Simplified Turbidimetric Assay of Lipase in Dosage Forms

By FREDERICK P. SIEGEL and BERNARD ECANOW

A simplified turbidimetric procedure for the standardization of lipase activity in dosage forms is described. The procedure compares in accuracy to the best titrimetric methods and simplifies the handling of a large number of samples to be assayed.

THE ASSAY OF lipase has practical consideration in the evaluation of enzyme activity in dosage forms and in serum as a diagnostic for acute pancreatitis.

The principle lipase procedures employed today are titrimetric, based on the hydrolysis of a glyceride substrate. The subsequent neutralization of the free fatty acids by a standard sodium hydroxide solution is usually expressed in lipase units. A lipase unit represents the enzyme activity resulting in the hydrolysis of glyceride equivalent to the release of 50 microequivalents of fatty acid.

Most techniques require from several to 24 hours of incubation to permit a titratable amount of fatty acid to be liberated and are further complicated by a difficulty in assessing a visual end point in opaque emulsion substrates. Lazo-Wasem (1) recently applied a nonaqueous titration procedure and circumvented the incubation time and end point problem. However, the required benzene extractions minimize its use with large numbers of samples and cut down its practicability in the laboratory.

The turbidimetric assay is a simplified procedure which can be conducted in the spectrophotometer and generally consumes not more than 30 minutes for the total routine. The application of this assay to dosage forms is discussed.

EXPERIMENTAL

Reagents.—The stock emulsion of 50% olive oil in water is prepared by the continental method employing powdered acacia, olive oil, and distilled water. The emulsion is homogenized in a colloid mill, stored in a cool place, and allowed to age for 3 weeks prior to use.

A working emulsion is prepared by placing 50 ml. of the stock emulsion with 300 ml. of a 0.2 *M* tris buffer, pH 8.0, in a separator. The diluted emulsion is shaken over a period of several hours; it is then allowed to sit for a period of about 12 hours to permit maximum creaming. The stable emulsion is carefully removed from the cream.

The stable emulsion is then carefully diluted in increments, with 0.2 *M* tris buffer, and read in the Coleman Jr. spectrophotometer to assess the ultimate final dilution required to produce an absorbance of approximately 0.65. The measurements are made in a 19-mm. cell at a wavelength of 600 mμ with a water blank.

The remainder of the stable emulsion is then diluted according to calculations and allowed to sit overnight to permit possible further creaming as the result of the dilution.

The diluted emulsion should yield an O.D. value of not less than 0.60.

Received April 10, 1963, from the College of Pharmacy, University of Illinois at the Medical Center, Chicago.
Accepted for publication October 16, 1963.
This study was supported by a grant from the University of Illinois Research Board.
The authors gratefully acknowledge the supply of lipase preparations from the Wilson Laboratories, Chicago, Ill.

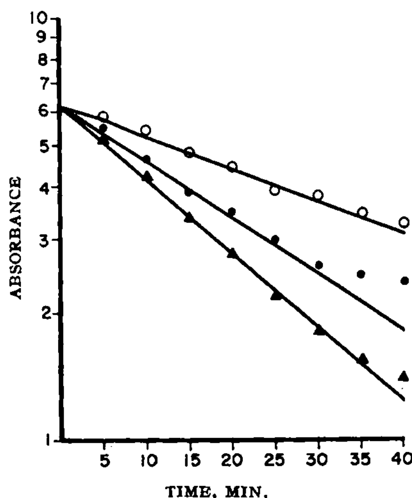

Fig. 1.—Semilogarithmic plot of absorbance of the reaction substrate *vs.* time. Key: ○, 0.6 u. lipase/ml.; ●, 1.0 u. lipase/ml.; and ▲, 1.4 u. lipase/ml.

Standard Curve.—Place 5 ml. of the working emulsion in 19-mm. Coleman cells. Add 1 ml. of a suspension containing 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 units of pancreatic lipase. Wilson's pancreatic lipase 3000 (3 u./mg.) was diluted in distilled water. The suspension was homogenized in a blender for about 1 minute.

A control is represented by 5 ml. of working emulsion plus 1 ml. of buffer. The point of addition of 1 ml. of enzyme suspension is designated zero time. The cell is mixed by inversion using a parafilm cover and read immediately as zero time. Absorbance readings are taken in intervals of several minutes at 600 mμ with a water blank. All operations were performed at 25°.

The logarithm of the absorbance will decrease linearly with time for approximately one half-time. This straight line region is employed to evaluate a half-time for each enzyme concentration. The half-time observed is inversely proportional to the enzyme concentration (Fig. 1 and Table I).

Methods.—In the analysis of an unknown, the lipase procedure entails the preparation of a suspension of the dosage form by homogenizing in the blender for about 1 minute, so as to yield a concentration of lipase in the approximate range of 0.5 to 1.0 u./ml.

