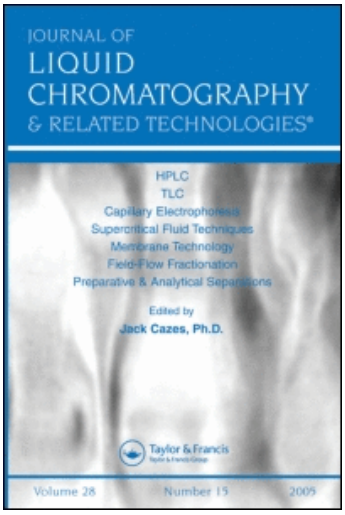

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Rod Chromatography—A New Approach to Automating TLC

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ROD CHROMATOGRAPHY- A NEW APPROACH TO AUTOMATING TLC

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ABSTRACT

A new technique for separating and quantifying materials using thin layer chromatography techniques is described. In this method the separation is done using quartz rods coated with adsorbing materials. Coating material selection and application uniformity are critical to the process.

Rods are spotted at one end with a solution of the unknown and dried. Development is similar to that used for TLC plates. Following development the rods are scanned in an apparatus designed expressly for the purpose which utilizes flame ionization detection. The output from the FID is amplified and integrated. A two pen recorder is used to record the resulting chromatographic and the integration curves.

Examples of applications are given in which such varied materials as olive oil, plasma lipids and surface active agents

are analyzed. Descriptions of unique and interesting practical applications are given for finger printing, crude oils and the forensic analysis of dyes.

INTRODUCTION

While rod chromatography has been known as a laboratory possibility ever since its discovery and patenting by Unilever in England, it was not until an instrument was built by Iatron Laboratories in Japan that it became a practical reality. This is due to the fact that a carefully designed and constructed mechanism is necessary to carry out the sequential steps in preparing, developing and scanning a rod without considerable time and careful manipulation.

Rod chromatography is relatively unknown in the U.S. but has been well developed in Japan, Europe and Canada. Since many may not be familiar with the technique, this paper will commence with an explanation of the principles and sequence of events involved in carrying out a thin layer chromatography/flame ionization detector analysis (TLC/FID) on a rod.

PRINCIPLES OF OPERATION

Separation in chromatography depends on selective adsorption on some medium. The medium may be a column or coated on another substrate and still adsorb selectively. Thus, plates of glass or

plastic film coated with silica or alumina separate dissimilar components over two dimensions.

Detecting the quantitive presence of the various components is a commonly desired end. A developed TLC plate may be read by a scanning densitometer and some quantitation achieved. A generally accepted quantifying method for other separation techniques such as GC has been a flame ionization detection. How to apply this to a plate separation is a design enigma. Lacking a flame that could scan a plate, the inventor of rod chromatography conceptualized a plate dimensioned to fit a flame and, thus, derived the TLC coated rod.

The basic technique is to use a rod coated with an adsorbing material that will withstand a hydrogen flame briefly. The construction decided upon was a quartz rod 0.9 mm in diameter and 155 mm long. The rod is coated with a 75 micron thick layer of a mixture of powdered glass and alumina or silica and the mixture fired onto the rod at 900^{o}C. About 5 mm are left uncoated on each end for handling.

In use, the sample to be separated is spotted onto the rod about 2 cm from one end using 0.1 to 3 ul of volume containing 5-10 ug of sample. Any solvent that will evaporate at temperatures that do not disturb the sample may be used as the sample carrier. The end of the rod bearing the sample is immersed in the developing solvent in a closed chamber to a depth of about 1.5 cm

Elution is carried out over 15 to 45 minutes, depending on the mobile phase used.

The rod is then dried until free of the developer solvent then passed through a hydrogen flame and scanned from top to bottom at a pre-selected speed. The combustion products are detected and a signal generated proportional to concentration as each eluted spot passes through the flame. Good linearity is found in the 3 to 30 ug sample weight range. The FID was chosen because it detects percent by weight of carbon. Greater accuracy or sensitivity is possible than with optical scanning of spots along with avoidance of problems of color development, wavelength selection for UV scanning or derivatization to generate fluorscence.

