25 µl Äthanol wurde das Hydrazid erhalten. Es wurde ohne Isolierung mit Raney-Nickel zum 3-Hydroxy-L-glutamin gespalten.[8] Das Filtrat wurde zur Abscheidung von Ni^{2+} mit alkoholischer Dimethylglyoximlösung versetzt, filtriert und eingedunstet (23°). Der Rückstand wurde mit Äther gewaschen und dann durch Papier- und Dünnschichtchromatographie in Butanol/Essigsäure/Wasser und Phenol/Wasser ein- und zweidimensional aufgetrennt. Neben nicht umgesetzter Hydroxy-glutaminsäure und einem unbekannten, mit Ninhydrin reagierendem Produkt wurde Hydroxyglutamin durch Vergleich mit der natürlichen Verbindung identifiziert.

1. Virtanen, A. I. und Hietala, P. K. *Acta Chem. Scand.* **9** (1955) 175.
2. Fowden, L. *Biol. Rev. Cambridge Phil. Soc.* **33** (1958) 398.
3. Spåre, C.-G. und Virtanen, A. I. *Suomen Kemistilehti* **B 36** (1963) 191.
4. Steward, F. C. und Pollard, J. K. in Holden, J. T. (Hrg.), *Amino Acid Pools*, Amsterdam, New York 1962, p. 40; *Plant Physiol. Suppl.* **31** IX (1956).
5. *vgl.* Cramer, F. *Papierchromatographie*, 5. Aufl. Weinheim 1962, p. 101.
6. Levy, A. L. *Nature* **174** (1954) 126.
7. Boisonnas, R. A., Guttmann, St., Jaquenoud, P.-A. und Waller, J.-P. *Helv. Chim. Acta* **38** (1955) 1491.
8. *vgl.* Greenstein, J. P. und Winitz, M. *Chemistry of the Amino Acids*, New York, London 1961, p. 1260.

Eingegangen am 14. November 1963.

Continuous Detection of Fractions in Effluents of Silicic Acid Chromatography *

E. HAAHTI** and T. NIKKARI**

Department of Medical Chemistry, University of Turku, Finland

The detection of fractions in chromatographic effluents forms a tedious everyday problem particularly in lipid work. Several methods are used for this purpose and these include (i) gravimetric estimation after evaporation of solvents, (ii) application of specific colour reactions, (iii) spot tests on glass-filter paper, followed by charring,[1] (iv) intermittent gas-phase detection,[2] and (v) refractometric monitoring.[3] From these methods only the last one can be applied for continuous detection. However, gradient elution and/or stepwise elution are usually employed in chromatographic separations; these techniques complicate the refractometric method.

The detection principle described here is based on the difference in volatility of the solvent and the eluted material; and the use of a hydrogen flame ionization cell for the detection of the higher boiling material. Continuity of operation is achieved through a running chain arrangement. In the course of the work it has been revealed that the same principle has been under consideration also in other laboratories.[4,5]

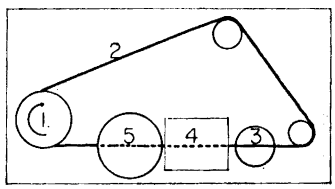

Fig. 1. Schematic diagram of the detector. For interpretation, see text.

* This work was supported by the PHS research grant HE-06818—02 from the *National Heart Institute*, Bethesda, Md., U.S.A.

** Preliminarily presented at the Societas Biochemica et Biophysica Fennica, Turku, Finland, April 17th 1963.

** Established Investigator of the *Finnish Medical Research Council*.

1. *Structure of the detector.* Fig. 1 illustrates the construction of the device. A synchronous motor (1) drives a circulating gold chain (2) of 1 mm diameter with a speed of 1 mm/sec in the direction of the arrow. The chain becomes wetted by the effluent of a chromatographic column (3) and enters a narrow heated tunnel (4) where air blows off the solvents at 120°C. Then the chain passes the flame of a regular hydrogen flame ionization detector (5). Higher boiling material on the chain is combusted and this gives rise to an ionization current, which is amplified to give a 50 mV output signal for a detector current of 1.5×10^{-9} A. The chain is used as anode at + 10 V and the flame jet as cathode. With a hydrogen flow of 20–30 ml/min, an appropriate heating of the chain is achieved when it runs at a distance of 1.5–2 mm above the jet. Due to the distance of column tip and flame there is a time difference of 50 sec. between effluent drop and its signal, respectively. Fig. 2 shows a photograph of the prototype under a normal silicic acid chromatography column.