A 1-ml. aliquot of the suspension is added to 5 ml. of the substrate. The tube is inverted several times and read as described under the *Standard Curve*. The half-time for the reaction of the un-

TABLE I.—STANDARD CURVE

u./1 ml. Aliquot	$T^{1}/_{2}$, Min.
1.4	17
1.2	20
1.0	24
0.8	30
0.6	40
0.4	65

TABLE II.—ASSAY OF LIPASE-CONTAINING DOSAGE FORMS

Dosage Form	U. Lipase per Dosage Form	U. Lipase[a] Titrimetric Assay (1)	U.[a] Turbidimetric
Lipase 3000 powder	3400 u./Gm.	3400	3400
Lipase 3000 powder	3000 u./Gm.	3000	3000
Enteric coated tablet A	120 u./tablet	110	108
Enteric coated tablet B	130 u./tablet	110	120
Capsules A	100 u./capsule	105	105
Capsules B	200 u./capsule	190	200
Capsules C	250 u./capsule	230	240
Powder paper A	100 u./paper	100	105
Powder paper B	200 u./paper	195	205
Powder paper C	250 u./paper	240	240

[a] Mean values ten assays.

known is inversely related to the half-time of the standards: ($T^1/_2$ unknown/$T^1/_2$ standard) = (concn. of standard/concn. of unknown). The following data are used to illustrate the assay.

A sample labeled to contain 200 u. of lipase was diluted to 200 ml. A 1-ml. sample of the above suspension produced a half-time of 20 minutes. The half-time for 1 u./ml. of standard enzyme is 24 minutes. Then $(20/24) = (1/X)$ and $X = 1.2$ u./ml. of lipase.

RESULTS AND DISCUSSION

Several commercial dosage forms were evaluated as well as several lipase concentrates employed in the manufacture of commercial dosage forms. Capsules and powder papers were also prepared from the concentrates in this laboratory and checked by this assay procedure (see Table II).

The rate of disappearance of the substrate turbidity is proportional to the release of fatty acids when compared to the method of Lazo-Wasem. Good recoveries of enzyme have been found with errors generally under 5%, but never exceeding 10%.

The substrate remains stable for a period of 2–3 weeks at 25°. It is advised that each new substrate batch be standardized with a standard lipase preparation, although duplications between batches have been good.

In assaying tablets of lipase the complete tablet is crushed and triturated to a fine powder. The material is then washed into a volumetric flask where it is brought to volume. The preparation is then homogenized in a blender.

One should be cautioned about the assay of lipase in a digestant tablet containing pepsin. The pepsin coat must be washed off prior to assay since the pepsin enzyme will inactivate lipase.

Caution in washing glassware is essential since it has been observed that certain surfactants will affect the rate of enzyme activity.

REFERENCE

(1) Lazo-Wasem, E. A., THIS JOURNAL, 50, 999(1961).

Sensitivity of Color Tests for Nitrites, Nitrates, and Glyceryl Trinitrate I. Solutions in Distilled Water

By JAMES C. MUNCH, MERCEDES FRANCO, and BERNARD FRIEDLAND

Threshold concentrations detected (limens) for nitrites, nitrates, and glyceryl trinitrate have been determined for solutions in distilled water with 16 reagents. Generally the most sensitive tests detected 10 to 100 parts per million of these products; except chromotropic acid detected 2 parts per million of nitrite.

NITROGLYCERIN (glyceryl trinitrate) investigations require delicate methods for the detection and determination of nitrites, nitrates, and glyceryl trinitrate in tissue fluids, viscera, and pharmaceutical products. Some hundred reagents have been suggested in the literature (1–22) but our investigations showed that only a few considered specificity and threshold limits for determination. Results with these reagents indicated that sixteen showed promise, although close attention to details are required for successful use with them. Based on findings in testing solutions in distilled water reported in Table I, additional tests are under way for determinations in various pharmaceuticals, as well as tissue fluids and viscera.

Preparation of Test Solutions.—A concentrated solution was prepared by dissolving: (*a*) 1.50 Gm. of reagent grade sodium nitrite; (*b*) 1.37 Gm. of reagent grade sodium nitrate; and (*c*) 10 Gm. of an analyzed mixture of 1 part glyceryl trinitrate and 9 parts of beta lactose, in distilled water and making up each volume to 100 ml. Just before use each stock solution was diluted from 1% (10,000 parts per million) to 5,000, 2,000, 1,000, 100, and 10 parts per million for test. The sodium nitrite solution was further diluted to 2 p.p.m. for the test with chromotropic acid. If a negative test was obtained within 5 minutes using a concentration of 10,000 p.p.m., additional tests were not undertaken. Such negative results are indicated by (10,000) in Table I. Otherwise, the figures represent the limen (threshold concentration detected) for each product with the chosen reagent. The colors recorded are the final colors observed at the end of 5 to 10 minutes.

Preparation of Reagent Solutions.—Reagent chemicals were obtained from Eastman Kodak Co., Rochester, N. Y. Solutions were prepared at the

Received July 3, 1963, from the Medical Department, Key Pharmaceuticals, Inc., Miami, Fla.
Accepted for publication October 22, 1963.
Presented to the Scientific Section, A.PH.A., Miami Beach meeting, May 1963.