A RAPID METHOD FOR CALCULATION OF THE TOTAL AMOUNT OF ABSORBING SUBSTANCE IN MICROEXTRACTS

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INTRODUCTION

The amount of a light-absorbing substance in tissue components can be determined *in situ* or after extraction by photographic or photoelectric measurement of the absorption. In the case of an extract, spheres (1) or lens-shaped droplets (2) can be used as microcuvettes. With spheres, a determination of the total absorption is straightforward since the volume of the absorbance per unit length can be determined directly. In the second case, it has so far been necessary to integrate the absorption determined for about 40 points (2). We propose to approximate the densitometer curve (Fig. 2) of the lens-shaped drops of RNA extract (Fig. 1) as a parabola which would permit the use of a simple equation based on three measurements for determining absorption in this paraboloid-shaped "cuvette" model.

METHODS AND MATERIALS

RNA determinations were carried out on a series of mouse mesencephalic V cells (3) using Edström's analytical technique. The integration of the optical density of the lens-shaped drop was carried out with an integrating device and was compared to the calculation obtained with the parabola-volume formula.

RESULTS

The formula for calculating the RNA content in a photographed extract using the parabola-volume is described in equation 1.

$$X_3 = \frac{\frac{\pi y_1^2 x_1}{2} f^2 r}{ad} \qquad (1)$$

where X_3 = $\mu\mu$g of RNA in the extract; $\frac{\pi y_1^2 x_1}{2}$ = volume in cubed centimeters of the parabolic cone which represents the integrated optical density of the extract; where x_1 = altitude of the parabolic cone in centimeters; y_1 = one-half the diameter of the base of the parabolic cone in centimeters; f = magnification factor in microns to which 1 cm along the y axis of the densitometer curve corresponds; r = difference in units of optical density between each step of the step sector reference (to be used only if a linear relationship is demonstrated over the range of optical densities within the image of the drop); a = height in centimeters per step of the step sector reference on the densitometer trace; $d_{257m\mu}$ = 2.93 $\mu^2/10^{-12}$g RNA, i.e., the optical density per square micron at 257 mμ per 1 $\mu\mu$g of ribonuclease-digested RNA.

In using this equation for calculation, it is practical to group it into two parts, one consisting of the constants and the other consisting of the variables which are measured.

$$X_3 = \left[\frac{y_1^2 x_1}{a}\right]\left[\frac{\pi f^2 r}{2d}\right]$$

$$X_3 = \frac{y_1^2 x_1}{a} C$$

where C is constant for a given magnification, reference system, and wavelength.

Both variables x_1 and a can be measured accurately, directly from the mean densitometer

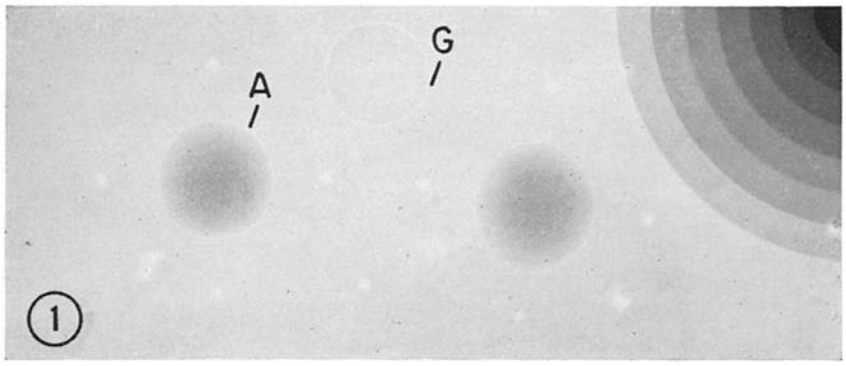

FIGURE 1 Two RNA extracts (*A*) from mouse mesencephalic V cells photographed with UV light at 257 mμ. Each drop contains the total RNA extracted from three cells. The glycerol drop (*G*) contains no RNA and is used for focusing and as a control for the density contribution due to light reflection by the liquid drop surface. The density reference, a step sector wheel, is seen at the edge. × 1.

curve.[1] However, it is difficult to get an accurate measurement of y_1 because of edge anomalies of the extract-densitometer curve (*a*, Fig. 2). A more accurate estimate can be made if y_0 is measured at some distance from the edge at x_0, and then the focal point, p, which is a constant for any given parabola, is calculated from equation 2 and substituted into equation 3.

$$p = \frac{y_0^2}{4x_0} \tag{2}$$

$$y_1 = 2\sqrt{px_1} \tag{3}$$

The use of $x_0 = \frac{3}{4}x_1$ has worked out satisfactorily for a wide variety of curves. Error in the measurement of y_0 due to curve asymmetry about the x axis is minimized by measuring $\pm y_0$ and dividing by 2 (Fig. 3).