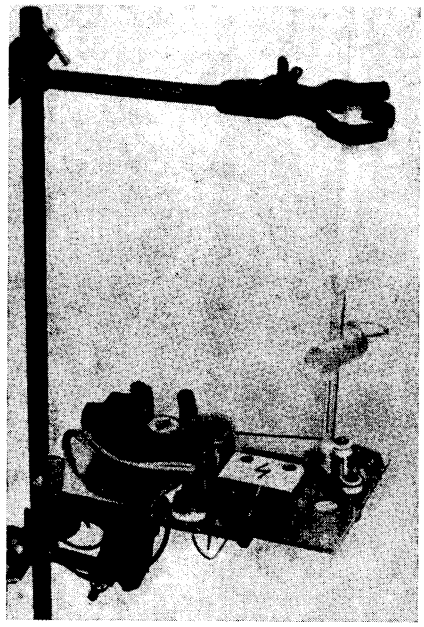

Fig. 2. Photograph of the detector, mounted below a 8 × 250 mm silicic acid column. Numbers on subunits refer to those of Fig. 1. Dimensions of chassis, 10 × 18 cm.

2. *Detection in preparative separations.* The detector is mounted so that the effluent drops from a chromatography column (about 1 drop per second) slightly touch the chain, whereafter the solution is led to a time calibrated fraction collector. Fig. 3 shows a record obtained from the

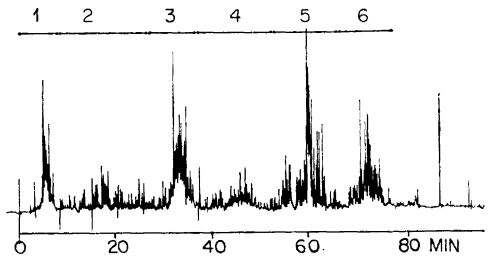

Fig. 3. Separation of 45 mg of a reference mixture containing (1) squalene, (2) impurities of squalene, (3) cholesteryl palmitate, (4) methyl oleate, (5) glyceryl tristearate and (6) oleyl alcohol. Fractions recombined as shown by arrows.

Conditions: Column: 8 × 250 mm, silicic acid 80–200 mesh, moisture 10 %.
Solvents: 0–15 min, benzene-hexane gradient 0–60 %;[6] 60 min → benzene. Flow rate, 1.3 ml/min.

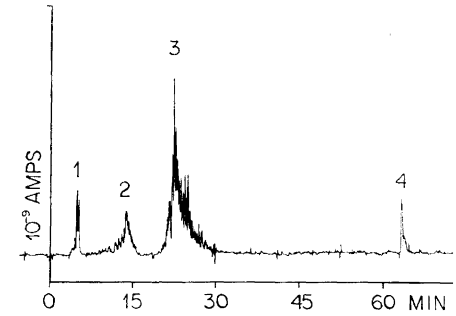

Fig. 4. Separation of 63 mg of human sebum neutral lipids. *Recorded and recovered fractions:* (1) Paraffins and squalene 6 mg, (2) waxes and sterol esters 18.5 mg, (3) triglycerides 32 mg and (4) cholesterol 3.7 mg. *Conditions: Column:* 8 × 200 mm, silicic acid 100–200 mesh, moisture 15 per cent.
Solvents: 0–5 min, hexane; 5–60 min, benzene-hexane gradient 0–50 %; 60 min → benzene. Flow rate 1.2 ml/min.

separation of 45 mg of a known reference mixture in a silicic acid column, 8 × 150 mm in size. The fractions can be located and combined with the aid of the record. Total recovery of the combined fractions in the presented curve was 38 mg, *i.e.* 84 %.