Thus, the steps are sample dissolution, sample spotting on a rod, solvent development, drying, flame scanning, curve plotting and peak integration. The tools needed are suitable rods, a suitable spotting method, a uniform and variable speed scanning set-up, an FID and a signal recorder.

Reducing this combination of operations for use as a routine method required the design and construction of a carefully specified and constructed instrument. This was the part contributed by Iatron Laboratories in Japan. Thus, we have, in effect, a black box that takes over after the rods are spotted, developed and dried and does everything else automatically. The rods are an item of supply also produced by Iatron and are, of

course, the heart of the system, and therefore, are carefully made and pretested. A rod is good for up to 300 scans and can therefore be looked upon as reusable. Only an accident or use of a compound that will not burn off without leaving a residue that cumulatively plugs the chromatographic surfaces limits its life. Such things are rare and are usually complex mixtures that perhaps should have been cleaned before analyzing.

INSTRUMENT DESIGN DETAILS

The device used is called the Iatroscan TH-10 and consists of the parts shown schematically in Fig. 1.

The rods are handled ten at a time in a rack for spotting, developing, drying and scanning thus avoiding finger contact with the rods. The rack is driven over a stationary hydrogen flame above which there is a collector electrode,the positive pole of the FID, while the burner sleeve is the negative pole. The rack of rods in indexed sideways from rod-to-rod and returns to the top of each rod while the flame is located between adjacent rods. The FID signal is amplified and fed to a recorder. An integrater is built into the instrument, thus, a two pen recorder is used giving the curves and their integration as a vertical distance plot between horizontal moves from peak-to-peak.

The instrument contains a variety of other features such as automatic zeroing between curves, an air curtain around the hydrogen flame to exclude air borne particles, flow controls for

