OPTIMUM OPERATION OF RECORDING COUNT RATE METERS FOR RADIOACTIVE PAPER CHROMATOGRAMS*

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Recording count rate meters are widely used for scanning paper strips on which radioactive compounds have been subjected to chromatography or electrophoresis. A strip is slowly pulled past a slit so arranged that the radioactivity in the region of the paper defined by the slit is counted with a suitable counter. The output of the counter is fed to a differentiating circuit, whose output is proportional to counting rate. This counting rate is recorded by a strip chart recorder, the chart of which is generally made to move at the same rate as the paper strip. Thus, the recorder plots radioactivity against position on the strip. The area under a peak in such a curve is proportional to the amount of radioactivity in the component responsible for the peak. Many types of scanning devices are commercially available. Many have been described. SHIPOTOFSKY[1] describes one and refers to many others. In the review paper of POCCHIARI AND ROSSI[2], various types of scanners are compared.

Because of statistical variations in count rate, the differentiating circuit of the ratemeter must average the count over a very appreciable interval in order to yield a satisfactory figure for count rate. This averaging time is controlled by varying the time constant of the differentiating circuit. The slit width and the strip speed may also be varied. There is very little information in the literature concerning optimal adjustment of these operating variables. WILLIAMS AND SMITH[3] have recommended that the slit width should equal four times the product of the time constant and the strip speed. GELBKE, HEITEFUSS AND FAIT[4] examined a number of sources of error in quantitation of radioactive paper chromatograms. They found that at the radioactivity levels they used, other errors were greater than the statistical count rate error, and that because of this there was little to be gained by the use of slow scanning rates.

In this paper, the optimum adjustment of operating variables will be quantitatively considered. A number of qualitative conclusions can readily be drawn. For example, a very wide slit will broaden the record of a peak, and decrease the apparent resolution of two closely-spaced peaks. On the other hand, a very narrow slit will result in such a low count rate that statistical error will be large. Too long a time constant will cause apparent broadening of peaks, while a very short time constant will give an unsatisfactory record because statistical count rate fluctuations will be recorded. A very fast strip speed will result in only a few counts from each peak to be recorded, resulting in a large statistical error. A very slow strip speed severely limits

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the number of strips that can be processed per day. The recorder range (*i.e.*, counts per minute for full scale) may also be varied. Thus, if a narrower slit is used, a lower range may be used. It is apparent that optimum results can only be obtained by quantitative consideration of the results of changes in operating variables. The recording obtained should have good fidelity and low error. Good fidelity means that the shape of the curve on the record is a close representation of the distribution of radioactivity on the strip. Low error means that the area of a peak on the record is strictly proportional to the amount of radioactivity in the corresponding zone on the strip.

TIME CONSTANT

Statistical fluctuations can be minimized by averaging the count rate over a long period. This is done by the use of a long time constant. A time constant long enough to reduce statistical fluctuations greatly is also long enough to reduce the fluctuations we wish to observe, those due to radioactive zones on the strip. Sensitivity (*i.e.*, the ability to detect a zone of weak radioactivity) is not affected by time constant. A long time constant will reduce the height of a small peak, but it will also reduce, by the same factor, variations due to statistical count rate fluctuation. The detectability of a small peak in the presence of background variation is therefore independent of time constant.

Choice of a time constant is aided by the following considerations. Suppose background counting rate is being recorded. The observed statistical count rate fluctuation will vary directly with the square root of the background counting rate, and inversely with the square root of the time constant, which is essentially the averaging time. The observed fluctuation will therefore be constant if the ratio of these two quantities is kept constant:

$$\text{Fluctuation} = \frac{\sqrt{b}}{\sqrt{tc}}$$

where b is the background counting rate (counts/min) and tc is the time constant (sec). This relationship holds at any recorder range, but if the range is changed, the fluctuation (expressed as per cent of full scale) will be constant only if the ratio is increased as the range is increased. Then

$$\frac{\sqrt{b}}{r\sqrt{tc}} = k$$

where r is the range (counts per min for full scale) and k is a constant. Too high a value for k gives an inordinate amount of fluctuation, and too low a value for k gives distortion (peaks will be lengthened and flattened). The value chosen for k is partially a matter of preference, but in general, values of more than 0.001 for k are objectionable. Hence, a convenient working rule for determining the minimum range that can be used with a given time constant is:

$$\text{Minimum range (counts per min full scale)} = \frac{1000\sqrt{b}}{\sqrt{tc}} \qquad (1)$$

SLIT WIDTH

While time constant affects fidelity but not sensitivity, slit width affects both fidelity and sensitivity. If a paper strip contains a series of adjacent similar peaks, the best sensitivity is obtained when the slit width is half the peak separation. This is illustrated (for triangular peaks) in Fig. 1. In the figure, curve A represents a series of adjacent triangular peaks. Curve B is the record that would be obtained with a slit width equal to half the peak separation (it is assumed that the time constant is short enough to cause no distortion). Curve C is the record that would be obtained with a slit width equal to $\frac{1}{4}$ the peak separation, and curve D is for a slit equal to $^3/_4$ the peak separation. It will be noted that the largest response (peak to valley difference) is obtained in curve B, but the greatest fidelity (peak-to-valley ratio) is obtained in curve C.

