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CENTRIFUGAL THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Centrifugal Thin Layer Chromatography is preparative chromatographic technique which make use of centrifugal force for separation of multi-component system. A typical instrument used in this technique is named as Chromatotron. The Chromatotron is a preparative, centrifugally accelerated, radial, thin-layer chromatograph. The sample to be separated is applied, as a solution, near the center of a spinning disk coated with a thin layer of sorbent. Elution by solvent forms circular bands of the separated components which are spun off from the edge of the rotor together with solvent. A novel collection system brings the eluate to a single output tube. The Cyclograph system is a centrifugally accelerated device for performing preparative chromatographic separations. The device spins a layer of adsorbent material coated as a flat ring on a glass backing. The stationary phase for the cyclograph instrument (a rotor) which is similar to a preparative TLC plate but it is round instead of rectangular. Centrifugal chromatography method can be applied in two versions: the linear and radial versions. In the radial version of the centrifugal TLC, the system chromatographer rotates about its axis so that the movement of the mobile phase is accelerated by centrifugal forces. The Cyclograph centrifugal chromatography system combines the advantages of both preparative TLC and Column Chromatography. It delivers fast, efficient separations. Most separations occur in twenty minutes or less. Fast separations are a result of the centrifugal action of the spinning Rotor driving the mobile phase through the adsorbent layer.

Keywords: - Chromatotron, Cyclograph.

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INTRODUCTION

Chromatography [1]

Chromatography is the collective term for a set of laboratory techniques for the separation of mixtures. The mixture is dissolved in a fluid called the "mobile phase", which carries it through a structure holding another material called the "stationary phase". The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus changing the separation.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for further use (and is thus a form of purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytes in a mixture. The two are not

mutually exclusive. Chemical integrity of sensitive sample components can be preserved even with the use of active silica gel adsorbents.

CENTRIFUGAL THIN LAYER CHROMATOGRAPHY [2]

The Chromatotron

The Chromatotron is a preparative, centrifugally accelerated, radial, thin-layer chromatograph. It replaces preparative TLC plates, small columns and HPLC. Overall dimensions are 30x35x30 cm.

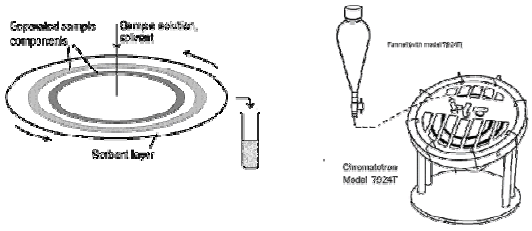

Figure 1: The Chromatotron

Principle of Operation

The sample to be separated is applied, as a solution, near the center of a spinning disk coated with a thin layer of sorbent. Elution by solvent forms circular bands of the separated components which are spun off from the edge of the rotor together with solvent. A novel collection system brings the eluate to a single output tube.

Capacity:	Upto 500 mg per component, 1-2 g total, e.g. dimethyl and diethyl phthalate in hexane – ethyl acetate 9:1 on a 4 mm layer of silica gel.
Sorbents:	Silica gel, alumina and silica gel – silver nitrate; not useful with RP sorbents.
Solvents:	Compatible with all chromatography solvents, including acetic acid; not suitable for use with mineral acids.

Special Advantages:

- No "spotting" of samples or scraping of bands.
- Separations are completed rapidly, typically within 20 min.
- A UV transparent lid allows direct observation of UV absorbing or colored compounds during the separation.
- Layer thickness of 1, 2, 4 or 8 mm gives high capacity. The sorbent layer is easily regenerated *in situ* for reuse.
- Solvents are used sparingly. Gradient elution is easy. A nitrogen atmosphere prevents oxidation of samples.
- Compact (easily moved from lab to lab), few controls, no high pressures.
- Low price. Half a dozen Chromatotrons cost less than a single prep HPLC.
- Compounds without a chromophore are detected in the eluted fractions by conventional TLC.
- Connection to refractive index and UV monitors is not recommended.
- May be used with a fraction collector although hand collection is adequate.