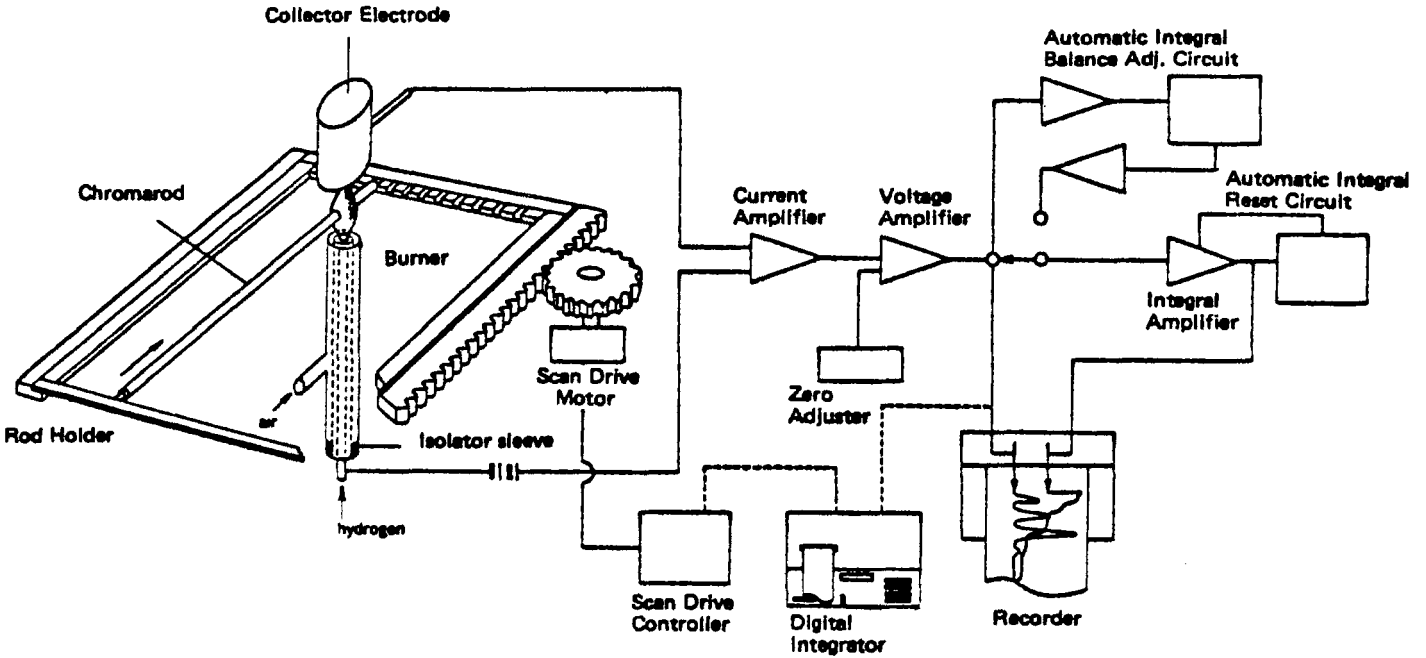


Figure 1

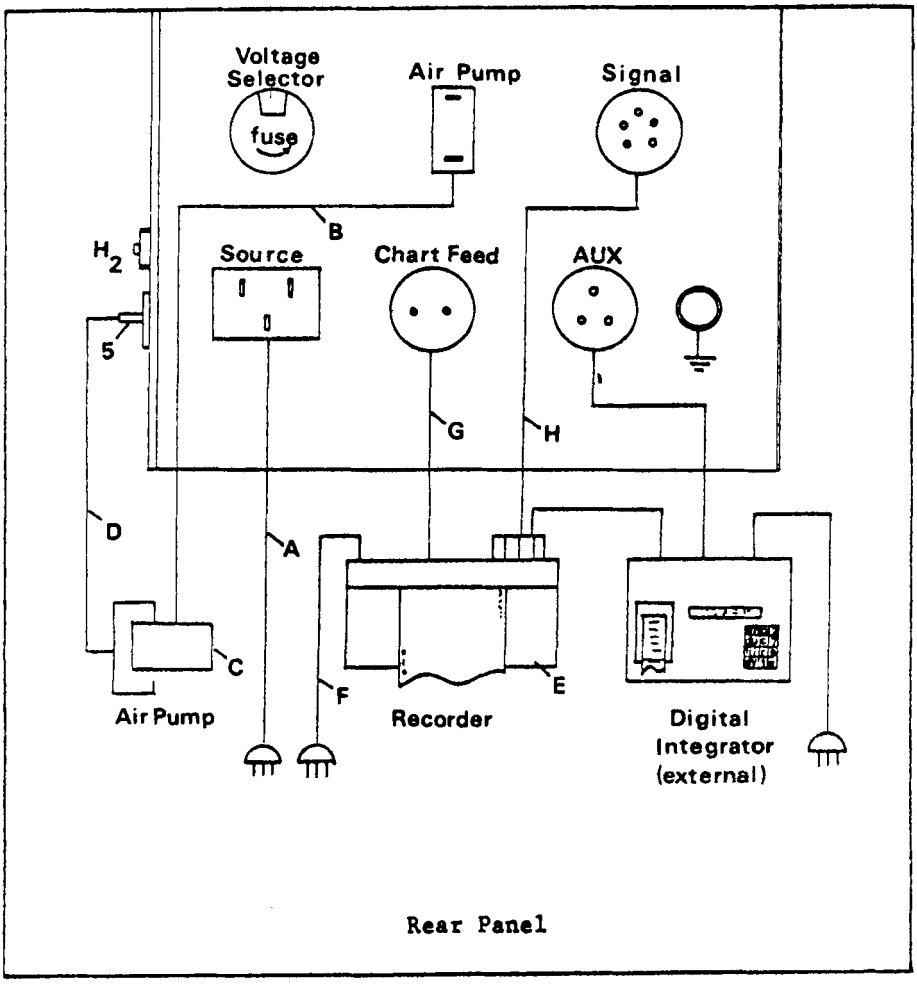


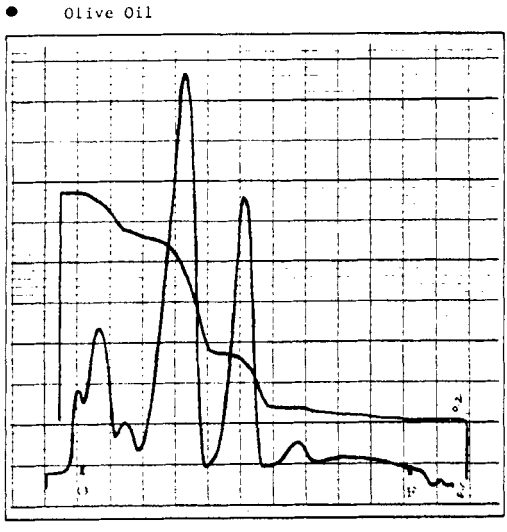
Figure 2

air and hydrogen, signal outputs for remote recording and a chart paper drive to shut off the strip chart recorder paper drive between rods or when a rack of rods is completely scanned. The layout of these features on the back of the instrument is shown in Fig. 2.

APPLICATIONS OF ROD CHROMATOGRAPHY

There have been literally hundreds of curves run successfully using compounds of the widest variety. A number of things that have been done using this technique that eluded TLC and HPLC methodology. However, since they were specialized areas they are simply noted in the references. A series of examples of applications in diverse areas but those of fairly broad interest are given below.

A great deal has been done with natural oils since it is often difficult to separate them and to identify the component compounds. Since a great deal of this work was done in Japan it is not surprising that a great emphasis was placed initially on seed and other food oils, especially those related to products of the sea. Thus, methods (Fig. 3) have been worked out and numerous analyses done on olive oil, coconut oils, sesame oil, peanut oil, shark liver oil, rape oil, soybean oil and safflower oil. The next most active area has been