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PROPORTIONAL CHAMBER DEVICE FOR THIN-LAYER RADIOCHROMATOGRAM ANALYSIS

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SUMMARY

A device consisting of proportional chambers has been constructed for the analysis of thin-layer radiochromatograms. The device enables one ot localize radioactive zones, to identify radionuclides and to measure their activities.

INTRODUCTION

Thin-layer radiochromatography has been widely used to study ^{3}H-, ^{14}C- and ^{32}P-labelled compounds. The analysis of radiochromatograms includes the localization of radioactive zones, measurement of their activities and radionuclide identification when several radionuclides are used.

Several methods of radiochromatogram analysis are available[1–4]:

(a) autoradiography (with a subsequent measurement of radioactivity by means of liquid scintillators);

(b) scanning of samples by gas discharge counters;

(c) localization of chromatographic zones by means of spark chambers.

Disadvantages of these methods are the time consuming radiochromatogram analysis (a and b above), the low accuracy of quantitative measurements (c above) and difficulties in radionuclide identification. In order to overcome these disadvantages, the possibility of using a proportional chamber (PC) in radiochromatogram analysis has been tested[5]. This detector can be used to perform rapid radiochromatogram analyses with good accuracy.

A new device has been developed and constructed on the basis of proportional chambers for the analysis of thin-layer chromatograms.

DESCRIPTION OF THE DEVICE

The device consists of a position-sensitive detector[6], readout electronics and a data output system[7,8]. A schematic diagram is shown in Fig. 1.

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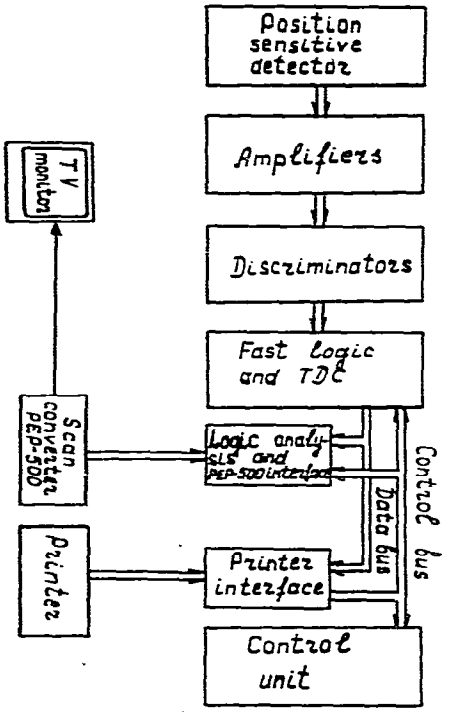

Fig. 1. Schematic diagram of the device.

Signals from the proportional chambers are supplied to "zero-crossing" discriminators through amplifiers. Fast logic units select good events and produce control signals for TDCs. Digital information from the TDCs is transferred to the control units and subsequently to a PEP-500 Lithocon solid-state image/scan converter[9], and finally it is presented to a TV monitor. In addition, the data can be printed out. A general view of the device is shown in Fig. 2.

A schematic diagram of the detector is given in Fig. 3. It consists of three proportional chambers with drift spaces (D). The chambers are placed in a vacuum vessel (Fig. 4). A thin-layer chromatogram with a maximum size of 200 × 200 mm^2 is placed in the detector volume. The chromatogram is directly connected to a sensitive volume of the PC1 by means of the drift spaces D0, D1. The electric field in the D0, D1 provides a full collection of secondary electrons in the PC1. The detector efficiency is determined by the sample thickness and by the radioactivity depth distribution. The PC3 placed at the greatest distance from the sample detects electrons with an initial energy of above 200 keV. Electrons with lower energy are stopped in a previous gas layer or in an Al filter of the D3. The PC2 detects electrons with an initial energy of above 100 keV. The PC1 detects electrons in the whole energy range. The three-fold coincidence between anode signals of the PC1, PC2 and PC3 ($A_1 \cdot A_2 \cdot A_3$) occurs when an electron emitted by ^{32}P is detected. If the third chamber does not count ($A_1 \cdot A_2 \cdot \bar{A}_3$), the electron is emitted by ^{14}C. Part of events of this type can be also due to electrons from ^{32}P. The type of events ($A_1 \cdot \bar{A}_2 \cdot \bar{A}_3$) can be considered as electrons from ^{3}H.