Cyclograph: Centrifugal Chromatography System [3]

Features

- Variable speed motor (100-1400 RPM).
- Integrated 4-watt UV lamp for hands-free viewing of the sample.
- On/Off switches for the motor and UV lamp.

- Adjustable plane angle setting of vessel(0-30 degrees).
- Ultra quiet & accurate low flow solvent pump (0-15ml/min).
- Compatible with all common chromatography solvents including acetic acid.
- Easily detachable chamber for submersible cleaning.

Cyclograph

The Cyclograph system is a centrifugally accelerated device for performing preparative chromatographic separations. The device spins a layer of adsorbent material coated as a flat ring on a glass backing. A solvent pump is used to apply the sample and mobile phase to the center of the spinning adsorbent ring. The centrifugal action accelerates the flow of the mobile phase through the adsorbent, separating the sample components as circular bands.

The mobile phase elutes continuously into a specially shaped collection channel inside the body of the instrument. Component bands are collected manually in test tubes or optionally by an automated fraction collector (not included). Separations occur quickly, usually within 20 minutes, versus the typical 90 to 120 minutes for preparative Thin Layer Chromatography (TLC) or Column Chromatography.

Principle of Operation

The stationary phase for the cyclograph instrument is called a rotor. A rotor is similar to a preparative TLC plate but it is round instead of rectangular. The round glass support of a rotor has a hole in the center so that it can be mounted on the motor of the cyclograph. The motor will spin the rotor at 720 RPM. The adsorbent material of the Rotor is coated as a ring that does not extend into the center hole of the glass. This clear zone of glass at the center is where the sample and mobile phase will be applied. The sample is applied first as a solution by way of the solvent pump or a hand held syringe. The spinning action of the rotor will force the sample liquid to flow as a uniform round band on the inner edge of the adsorbent ring. The solvent pump is then switched to the mobile phase chosen to separate the components of the sample. The spinning action of the rotor, and to a lesser degree the pump flow rate, provides the motive force of the mobile phase. The sample components will migrate through the Rotor adsorbent as circular bands and will have differing affinities for the adsorbent versus the mobile phase. This causes them to migrate at different rates affecting separation. The circular bands eventually migrate to the outer edge of the adsorbent ring. The flowing mobile phase stream containing the component bands is ejected from the edge of the Rotor much like water from a rotating wet tire. A specially shaped collection channel around the outer perimeter of the Rotor collects the eluting mobile phase. The entire collection vessel is angled so that gravity drains the eluent to the exit port where it can be collected. The design of the vessel preserves the integrity of the sample bands so as to prevent remixing of the sample component bands.

Factors Affecting [4]

Centrifugal chromatography, first proposed as a variant of paper chromatography, was subsequently used in TLC[5]. This method is applied in two versions: the linear and radial versions. In the radial or circular version of the centrifugal TLC, the system chromatographer rotates about its axis so that the movement of the mobile phase is accelerated by centrifugal forces, as in the linear version. In the radial centrifugal TLC, the mobile phase is supplied to the centre of a circular sorption

system. The flow rate of the mobile phase can be regulated by means of a micropump. According to the authors[6], centrifugal forces play the same role as pressure in HPLC. In linear centrifugal TLC, the quantity equivalent to pressure P in HPLC can be expressed by the relation[6]: -

$$P = \rho\omega^2 Z_f / 2 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, ρ is the density of the mobile phase, ω the angular velocity of the plate, and Z_f the distance from the point of supply to the front of the mobile phase.