in the analysis of lipids including neutral and phosolipids and has included both those occuring in human systems and other forms of life. Some very interesting research has been done in analyzing complex lipid mixtures from both serum and tissue lipid sources by Shishido at Nipon Roche in Japan (4) and Sipos and Ackman at the Fisheries and Marine Service in Halifax (24). One interesting sideline in the latter group's work was an experiment where a single



SAMPLE:
① Olive Oil

CONDITIONS:
Stationary Phase:
CHROMAROD-S
$AgNo_3$
Impregnated
Mobile Phase:
Benzene: Ethyl Ether
97 : 3
Gas Flow: H_2 160ml/min
Air 2000ml/min
Scanning speed: 32sec/scan
Chart speed: 240mm/min

Figure 3

indistinct spot was removed from a TLC plate following plate separation of fluid from a sea mussel. This was applied to a rod and eluted along it into three distinct peaks that were identified., one being a hydrocarbon. Finding the later was a vital point since the research was directed at whether or not mollusks ingest oil from oil spills and it seems they do.

It was in this work that it was learned that rod life could be extended by storage in chromic acid cleaning solution. The normal recommended storage in a humid atmosphere gave a shorter rod life with these complex materials that tended to plug the rod surfaces even after flaming. The cleaning solution overcame this problem.