In Fig. 2, the heavy line represents a series of peaks, shaped like sine waves, the peak separation being twice the peak width at half height.

If the peak separation is regarded as being 360°, we may speak of the slit angle, θ, being 90° if the slit width is $\frac{1}{4}$ of the peak separation. It can be shown that at a given strip speed, the relative response obtained with a given slit width is equal to

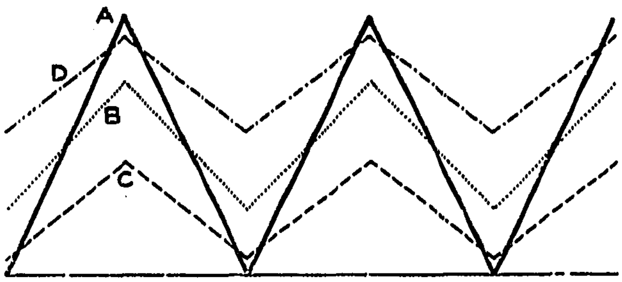


Fig. 1. Effect of slit width on response and fidelity. Curve A: a series of triangular peaks. The remaining curves show the records that would be obtained from these peaks with slit width equal to half the peak separation (curve B), one-fourth the peak separation (curve C) and three-fourths the peak separation (curve D).

Fig 2. Effect of time constant on fidelity. Heavy line: a series of peaks shaped like sine waves. Light line: record obtained when the time constant is such that the amplitude is reduced by 50%. (This occurs when the time constant is about 28% of the peak separation time.)

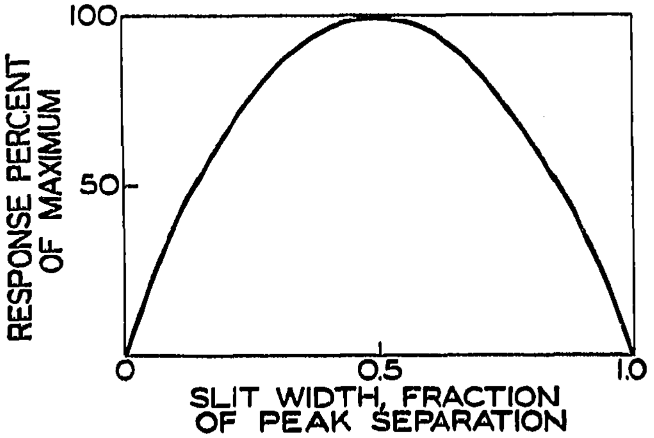


Fig. 3. Effect of slit width on response (sine wave peaks).

$2(\sin(\theta/4) + \cos(\theta/4) - 1)$. A plot of this function is shown in Fig. 3. It will be noted that, as was the case with triangular peaks, maximum response is obtained when the slit width is equal to half the peak separation.

FIDELITY

When a long time constant is used, peaks will be flattened and valleys filled. In Fig. 2, the light line represents the case where the time constant is such that the peak height is reduced to $^3/_4$ of its original value, and the valley is "filled in" by a like amount. The result is a sine wave having half the amplitude of the original. (Accompanying this reduction in amplitude will be a time delay. Since this delay is, as a first approximation, the same for all peaks, and since it has no effect on the factors to be considered, it is ignored in Fig. 2 and in subsequent discussion.) The longer the time constant the greater is the reduction in amplitude. It is convenient to speak of a "fidelity factor", f_t, where f_t is the ratio of the amplitude of the distorted wave to that of the original wave. In the example of Fig. 2, $f_t = 0.5$. It is also apparent from Fig. 2 that v, the valley ordinate, is equal to $(1 - f_t)/2$, and p, the peak ordinate, is equal to $(1 + f_t)/2$. The peak-valley ratio p/v, is $(1 + f_t)/(1 - f_t)$.

It can be shown that for sine wave peaks

$$f_t = \frac{1}{\sqrt{[1 + (2\pi t)^2]}} \tag{2}$$

where t is the time constant divided by the time separation of the peaks. (Time separation of peaks, of course, depends on strip speed.) This relationship is plotted in Fig. 4. Also shown in the figure is the peak–valley ratio corresponding to various values for t. It is apparent that distortion is very appreciable unless the time constant is less than 0.2 times the peak separation time.