On the basis of the foregoing, centrifugal TLC can be classified as a TLC method with a controlled forced flow. The flow is stabilized by employing continuous TLC conditions and the rate of elution is regulated by the rate of rotation of the support. We may note that the rate of elution is directly proportional to the square of the angular velocity[6]. A detailed critical review on centrifugal chromatography[7], written in 1964, has not lost its importance today. The authors[7] believe that the economy of time in the elution of thin-layer chromatograms by centrifugal TLC is not very great. It is impossible to agree fully with this view. For very long separations, this method is undoubtedly economically justified and it has continued to be used in analytical practice in recent years: the reversed phase centrifugal TLC has been used successfully for the separation of polar compounds of plant origin and the ion-exchange version of centrifugal TLC has been employed for the determination of Fe, Co, Ni, Cu, Hg, and other metals. Different aspects of the application of centrifugal TLC have been discussed in a number of studies[8]. The possibilities of centrifugal TLC for preparative separation have been demonstrated by Stahl and Muller [9], who showed that the specific sample charge in this method can amount to between 10 and 1000 mg per millimeter of the thickness of the absorption layer for one compound. The results of a detailed study are of interest not only for preparative but also for analytical chromatography. Fig.2[9] illustrates the dependence of the separation time on the overall flow rate of the mobile phase. It follows from the data presented that an increase in the flow rate of the mobile phase leads to a significant shortening of the chromatographic process, which is particularly pronounced in the region of low rates. Fig. 3[9] presents the dependence of the separation factors for hydrazones (dyes) on the volume rate of supply of the mobile phase and the rate of rotation of the chromatographic system. The maximum resolution for the average flow rate of the mobile phase (2—3.5 ml min^{-1}) was obtained in the region of relatively low rates of rotation of the chromatographic system (~750 rev min^{-1}). In comparing preparative centrifugal TLC with traditional preparative TLC and column chromatography, the authors[9] arrived at the justified conclusion that this method has definite advantages.

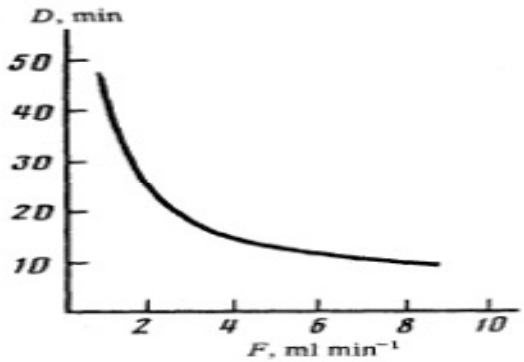

Figure 2. Dependence of the separation time *D* on the volume flow rate of the mobile phase F; experimental conditions: stationary phase—silica gel with 1.5% of acronal, mobile phase—80: 20 hexane—ethyl acetate mixture, rate of rotation 750 rev/ min [9]

Attention should be drawn to the recent appearance of several new methods and variants of apparatus involving centrifugal TLC[10]. A new method of planar preparative chromatography is sequential centrifugal layer chromatography (SCLC)[10]. According to the authors[10], it combines the advantages of preparative and analytical centrifugal, anti radial, and sequential TLC.

The apparatus for SCLC makes it possible to carry out circular chromatography with centrifugation at different rates of rotation. The supply of the eluent to any point on the plate makes it possible to separate additionally the individual components using individual mobile phases. Furthermore, there is a possibility of carrying out anti radial chromatography under the influence of capillary forces. Thus the SCLC method is in fact a hybrid procedure which expands the possibilities of centrifugal TLC. A new centrifugal countercurrent chromatograph for partition chromatography has been proposed by Japanese investigators[11].

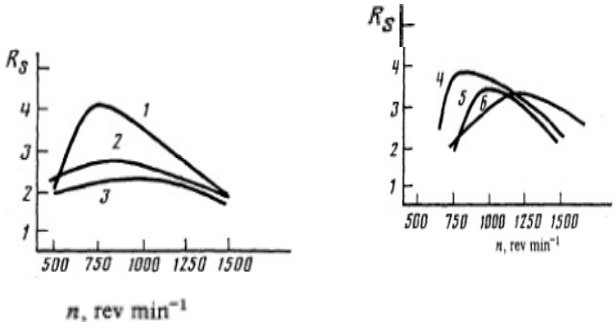

Figure 3. Dependence of the separation factor *Rs* for hydrazone dyes on the rate of rotation of the chromatographic system n ; experimental conditions: stationary phase—silica gel 60 with 1.5% of acronal, mobile phase—80: 20 hexane-ethyl acetate mixture, volume rate of supply of mobile phase to the chromatographic system (ml/ min1):
1) 3.5; 2) 2.1; 3) 1.1; 4) 5.2; 5) 7.1; 6) 8.8.