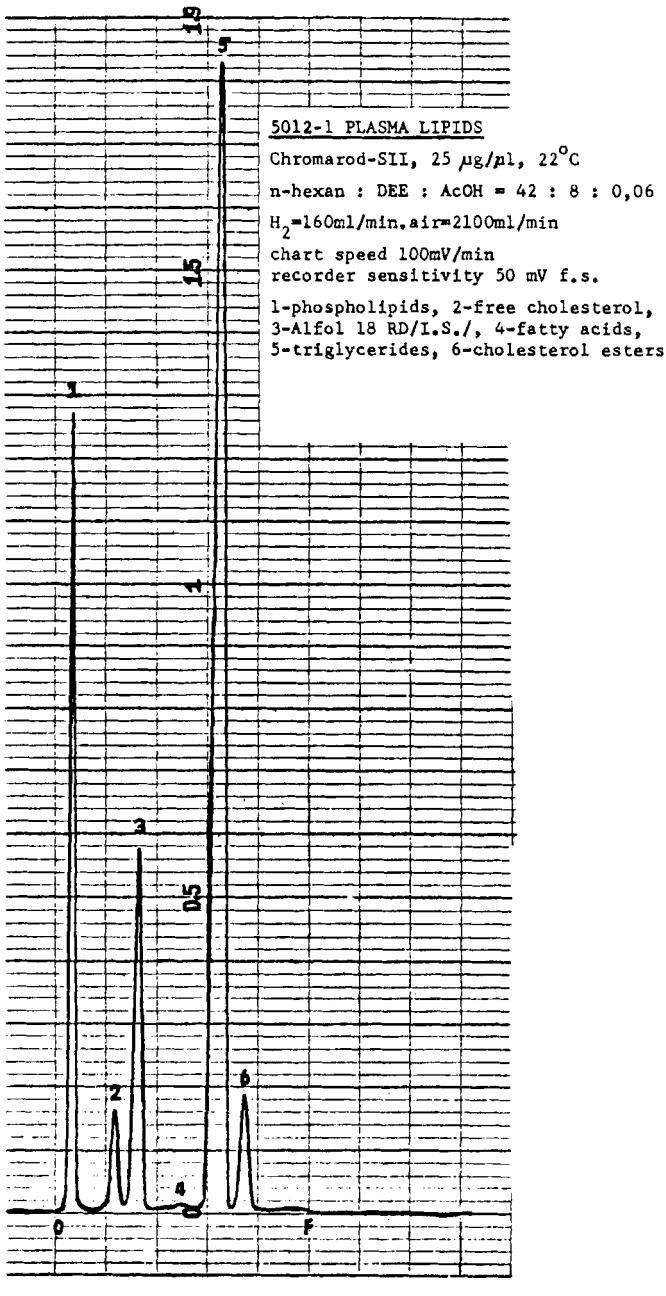


Figure 4

The work in Japan with serum (6)is shown is Fig. 4. This provided an excellent opportunity to evaluate the response in a natural mixture to the spotting quantity on the rod.

A variety of plasma lipid samples were used from patients with various types of hyperlipidema. The amount of of material in the spot is plotted against the cell volume. A mean is drawn in and it shows that the volume percent shoots up sharply below about 0.5 microgram. See Fig. 5.

Much has been done with pharmaceutical compounds (6) on the TH-10. Results have been published in which analyses have been done on psychotropic drugs in which studies were made to relate dosage size with blood content at time intervals. Other analyses have included quantitation of the active component in natural medicants such as saponins from ginseng root and glycyrrhizin from licorice root.

Leaving natural substances, an area with surprising success has been polymer separation and molecular weight distribution determinations. Also, chain length separation is readily accomplished from mixed alkyls used in making synthetic detergents. Following is an example of a polyoxyethylenenonyl phenyl ether. Note the closeness and sharpness of the peaks. It should be noted here that a strip chart recorder with fast response is necessary to catch all the peaks. The common response time of 1/2 second for recorders used in such analyses