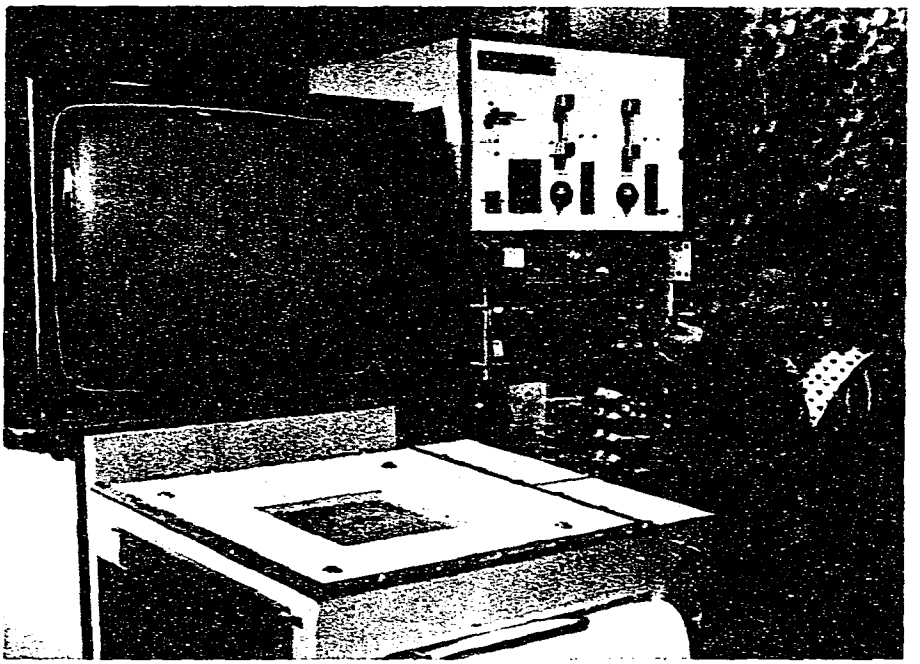

Fig. 2. General view of the device.

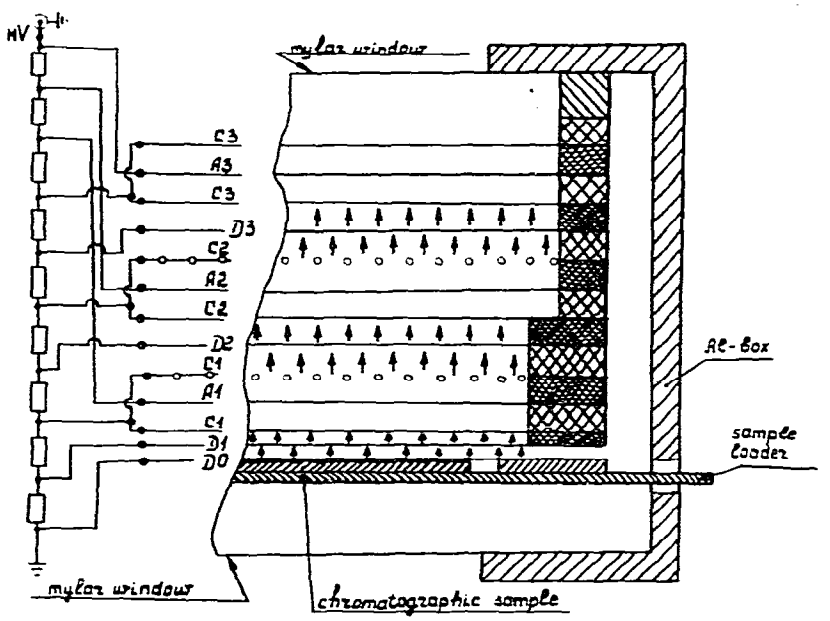

Fig. 3. Schematic view of the detector. The direction of electron motion in drift spaces D0, D1, D2 and D3 is denoted by arrows. C_1, A_1, C_1 are the PC1 electrodes, C_2, A_2, C_2 the PC2 electrodes and C_3, A_3, C_3 the PC3 electrodes. The distance from the chromatogram surface to the nearest cathode of the PC1 (C_1) is 3 mm.