A rapid preparative separation of a series of mixtures of amino acids, sugars, and saturated aliphatic acids has been achieved with its aid. The possibilities of centrifugal TLC for a significant increase of the flow rate of the mobile phase in HPTLC have been demonstrated. It has been shown that the results obtained in three-minute separation with centrifugation are equivalent to 12-minute separation with the aid of the usual circular TLC. The method of centrifugal paper chromatography, its possibilities, and the prospects for its development have been considered in a review. The possibilities of obtaining high flow rates of the mobile phase in centrifugal chromatography are limited. It is believed that the use of rates of rotation at which pressures above 1 atm are created [see Eqn. (1)] would cause the rupture of the column of solvent and the mixing of air with the solvent at the point of its introduction. Yet another limitation is associated with the application of an open layer of sorbent in centrifugal chromatography: the increase in the resistance of the layer of sorbent with increase in the rate of elution can lead to the "spilling" of the eluent through the sorbent. This is in fact why centrifugal chromatography does not permit the attainment of the same high flow rates of the mobile phase as in HPLC.

Advantages [12]

1) The Cyclograph centrifugal chromatography system combines the advantages of both preparative TLC and Column Chromatography. It delivers fast, efficient separations. Most separations occur in twenty minutes or less.

2) Fast separations are a result of the centrifugal action of the spinning Rotor driving the mobile phase through the adsorbent layer. The velocity of the mobile phase enables the use of smaller particle adsorbents. The Rotors for the Cyclograph use a 15 micron average particle adsorbent bed, similar to TLC. This smaller particle bed versus the 35-75 micron cut typical of low pressure column

chromatography allows a higher degree of separation efficiency.

3) Centrifugal action combined with the use of a solvent pump to apply the mobile phase allows complete control of solvent velocity profile. Typical flow rates are in the range of approximately 2 - 3 ml/min per millimeter of adsorbent thickness. Tight bands mean eluted fractions can be collected in a minimum volume of solvent.

4) The solvent metering pump also makes the use of step gradients easy to perform with the Cyclograph. The inlet of the pump may be switched from a weak solvent to a more polar solvent during a run. This enables compounds with a wide range of polarities to be separated. Complex samples such as natural products are quickly separated into component classes.

5) Sweeping the polarity of the mobile phase from weak to strong makes it easy to reuse a Rotor for many sample separations. By concluding the run with a strong solvent, otherwise strongly adsorbed compounds will be flushed from the Rotor. This enables a Rotor to be reused for dozens of samples.

6) Separations on the Cyclograph scale easily and quickly. Rotors are available from 1 mm thick up to 8 mm thickness. For difficult cases, eluted components that are only partially resolved, can be cycled back onto the spinning Rotor. This effectively allows the chromatographer to increase "the length of the column" without the associated disadvantages.

7) Separations on the Cyclograph can be monitored as they happen with the built in UV254 lamp. Separated substances which absorb 254 nm light will be visible against the fluorescent green background of the Rotor. Optionally a UV366 source may be used to detect intrinsically fluorescent components.

8) Chemical integrity of sensitive sample components can be preserved even with the use of active silica gel adsorbents. Unlike TLC where the sample solvent is evaporated before chromatography, the sample introduced on Cyclograph need never be exposed to dry adsorbent. This minimizes the possibility of reaction of sample components on the active adsorbent surface. Further control of the atmosphere is possible by flushing the vessel with nitrogen gas during chromatography.

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