Fig. 4 shows the fractionation of 63 mg of human sebum neutral lipids. The peaks represent (1) squalene, (2) waxes and sterol esters, (3) triglycerides and (4) cholesterol. The total recovery in this experiment was 60.5 mg, *i.e.* 95 %.

3. *Detection in "analytic" separations.* Silicic acid column of 2 × 150 mm are used. The column tip is made of 0.03 inch i.d. teflon capillary to touch the chain. The effluent solution (about 0.1 ml/min) is all caught up by the chain and passed to the detector.

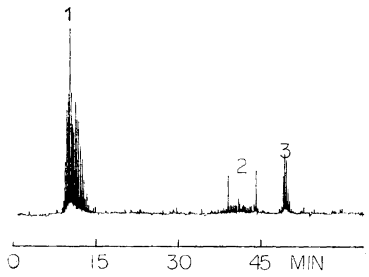

Fig. 5. "Analytic" separation of 2 mg of neutral lipids of fasting human serum. Fractions: (1) sterol esters, (2) triglycerides, (3) cholesterol. *Conditions: Column:* 2 × 150 mm, silicic acid 100–200 mesh, moisture 15 %.

Solvents: 0–12 min, 10 % benzene-hexane; 12–40 min, 40 % benzene-hexane; 40 min → benzene. Flow rate 0.1 ml/min.

Fig. 5 shows an "analytic" record of 2 mg of fasting human serum neutral lipids. The sample was injected with a microsyringe into the column while running on hexane. Hereafter a stepwise elution was carried out using benzene-hexane mixtures as given in Fig. 5.

Fig. 6 shows the fractionation of human serum sterol esters on unsaturation basis. 5 mg of the sample were injected in 20 µl of hexane into a silver nitrate impregnated silicic acid column[7-9]. Stepwise elution was carried out as given in Fig. 6. Four fractions were resolved, corresponding to 0, 1, 2, and 3 double bonds in the respective fatty acids. The total solvent consumption of the run was less than 5 ml.

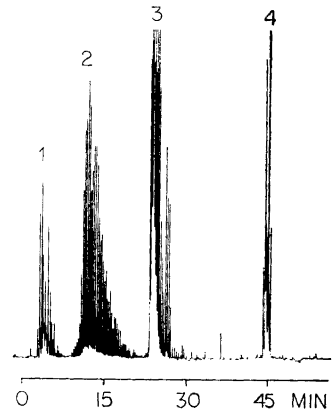

Fig. 6. Analytic fractionation of human serum sterol esters on unsaturation basis. Recorded fractions: (1) Saturated esters, (2) monounsaturated esters, (3) diunsaturated esters, (4) triunsaturated esters.

Conditions: Column: 2 × 150 mm, silicic acid 100–200 mesh, impregnated with 20 % $AgNO_3$-solution.[9]
Solvents: 0–7 min, 10 % benzene-hexane; 7–20 min, 40 % benzene-hexane; 20–40 min, 10 % chloroform-benzene, 40 min → chloroform. Flow rate 0.1 ml/min.

4. *Quantitation of the detection.* As can be seen from the presented curves, this prototype work has been carried out with an electronic equipment not adequate for the present purpose. Only slow signals are to be recorded and it seems that amplifier time constant of about 3 sec would give a curve free from the counting noise caused by chain meshes and/or drop count (*e.g.* sterol esters, Fig. 6). Volatile compounds (*e.g.* methyl myristate) give a smaller signal than less volatile (*e.g.* triolein) due to partial evaporation of the compound immediately before entering the flame. It seems advisable to turn to the study of quantitation after a more exact establishment of the optimal conditions, materials and dimensions of the device. So far the use of the detector has been limited to localization of fractions in preparative work followed by gravimetric estimation.