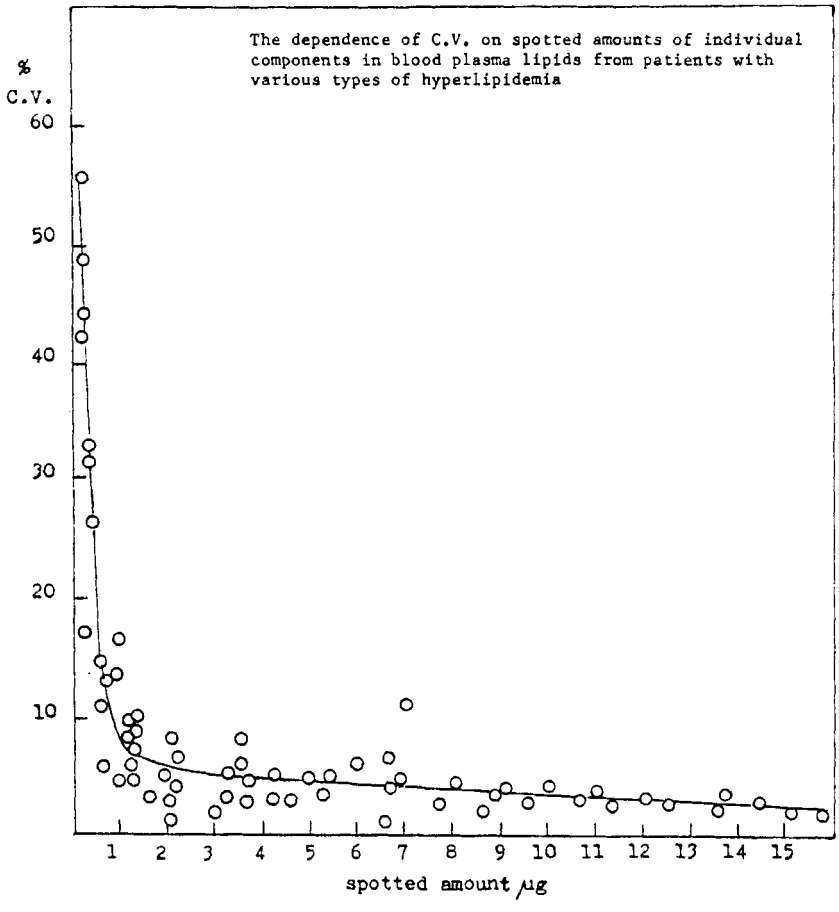
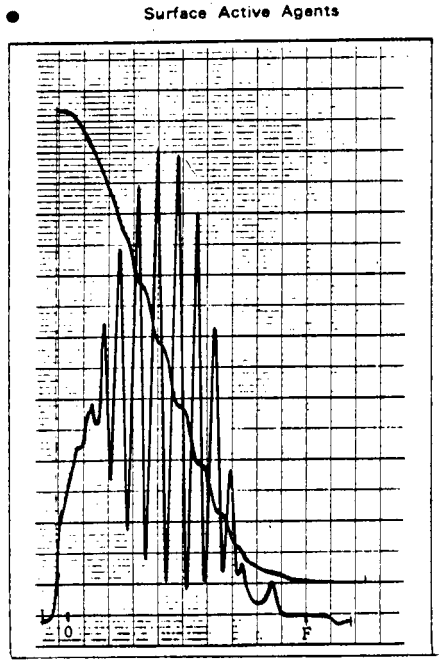


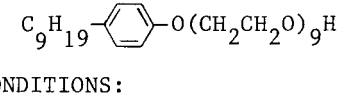
Figure 5

may not be acceptable and a recorder responding full scale in 1/4 second is required. This is shown in Fig. 6.

This description would not be complete without mentioning that rod chromatography, although a unique and independently useful tool, will inevitably be compared with TLC, HPTLC and HPLC. It cannot be claimed at least as yet that it will replace



SAMPLE:
polyoxyethylenenonylphenyl-ether
(EO = 9 added molecules)



CONDITIONS:
Stationary phase:
CHROMAROD-SII
Mobile phase:
Ethyl Acetate: Acetone: Water
70 : 20 : 4
Gas Flow: H_2 160ml/min
Air 2.0l/min
Scanning speed: 32sec/scan
Chart speed: 240mm/min

Figure 6

them. It does appear quicker, easier to use, more sensitive and less costly to use repetitively in certain applications. A comparison is made among the plate methods in the Fig. 7. Its limitations preclude the use of volatile compounds which could be handled readily in GC or HPLC and in materials that are substantive to the rod material and develop very low R_f values such as aflatoxins. There are, however, very few such compounds.

