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THE ENHANCEMENT OF DATA DERIVED FROM A THIN-LAYER CHROMATOGRAPHY RADIOACTIVITY SCANNER BY ACCUMULATION AND FOURIER TRANSFORMATION

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SUMMARY

A method of enhancing data, derived from the ratemeter of a thin-layer chromatography radioactivity scanner, for low levels of activity, is described. The averaging of data from several "rapid" scans followed by a smoothing operation involving Fourier transform results in an improvement of signal-to-noise ratio and resolution when compared with a single scan of the same total duration and using the smoothing components incorporated in the ratemeter.

INTRODUCTION

The enhancement of experimental data by accumulation of successive runs is applied in many fields, particularly spectroscopy. Noise, being random in nature, will effectively cancel out, whereas the signal information will be reinforced as data are accumulated. For a set of N observations which are summed, an enhancement of $\sqrt{N}$ is expected[1].

The use of radioisotopes in thin-layer chromatography (TLC) to establish the origin of, or to assist in the location of, components on the plate is well known. Two methods for detection are available: spots may be removed and their activity measured by counting or, if preferred, the plate may be preserved intact but scanned by means of a suitable detector. In the former case, extended counting periods allow the detection of relatively small amounts of activity, whereas in the latter, the signal-to-noise ratio to the recorder may be improved either by prolonging the scanning time or by accumulation of successive runs. If the noise pulses from the scanner have a constant amplitude throughout their frequency spectrum (white random noise), then the signal-to-noise ratio obtained from a single scan will be the same as that for the time-averaged result from a number of scans over the same total time. The output from the scanner used in the present work was derived from a ratemeter but, in general, instruments of this type are expected to incorporate low-pass filtering in the output stages. Thus the observed noise spectrum will no longer correspond to that of white random noise but there will be an increase in amplitude towards the lower frequencies.

It may be shown that, in such a case, a considerable improvement in signal-to-noise ratio occurs if the data are time-averaged from a number of scans rather than being derived from a single observation of the same total duration.

Of course, the performance of the scanner will also be improved by increasing the amount of radioactive tracer applied to the chromatographic plate, although this is limited by the capacity of the chromatographic medium and the specific activity of the sample to be analysed.

The use of Fourier transformation in the smoothing of experimental data is becoming increasingly popular and several detailed publications are available in this field[2–4]. This paper describes the advantage to which accumulation of data followed by Fourier transformation can be used to improve the data obtained from a TLC scanner for plates containing low levels of low-energy β-activity.

EXPERIMENTAL

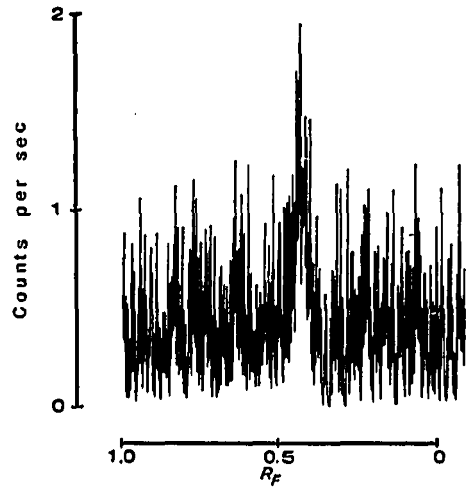
An aliquot (5 μg) of the 2,4-dinitrophenylhydrazones of an unknown β-sulphocarbonylic species (labelled with ^{35}S) admixed with other carbonyl compounds was applied to a silica gel G thin-layer plate (250 μm) and developed with butan-1-ol–0.880 ammonia solution (4:1). The plate was scanned on a Panax RTLS-1A thin-layer scanner, using a 2 mm slit, in conjunction with a P7900A ratemeter (range, 3 cps; time constant, 3 s). The scan speed was 12 cm/h. The output of the ratemeter was digitized at intervals of 4 s and punched on to paper tape. The total number of points per run was usually in the region of 1000. The data were processed on an IBM 1130 computer (16k store × 16 bit words) and Fourier transformations were calculated for an array, 1024 data points long, using the "Cooley–Tukey fast Fourier transform" method[5]. The results were displayed on an X–Y plotter. The results were compared with data originating from the scanner operating under more appropriate conditions for measuring low activities (scan speed, 3 cm/h; time constant, 30 s). The output was digitized at intervals of 2 s in order to simplify the calculation of signal-to-noise ratios.