1. Ways, P. *J. Lipid Res.* **4** (1963) 101.
2. Karmen, A., Walker, T. and Bowman, R. L. *Ibid.* **4** (1963) 103.
3. Hirsch, J. *Ibid.* **4** (1963) 1.
4. Meijer, J. W. A. *Personal communication.*
5. Bowman, R. L. *Personal communication.*
6. Hirsch, J. and Ahrens, E. H., Jr. *J. Biol. Chem.* **233** (1958) 311.
7. De Vries, B. *Chem. Ind. (London)* **1962** 1049.
8. De Vries, B. *J. Am. Oil Chemist's Soc.* **40** (1963) 184.
9. Haahti, E., Nikkari, T. and Juva, K. *Acta Chem. Scand.* **17** (1963) 538.

Received November 14, 1963.

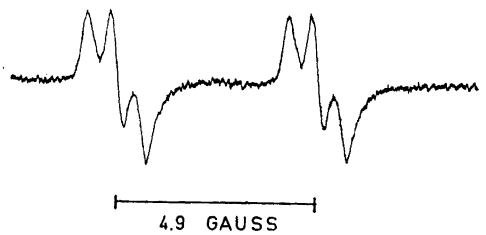

Fig. 1. ESR-spectrum of the free radicals formed from 7-chlorotetracycline hydrochloride dissolved in 1 N NaOH in H_2O.

Free Radicals in Aqueous Alkaline Solutions of Tetracycline Derivatives

CARL LAGERCRANTZ and MARGARETA YHLAND

Department of Medical Physics, University of Göteborg, Göteborg, Sweden

Using the technique of electron spin resonance (ESR), it has been observed that solutions of several tetracycline derivatives in aqueous sodium hydroxide contain a relatively large concentration of free radicals. The ESR-spectra of these radicals have a characteristic appearance. The ESR-spectrum of the radicals present in a 0.02 M solution of 7-chlorotetracycline (aureomycin) hydrochloride in 1 N NaOH thus exhibited a doublet splitting with a hyperfine coupling constant of 4.9 gauss. Each one of the two lines was further split into a triplet with a coupling constant of about 0.5 gauss (Fig. 1).

Immediately after dissolving the substance, the radical concentration was found to increase slowly from zero up to a maximum value after about 2 h. An optimal resolution of the spectrum was obtained about 20 min after the substance had been dissolved. In this case the freshly prepared solution had been kept in an open vessel for 5 min before being filled into the sample cell. The resolution was found to increase with a decreasing microwave power applied to the sample, but was not improved by degassing of a solution in which radicals had already been formed.

On dissolving the substance in 2 N or 5 N NaOH, the formation of radicals was found to be more rapid than in 1 N NaOH, especially when the solution was kept in an open vessel. Primarily, the ESR-spectrum was identical with that obtained in 1 N NaOH (Fig. 1), but after some time a new line appeared which was located in the center between the original two lines. The center line gradually increased in magnitude and finally dominated the entire spectrum. No center line of this type could be observed in solutions of tetracycline derivatives when prepared from 1 N NaOH. When the experiments were performed with 0.1 N NaOH, there were no radicals to be observed.

ESR-spectra, identical with that obtained with 7-chlorotetracycline (Fig. 1), were also observed with tetracycline (achromycin), 5-hydroxytetracycline (terramycin) and 6-demethyl-7-chlorotetracycline (Ledermycin).

When 7-chlorotetracycline hydrochloride was dissolved in 1 N sodium hydroxide (NaOH or NaOD) prepared with D_2O, a new structure appeared in the center part of the spectrum between the original 2 × 3 lines. This structure seemed to be of a nature different from that observed with an aureomycin solution prepared with 2 N or 5 N NaOH in H_2O, and consisted of five lines, each one of which was further split into at least four lines. The magnitude of this central structure slowly increased and dominated the spectrum after about 1 h (Fig. 2). At the same time it was noted that the intensity of the original 2 × 3 line structure had been reduced.