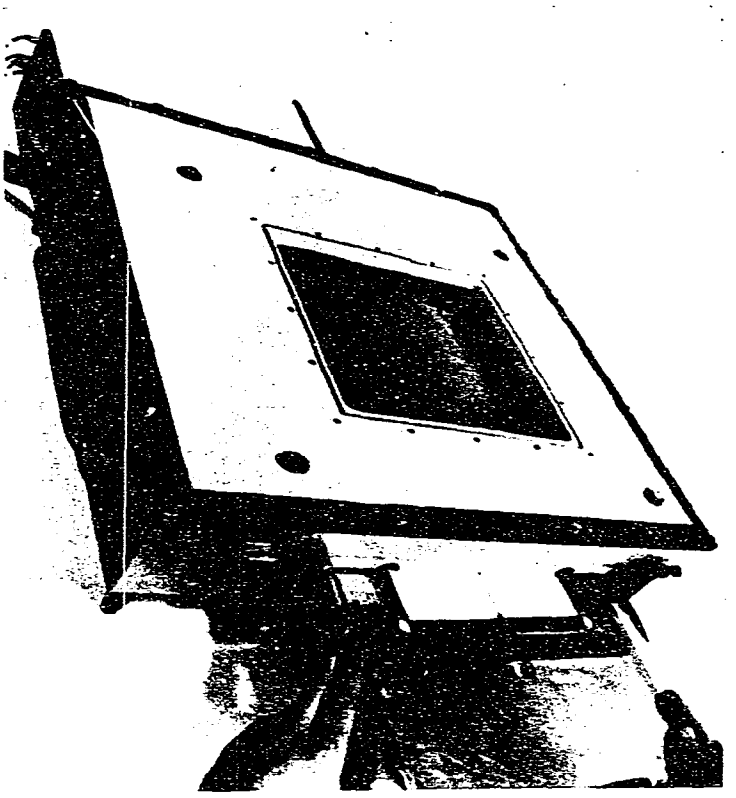

Fig. 4. General view of the detector. Placing the sample into the detector.

Thus, for each radionuclide there exist definite ralationships between the number of events of various types:

for ^{3}H: $N_2 = N_3 = O$

for ^{14}C: $C_1N_1 = C_2N_2$, $N_3 = O$

for ^{32}P: $P_1N_1 = P_2N_2 = P_3N_3$

Here N_1 is the number of events of the $(A_1 \cdot \bar{A}_2 \cdot \bar{A}_3)$ type, N_2 that of the $(A_1 \cdot A_2 \cdot \bar{A}_3)$ type and N_3 that of the $(A_1 \cdot A_2 \cdot A_3)$ type. The empirical coefficients C_i, P_i are mainly dependent on the method of preparing the thin-layer chromatograms.

The detection efficiency, ε, of a given radionuclide is determined as follows:

$$\varepsilon_H = 2 \cdot \frac{N_1}{I_H}$$

$$\varepsilon_C = 2 \cdot \frac{N_1 + N_2}{I_C} = \varepsilon_{C1} + \varepsilon_{C2}$$

$$\varepsilon_P = 2 \cdot \frac{N_1 + N_2 + N_3}{I_P} = \varepsilon_{P1} + \varepsilon_{P2} + \varepsilon_{P3}$$

Having measured N_1, N_2 and N_3 and knowing the partial efficienceis ε_{ij} ($i = H, C, P$; $j = 1, 2, 3$), it is easy to identify a radionuclide in the radioactive zone and to deter-

mine its quantitative content. Changes in the quantitative relationships between N_1, N_2 and N_3 in the radioactive zone show that there are several radionuclides present. From an analysis of these relationships the number of different radionuclides can be estimated in this radioactive zone.

During one analytical run the device makes possible the presentation of the three-dimensional information on radioactive ^{3}H-, ^{14}C- and ^{32}P-labelled zones to the TV monitor, the identification of the radionuclides indicated and the printout of integrated counts of any selected chromatogram zone, coordinates of this zone, exposure and dead time.