RESULTS AND DISCUSSION

The total activity applied to the TLC plate was 5 counts/s, which was also the maximum possible for the mixture in question. Preliminary experiments showed that the instrument settings were close to the optimum working conditions for the technique adopted.

A typical thin-layer radioactivity scan is shown in Fig. 1, in which very little "peak" information is evident. A major component appears to lie at R_F 0.43. An improved result, obtained by accumulating the data from eight such runs, is shown in Fig. 2. It is now clear that the chromatogram consists of one major radioactive component under these conditions.

Data smoothing by means of analogue filters is a well known feature of many scientific instruments; however, the effect can be reproduced in a digital form through the use of Fourier transform. The algorithm allows one to calculate the frequency spectrum of the signal to be analysed; it consists of components of low frequency corresponding to the peak information and a high-frequency component associated with



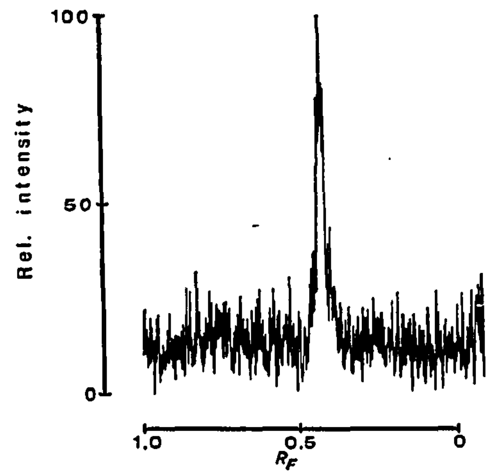


Fig. 1. A typical single radioactive scan.

Fig. 2. Radioactive scanning record obtained as a result of accumulating data from eight scans.

the noise pulses. If transformation is reversed then the information held in the frequency spectrum will be converted back into the original data. If, however, prior to reversing the Fourier transform, the components above a certain frequency are attenuated, then the data which are returned will contain the original low-frequency components associated with the radioactivity maximum, but the noise will be considerably reduced. Too early a cut-off will result in some broadening of the peak although, under some circumstances, resolution may need to be sacrificed in order to obtain a smoother trace. The manner in which the attenuation of unwanted frequencies is carried out is of some importance. An abrupt setting of all values beyond a certain point to zero has disadvantages in that it may lead to the generation of a periodic waveform ("ringing") superimposed on the baseline. A better method is to "mask" the unwanted data by means of a suitable function such as a linear ramp, exponential or Gaussian curves. Fig. 3 shows the data of Fig. 2 after modification by Fourier transformation and masking by a Gaussian function. This was chosen such that after 101 points the attenuation was 85%.

It is evident that the final operation has resulted in a considerable enhancement of the signal-to-noise ratio. Earlier attenuation of the frequency spectrum caused peak broadening, whereas the inclusion of higher frequencies had little effect on peak width but increased the noise. A comparison of the smoothed data accumulated from four scans with that derived from a single scan of the same total duration, but with a more "appropriate" time constant, revealed that the operation described above resulted in a three-fold improvement in signal-to-noise ratio (defined as the ratio of the peak value of the signal intensity to the r.m.s. value of the noise intensity[1]) over the single observation. In addition, the peak width (as measured at half the peak amplitude) was 40% less, and more realistic, when the method of accumulation and Fourier transformation was compared with the single scan measurement. This improvement in performance was considered worthwhile in terms of the added complications which the technique involved and, in experiments containing lower levels of activity, accept-

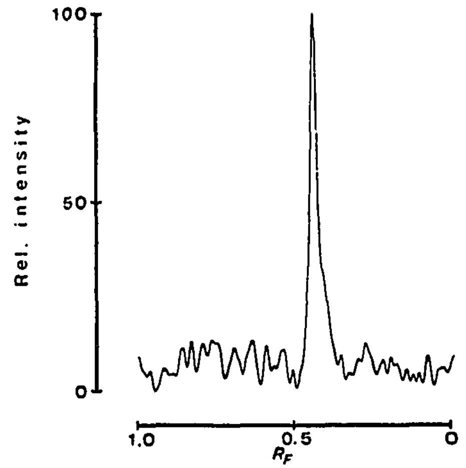


Fig. 3. Radioactive scanning record obtained as a result of accumulating data from eight scans followed by modification by Fourier transformation.

able traces were obtained after successive accumulation of data which revealed no peak information on single scans under any conditions.

This technique is being used to study the sulphur-containing products formed during the reaction of ascorbic acid and sulphite in the presence of glycine.

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