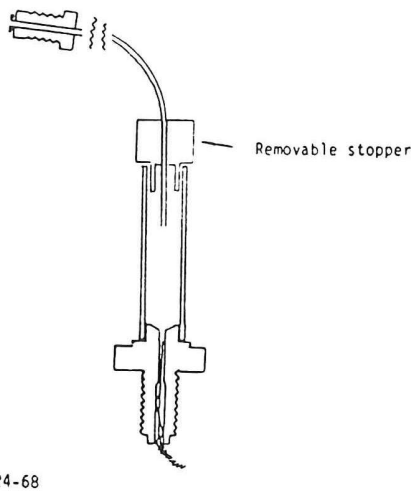
CHROMATOTRON PARTS LIST

Part Number	Description
8924-03	Instruction manual for model 8924 Chromatotron
8924-06	Glass rotor
8924-070	Scraping tool
8924-071	Scraper blade, 1 mm
8924-072	Scraper blade, 2 mm
8924-074	Scraper blade, 4 mm
8924-07-X	Scraper blade, X mm Give required X in range 0.3 to 4.0
8924-079	Finishing scraper blade
8924-16	Coating arbor
8924-19	Collar endplate (with Teflon washer)
8924-24	Teflon washer for collar endplate
8924-34	Key for endplate
8924-36	Output tube
8924-40	Shoe horn
8924-48	Lid retaining clips, set of 8
8924-53-1	Tubing with one end fitting for connection between filter and coupling. See diagram, next page.
8924-53-2	Stopper. See diagram, next page.

8924-53-3	Coupling. Two required. See diagram below.
8924-53-4	Tubing with fittings for connection between pump and coupling. Two required. See diagram below.
8924-54	Solvent filter
8924-55	Solvent pump model RH (specify voltage and frequency). Includes solvent inlet, tubing with end fittings, couplings, stopper and filter. This is a modified Fluid Metering Inc. pump, not available directly from FMI.
8924-65	Wick and 2 wire wick holders
8924-67	Solvent inlet
8924-68	Solvent inlet for direct introduction of sample, by-passing pump. See diagram below.
8924-90	Gilmont flowmeter, 10-2100 ml/min, for nitrogen
8924-92	Teflon lid with solvent inlet
8924-93	Replacement Teflon sheet for Teflon lid





8924-68
Solvent inlet for direct introduction of sample, by-passing the pump.

APPENDIX 1

REGENERATION OF SILICA GEL - GYPSUM

Some batches of silica gel PF with calcium sulfate do not bind well to glass rotors. This is probably caused by the binder (partly hydrated) and by organic impurities. To regenerate, heat at about 160° for 3 hr. Place the absorbent in a dish or tray, preferably metal, to a depth of not more than 7 cm and cover loosely with aluminum foil. During the cooling, press the foil on tightly to keep out moisture.

If the absorbent is weighed out after regeneration then reduce the quantity in the recipe by about 3% to allow for the loss in weight during the heating. The mixture will be too viscous to mix well if this allowance is not made.

Absorbent layers formed from regenerated silica gel PF are harder and more firmly attached to the glass rotor. Resolving power is also improved.

Regenerated silica gel PF gains about 10% in weight when exposed to air for 2 weeks but still binds well to glass.

APPENDIX 2

CELLULOSE LAYERS

Rotors can be coated with cellulose but the performance of the layers is not satisfactory with aqueous solvents. Bands of compounds become non-circular and streak as the water content of the solvent is increased. The procedure given below is not necessarily optimum.

Procedure for 1mm layers. Set up a rotor as in the procedure for glue-bound layers, pages 29-30. In a wide mouth jar disperse 2 g of Elmer's Glue-all or clear Polyseamseal (the latter is recommended, see page 29 for further information) in 106 ml of water by stirring, add 25 g of Sigmacell Type 50 (Sigma Chemical Company, cat. no. s5504). Stir well with a spatula and allow to stand for 30 min with occasional stirring and bumping to encourage bubbles to rise. Cover with aluminum foil without removing the spatula. Add a further 6 g of Sigmacell in portions of about 1 g during 30 min with stirring and bumping. The mixture becomes creamy as the final portions are added and bubbles rise less readily. After a further 1 hr or more with stirring and bumping at intervals, pour the mixture in the usual way and allow the coated rotor to air dry without rotation. A 60 watt lamp 20 cm above the center of the layer will shorten the drying time.

After air drying is complete, heat the rotor in an oven at 70° then raise the temperature to 100° for 1 hr. Cool slowly to avoid cracking the glass.

A crust which forms on the layer will interfere with scraping. Stop the scraping process to remove pieces of loose crust trapped under the blade. Cracking of the layer during the drying will be less of a problem if the quantity of glue in the recipe is increased but scraping then becomes more difficult.

Before use, the scraped layer must be washed with at least 120 ml of acetone to remove large amounts of unpolymerized glue.

APPENDIX 3

THE TEST MIXTURE

A mixture of high Rf colored compounds is useful for testing absorbent layers. "The test mixture" refers to a mixture of the 2,4-dinitrophenyl-hydrazones 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 Rf impurities that remain on the absorbent.

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