EXPERIMENTAL RESULTS

The properties of the device were investigated using different test radiochromatograms.

Table I presents the results of measurement of the efficiencies of the device for radiochromatograms with silica gel TLC-G and an infinitely thin layer (ε_i^*). For tritium this quantity is a calculated one.

TABLE I

EFFICIENCY OF THE PROPORTIONAL CHAMBER FOR RADIOCHROMATOGRAMS WITH SILICA GEL TLC-G AND AN INFINITELY THIN LAYER (ε_i^*)

i	ε_{ij}			$\varepsilon_i = \sum_{i=1}^{3} \varepsilon_{ij}$	ε_i^*
	$j=1$	$j=2$	$j=3$		
H	0.01	0	0	0.01	0.5
C	0.25	0.15	0	0.40	0.8
P	0.25	0.20	0.15	0.60	0.85

Analyses performed on a series of samples with ^{3}H and ^{14}C of known absolute activities showed a good reproducibility of the results. The error in measuring the intensities is about 10%. When studying ^{3}H-, ^{14}C- and ^{32}P-labelled radiochromatograms (on one sample), a reliable radionuclide identification has been achieved.

The resolution of the device was studied by means of test radiochromatograms with spots of the same activity (Figs. 5 and 6). The spots are completely separated at the following distances between their boundaries: 2–3 mm for ^{3}H, 5–6 mm for ^{14}C and about 12 mm for ^{32}P.

The autoradiographs of seven ^{3}H-labelled spots of different activities (and diameters) obtained by means of the device are shown in Fig. 7. The activity of the weakest spot is about 5 pCi; its diameter is about 3 mm. The sensitivity of the device (without taking into account absorption of radiation by compounds in the radiochromatogram) is determined from the detector noise to be about 0.1 Hz/cm^2. The maximum rate of the device is of the order of 10^5 I/sec.

The time required to place a radiochromatogram in the detector volume and to prepare the device for measurements was 10–15 min. The time of analysis of a test sample did not exceed 15 min.

Radiochromatograms with activities $I_H = 1500$ dpm, $I_C = 200$ dpm and $I_P = 150$ dpm were successfully analysed by means of the device.

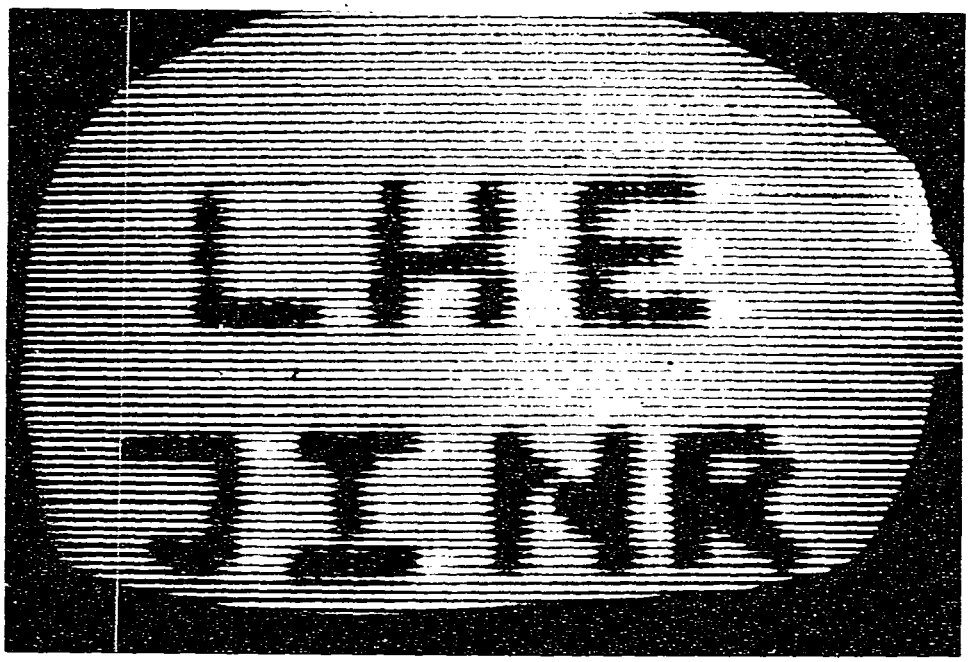

Fig. 5. Autoradiograph (TV picture) characterizing the resolution of the device. The label is painted on glass with ^{3}H-labelled ink. The diameter of the spots of which the label is made and the distances between the spot boundaries are about 2 mm.