In a practical case, there is distortion due to slit width as well as distortion due to time constant. Total distortion can be estimated if slit width distortion is also expressed as a fidelity factor, f_s. Then

$$f = f_s \cdot f_t$$

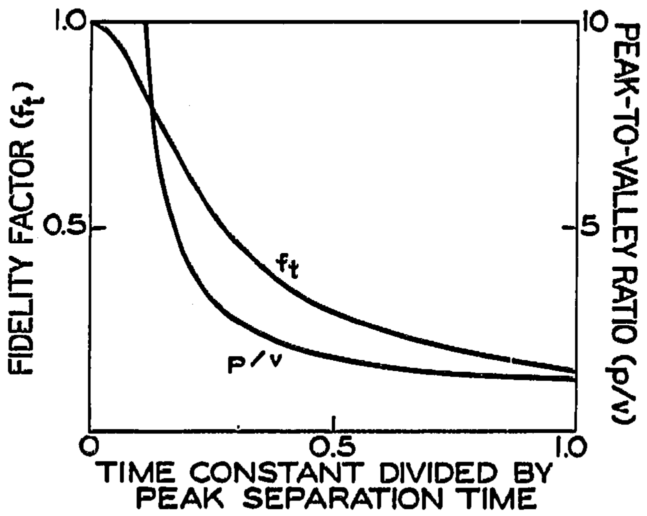
where f is the net fidelity factor, which is the product of the fidelity factors due to slit width and to time constant and the net peak-to-valley ratio

$$\frac{p}{v} = \frac{1 + f}{1 - f} \tag{3}$$

For sine wave peaks, it can be shown that

$$f_s = \frac{\sin\left(\frac{\theta}{4}\right) + \cos\left(\frac{\theta}{4}\right) - 1}{\sin\left(\frac{\theta}{4}\right) - \cos\left(\frac{\theta}{4}\right) + 1} \tag{4}$$

where θ is the slit angle defined as before. For triangular peaks (Fig. 1), $f_s = 1 - s$, where s is the slit width expressed as fraction of peak separation. Fig. 5 shows, for



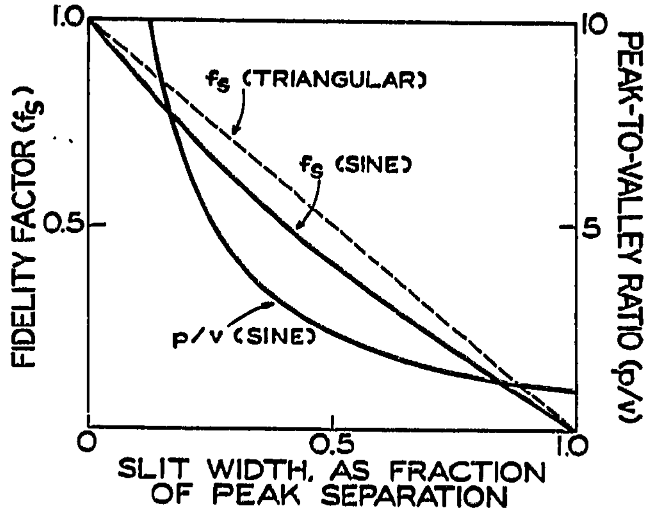


Fig. 4. Effect of time constant on fidelity.

Fig. 5. Effect of slit width on response and fidelity, for triangular and sine wave peaks.

triangular and sine wave peaks, the value of f_s for various slit widths. It will be noted that the relationship is similar for both peak shapes. In the figure, peak–valley ratios for sine wave peaks are also plotted. It will be noted that at a slit width equal to half the peak separation (the width that gives optimum sensitivity) there is rather severe distortion.

ERROR IN PEAK AREA

The standard error of the area of a recorded peak is equal to the square root of the number of counts recorded in it. The number of counts recorded is equal to the product of the number of counts per second resident in the peak and the slit time, the number of seconds required for a given point on the paper strip to traverse the slit. The counts per second resident in the peak can be defined as the recorder scale reading (in counts per second) that would be obtained if the peak was positioned in a slit wide enough to accommodate the entire peak, and the strip speed was zero. The standard error expressed as per cent error is

$$\text{Per cent error} = \frac{100}{\sqrt{(\text{counts per sec} \times \text{slit time})}}$$

Since slit time is slit width divided by strip speed, we may restate the above (using counts per min, the more usual unit, rather than counts per sec).

$$\text{Standard error (\%)} = \frac{775\sqrt{(\text{strip speed})}}{\sqrt{(\text{slit width})}\sqrt{(\text{c.p.m.})}} \tag{5}$$

where strip speed is expressed in cm/sec and slit width in cm, and where c.p.m. is the number of counts per min resident in the peak.