$$y_0 = \frac{\pm y_0}{2} \tag{4}$$

Equation 1 can be simplified by combining equation (2) and (3):

$$y_1^2 = \frac{y_0^2 x_1}{x_0};$$

if $$x_0 = \tfrac{3}{4}x_1,$$

then $$y_1^2 = \tfrac{4}{3}y_0^2.$$

Substitution into equation 1:

$$X_3 = \frac{y_0^2 x_1}{a}\frac{4}{3}C. \tag{5}$$

This is compared to the equation used with the integration device (2):

$$X = \frac{\Sigma h s f^2 r}{ad} \tag{6}$$

where Σh = the sum of the mean zone heights (millimeters); s = the value in square centimeters of the central circular surface of the integration device; f, r, a, and d = same as in equation 1. Σh represents approximately 40 individual measurements per extract. Obtaining these is a time-consuming chore when this equation is used (see Fig. 3, reference 2).

Equation 5 was compared to equation 6 in three series of RNA determinations. This comparison is summarized in Table I. There is no significant difference either in the mean value for RNA content or in the variation, measured by the standard error of the mean, between the value obtained with the integrating device and the values obtained with the parabola-volume formula. The correlation coefficient between these two methods is essentially one. The application of the parabola-volume equation 5 is shown to be a simplified and useful method for calculating the total absorption in a lens-shaped cuvette.

[1] The mean densitometer curve is the curve of best fit, graphically drawn between two densitometer scans (Fig. 2) made perpendicular to each other across the circular extract (Fig. 1).

FIGURE 2 Two densitometric traces across perpendicular diameters of drop A seen in Fig. 1. The dip in the densitometer curve (*a*) is an edge anomaly produced by light refraction. × 1.

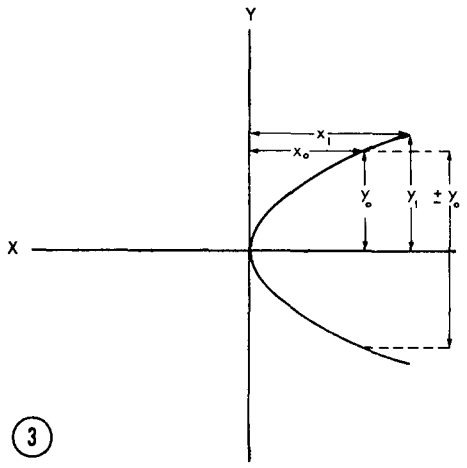

FIGURE 3 Diagram of parabola illustrating dimensions used in the parabola-volume equation.

DISCUSSION

One shape of curve for which the calculation obtained with the parabola equation does not correlate closely with the results obtained with the integrating device is that in which x_1 is greater than $3y_1$. That this could be due to difficulties of accurately measuring the mean zone heights (Σh) of steep curves implies that the parabola equation may give higher accuracy in these cases. The differences between the integration device and the parabola-volume equation range from 20 to 90 $\mu\mu$g RNA for these curves.

The shape of the densitometer curve is directly related to the absorbance of the drop of ribonucleic acid. From the Beer-Lambert law the absorbance varies with RNA concentration and path length through the drop. The path length through the drop is in turn a function of the contact angle.

TABLE I

Comparison of RNA Content Calculated by Parabola-Volume Formula and by Integration Device in Three Groups of RNase Extracts from Mouse Mesencephalic V Cells

	RNA content* $\mu\mu$g					
	Control		IDPN treated 2 wk		IDPN treated 5 wk	
	X‡	X_3§	X	X_3	X	X_3
n	38		24		30	
Mean ± SEM	700 ± 29	703 ± 29	689 ± 26	680 ± 27	714 ± 26	710 ± 28
Correlation coefficient, r	0.99		0.97		0.99	

* RNA content extracted from three mesencephalic V cells.

‡ X is the RNA content calculated with the integration device.

§ X_3 is the RNA content calculated with the parabola-volume formula. IDPN-iminodipropionitrile.

These two variables, RNA concentration and path length, determine the profile of the densitometer curve.

The contact angle is calculated from the Young-Dupré equation which is derived by equating the horizontal component of the surface tension forces.

$$\gamma_{1v} \cos \theta = \gamma_{sv} - \gamma_{s1}$$

Theoretically the contact angle and thereby the path length are constant under given conditions of liquid, surface, and vapor phases. However, in practice the contact angle varies over a given range (4). Presumably this is due to nonideal surface conditions. Experimentally we have measured the height at the apex of two drops of glycerol-phosphate buffer which have the same diameter. One, drop A, had a contact angle near the high value of the variation range, whereas the other, drop B, represented its low value. Measurement with the fine focus microscope control shows that drop A had a thickness of 36 μ at its apex while drop B had a thickness of 19 μ. The variation of path length through the drop, due to the variation of contact angle, is on the order of 1.5–2 times. Since the variation of height of the densitometer curve at its apex is on the order of 10 times, we conclude that the shape of the densitometer curve is principally due to a large variation of RNA concentration from drop to drop rather than to large differences in path lengths. Curves of $x_1 = 3y_1$ arise because the drop has an increased RNA concentration.

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