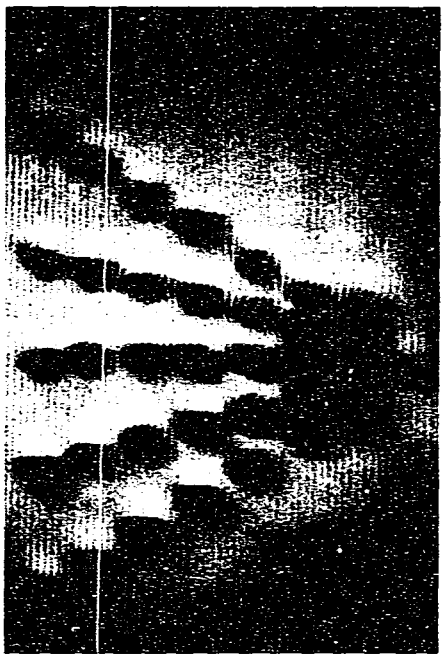

Fig. 6. Autoradiograph (TV picture) characterizing the resolution of the device. The minimal distance between the spot boundaries is about 1 mm.

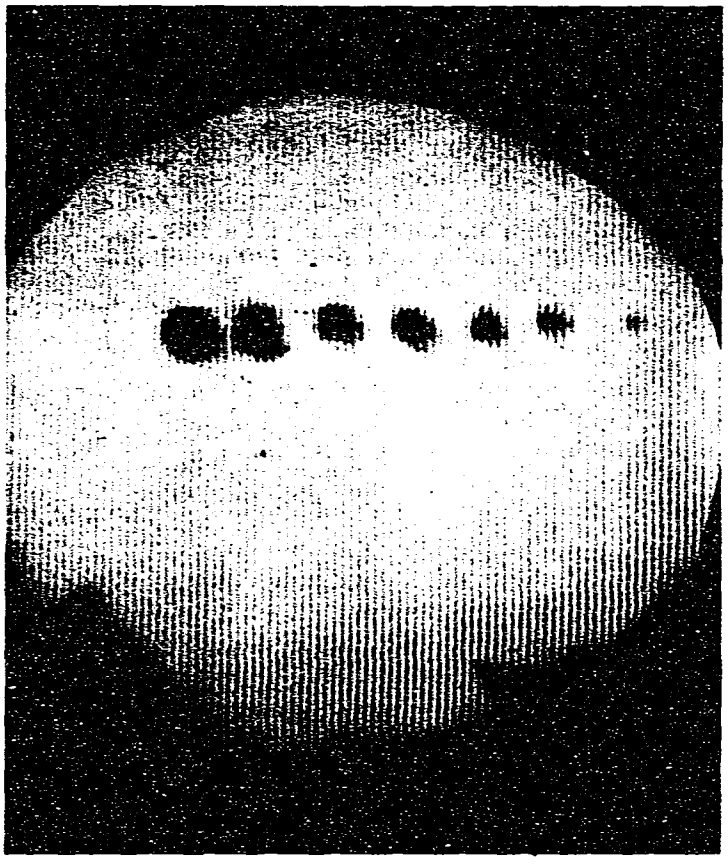

Fig. 7. Autoradiograph (TV picture) of a ^{3}H radiochromatogram with spots of different activity and diameter. The activity of the weakest spot is *ca.* 5 pCi; the diameter is *ca.* 3 mm.

CONCLUSION

The device described here is a high-efficiency automatic system for carrying out quantitative and qualitative analyses of thin-layer radiochromatograms (electropherograms). The determination of the localization of radioactive zones, radionuclide identification and measurement of zone activities are performed during one measurement run. The device has a high sensitivity, a small dead time, good space resolution and high reproducibility. In addition, after analysing a radiochromatogram by means of the device, the investigated compound can be used for further studies. The possibility exists of increasing the topographic resolution to 1–2 mm for all radionuclides. Complete automation of the measurements is possible.

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