OPTIMUM OPERATING CONDITIONS

The operating conditions selected for strip scanning will depend on the relative importance of sensitivity, fidelity, and scanning time. Best results are obtained when the distortion due to time constant is approximately equal to the distortion caused by slit width. This is apparent from the following: no great increase in fidelity can be obtained by using a narrower slit if the distortion due to time constant is already greater than that due to slit width. Conversely, no great increase in fidelity can be obtained by reducing time constant distortion if the slit width distortion is already greater than the time constant distortion. Selection of proper operating conditions may be done by first selecting a slit width that gives the desired compromise between sensitivity and fidelity. A suitable strip speed can then be selected, low if the radioactivity of the sample is low, and high if ample radioactivity is present.

TABLE I

OPTIMAL CONDITIONS FOR OPERATION OF SCANNERS FOR RADIOACTIVE PAPER CHROMATOGRAMS

The figures have been calculated for sine wave peaks with a peak separation of 2 cm. Values for other peak shapes are not greatly different. Values calculated for a 4 cm peak separation are also not greatly different from those above, except that the peak-to-valley ratios are roughly twice as great as those shown.

Slit width (cm)	*0.125*	*0.125*	*0.25*	*0.25*	*0.5*	*0.5*	*1.0*	*1.0*
Strip speed (cm/sec)	*0.001*	*0.1*	*0.001*	*0.1*	*0.001*	*0.1*	*0.001*	*0.1*
Time const. (sec)[a]	150	1.5	220	2.2	350	3.5	700	7.0
p/v[b]	10	10	5	5	2.7	2.7	1.4	1.4
Min. range/$\sqrt{b}$[c]	83	830	67	670	53	530	39	390
% std. error $\times \sqrt{(\text{c.p.m.})}$[d]	69	690	49	490	35	350	25	250

[a] The time constant at which distortions due to slit width (eqn. 4) and time constant (eqn. 2) are equal.
[b] Peak-to-valley ratio (eqn. 3).
[c] Minimum full-scale range, divided by square root of background counting rate (eqn. 1).
[d] Per cent standard error of peak area, times the square root of the number of counts per minute resident in the peak (eqn. 5).

Table I gives data for operation at two strip speeds and four slit widths. The table is not intended to be used as it stands. It should be used to construct a series of graphs, on logarithmic graph paper, that are useful in selecting operating conditions. Fig. 6 is an example of such a graph, for a slit width of 0.5 cm. It was constructed, from Table I, for use with a scanner in our laboratory. This scanner has a background counting rate of 25 counts/min, and the line for minimum range was constructed accordingly. Strip speeds on the scanner are expressed as inches per hour; therefore the abscissal scale is in these units (0.1 cm/sec = 142 in./h). For convenience, data on error (last line of Table I) are shown for peaks having 100 and 1,000 resident counts per minute. Similar graphs, for other slit widths, can be easily made. It is apparent from the graph that if the standard error is to be kept below 5 % the maximum strip speed is about 30 in./h for a zone containing 1,000 counts/min. It is also apparent that strips of low activity, which require low ranges, also require long time constants. If the longest available time constant is 100 sec, ranges below 500 counts/min for full scale will give undesirable statistical count rate fluctuation.

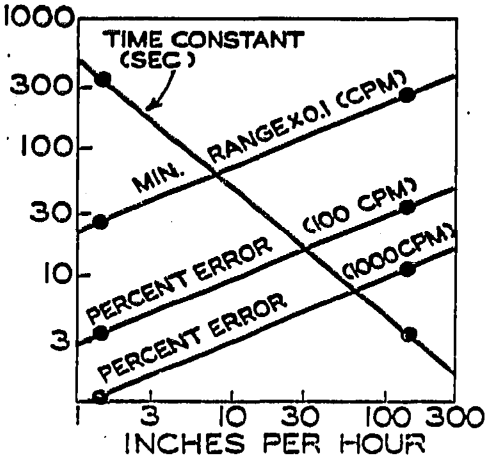


Fig. 6. Optimum operating conditions for a slit width of 0.5 cm. The curves are straight lines drawn as described in the text through points from the data in Table I for a slit width of 0.5 cm.

SUMMARY

Operating variables in the use of recording ratemeters for scanning radioactive paper chromatographic strips are discussed. Best sensitivity, and least statistical error in estimation of peak area, is obtained with a wide slit. However, wide slits give poor fidelity, that is, the shape of the record is not an accurate representation of the shape of the actual peak. Short time constants give a record showing undesirable fluctuations due to statistical count rate variations, while long time constants give poor fidelity. Slow strip speed is required for strips of low activity. Optimal selection of operating conditions is considered from a quantitative standpoint.

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