One very interesting example of a practical usage that is a forensic application. In detecting forgeries, analysis of ink is often required. The ink may be present as only a short line or

as a dot, such as in a figure or decimal alteration. A figure that had been written as a straight line figure one was believed altered into a seven by addition of a cross bar. To the eye the seven appears as an authetic figure. Since the chances are extremely remote that the same ink would have been used in making the cross bar as used in making the vertical line, an analysis of the inks was desired.

A spot from each line was cut from the paper using a square end syringe needle. This spot was placed in a chloroform and methanol mixture. The resulting ink solution was spotted on the rod then developed in a methanol-hexane mixture. The resulting elutions were scannned and two very dissimlar sets of curves

COMPARISON OF TLC (1) HPTLC (2) AND RTLC

	TLC	HPTLC	RTLC
Plate Size	20 x 20 cm	10 x 10 cm	1 mm x 155 mm
Sample Volume (capiliary application)	1 - 5 µL	0.1-0.2 µL	0.1-3 µL
Diameter of spots	3 -6 mm	1.0 mm	1 -3 mm
Diameter of separated spots	6-15 mm	2-5 mm	N/A*
Solvent migration	10-15 mm	3-6 mm	N/A*
Detection limits	-	-	
Flame Ionization			<1 ng
Absorption	~5 ng	~0.5 ng	
Fluorescence	~0.1 ng	~.01 ng	

(1) ANALYTICAL CHEMISTRY, Vol. 53, No. 2, p.254A, February 1981

(2) From Hezel, U.B. (3)

* Not Applicable

Figure 7

resulted. In this case the differences could be seen qualitatively by eye but the scan quantifies the components and produces curves that can be used as hard evidence. Such curves in the hands of an ink chemist can result in the identification of the ink by manufacturer and by the type applications such as ball point, fountain pen, or office machine ribbon. Thus, pen alteration of office machine produced records can be distinguished, however skilled the forger. Such applications are limited only by the imaginations of those who put rod chromatography to work.

Rod chromatography is in its infancy. The next five years will likely produce a geometric increase in publications in this field expanding its uses into what will likely be routine control uses.

REFERENCES

1. T. Contgreave & A. Lynes, J. Chromato., **30**,117 (1967)

2. F.B. Padley,J.Chromatog.,**39**,37-46 (1969)

3. J.J. Szakasits, P.V. Peurifoy, L.A. Woods,Anal. Chem. **42** (1970)

4. S. Araki, S. Suzuki, T. Hobo, M. Yamada, Japan Analyst, **19**, 493 (1970)

5. T. Okumura, T. Kadono, ibid, **22**,980 (1973)

6. T. Kawai,S. Hasunuma,E. Nakano,I. Sakurabayashi, N. Okkubo, S. Yoshioka, J. Ishii, The Japanese Journal of Clinical Pathology, **19**,293 (1971)

7. E. Nakano, I. Sakurabayashi,S. Hasunuma,T. Kawai,T. Tsuchia, N. Okkubo, ibid, **20**,186 (1972)

8. E. Nakano,T. Kawai,VII World Congress of Anatomic and Clinical Pathology, Munich,12-16 Sept. 1972.

9. M. Tokunaga,S. Ando, N. Ueda, Proceedings of Japanese Conference on the Biochemistry of Lipids, **15**, 195 (1973)

10. H. Ganshirt, Quantitative evaluation of thin layer chromatograms. In Thin-Layer Chromatography, 2nd Ed.,E. Stahl, ed.,Springer-Verlag, Berlin & New York, 1969, p.133.

11. H.K. Marigold and K.D. Mukherjee. New Methods of quantitation in thin-layer chromatography: tubular thin-layer chromatography(TTLC). J. Chromatogr.Sci. **8**:379 (1970).

12. J.L. Hojnacki,R.J. Nicolosi, K.C. Hayes. Densitometric quantitation of neutral lipids on ammonium sulfate impregnated thin-layer chromatograms, J. Chromatogr. **128**: 133-39 (1976)

13. E. Haahti, R. Vihko, I. Jaakonmaki and R.S. Evans. Quantitative detection of solute in thin-layer chromatography. J. Chromatogr.Sci. **8**:370-374 (1970).

14. H.P. Kaufmann and K.D. Mukherjee.Die dunnschicht-chromatographie auf dem fettgebiet XXI: Die DC-analyse der lipoide mit hilfe eines flammenionisations-detektors.Fette.Seifen. Anstrichmittel. **71**: 11-17 (1969).

15. K.D. Mukjerje,H. Spaans and E. Haahti. New Detector systems for thin-layer chromatography. J. Chromatgr.**61**:317-21 (1971).

16. F.B. Padley. The use of flame-ionisation detector to detect components separated by thin-layer chromatography. J. Chromatogr. **30**:117-24 (1967).

17. J.J. Szakasits, P.V. Peurifoy, and L.A. Woods. Quantitative thin-layer chromatography using a flame ionization detector. Anal. Chem. **42**:351-54 (1970).

18. T. Okumura and T. Kadano. Thin-layer chromatography on pre-coated adsorbents fixed with fused glass.

VIII. Thin-layer chromatography on silica gel and alumina sintered sticks. Bunseki Kagaku (Japan Analyst) **22**:980-87 (1973).

19. T. Okumura and T. Kadano, and A. Iso'o. Sintered thin-layer chromatography with flame ionization detector scanning. J. Chromatogr. **108**:329-36 (1973).

20. M. Tanaka, T. Itoh, and H. Kaneko. Quantitative determination of neutral lipids on thin-layer-FID chromatography. Yukagaku (Oil Chemistry) **25**:263-65 (1976).

21. M. Tanaka, T. Itho, and H. Kaneko. Quantitative determination of polar lipids on thin layer-FID chromatography. Yukagaku. **26**:454-457 (1977).

22. E. Gantois, F. Mordret and N. LeBarbancho. Uilisation de l'appareil Iatroscan TH-10 pour la separation et le dosage par chromtographie sur couche mince de quelques constituants des corps gras. Rev. France.Corps. Gras. **24**:167-69 (1977).

23. D. Vandamme, V. Blaton, and H. Peeters. Screening of plasma lipids by thin-layer chromatography with flame ionization detection on Chromarods. J. Chromatogr.**145**:151-54 (1978).

24. R.G. Ackman, Fundamental groups in response of flame ionisation detectors to oygenated aliphatic hydrocarbons.J. Gas Chromatogr. **2**: 173-79 (1964)

25. R.G. Ackman and J.C. Sipos. Flame ionization detector response for the carbonyl atom in the carboxyl group of fatty acids and esters. J. Chromatogr. **16**:298-305 (1964).

26. R.F. Addison and R. G. Ackman. Flame ionization detector molar response for methyl ester of some polyfunctional metabolic acids. J. Gas. Chromatogr. **6**:135-38 (1968).

27. E.G. Bligh and W.J. Dyer. A rapid method of total lipid extraction and purification. Can. J. Biochem.Physiol. **37**:911-17 (1959).

28. R.F. Addison, R.G. Ackman, and J. Hingley. Distribution of fatty acids in cod flesh lipids. J. Fish. Res.Bd. Canada. **25**: 2083-90 (1968).

29. E.G. Blish and M.A. Scott. Lipids of cod muscle and the effect of frozen storage. J. Fish. Res. Bd. Canada. **23**: 1025-36 (1966).