Timed-Release Tablets Employing Lipase–Lipid–Sulfamethizole Systems Prepared by Spray Congealing

KARAMAT A. JAVAID*, JULIAN H. FINCHER, and CHARLES W. HARTMAN†

Abstract □ *In vitro* dissolution profiles of some spray-congealed formulations of sulfamethizole, incorporated in a lipid–lipase matrix and compressed into tablets, are reported. The formulations employ a pancreatic lipase–glyceryl trilaurate and glyceryl tristearate system, with enzyme–substrate combinations serving as a release-controlling vehicle to produce a timed-release effect. The granules made by spray congealing were compressed into tablets, and *in vitro* dissolution tests were carried out in simulated fluids. The main portion of the drug was released through the hydrolysis of substrate by lipase, and the amount of drug released after 1.5 hr. was dependent upon the lipase activity measured in the form of percent hydrolysis of substrate. Effects of lipase accelerators such as calcium ions and glyceryl monostearate on lipase activity were evaluated in terms of drug release. Different concentrations (2, 5, and 8% w/w) of calcium carbonate increased the drug release. Lower concentrations (1, 2, and 5% w/w) of glyceryl monostearate increased while higher concentrations (7 and 10% w/w) decreased the drug release.

Keyphrases □ Timed-release tablets—spray-congealed lipase–lipid–sulfamethizole systems □ Lipase–lipid–drug system—effect of lipase accelerators on drug-release rates □ Drug-release rates—lipase–lipid–sulfamethizole systems □ Calcium—as lipase accelerator, effect on drug release from timed-release tablets □ Glyceryl monostearate—as lipase accelerator, effect on drug release from timed-release tablets

The physiological functions of the GI tract are the digestion and absorption of nutrient materials and medicaments. Many physiological factors may influence the release and absorption of drug from the GI tract; one such factor is the effect of digestive secretions of the alimentary canal. The overall problem in standardizing absorption from oral dosage forms involves overcoming the variability of the physiological, physical, and chemical properties of the GI tract. The most practical and successful methods of controlling intensity and duration of drug action depend on the control of release and absorption. Timed release of a drug can possibly be obtained by incorporating the drug in an enzyme–substrate matrix. Brewton (1) showed that triamcinolone release can be controlled from encapsulated granules employing lipase–lipid–drug combinations. Lybrand (2) demonstrated the effect of proteinase–protein–drug systems on the dissolution rates of sulfanilamide tablets.

The purposes of this investigation were: (*a*) to prepare a timed-release sulfamethizole tablet employing a spray-congealed lipase–lipid–drug system, and (*b*) to study the release of drug controlled by the digestion of substrate by lipase in the formulation.

Table I—Quantities, in Milligrams, of Ingredients in 200 mg. of Spray-Congealed Drug–Lipid Granules

Formulation	Mesh Size	Sulfamethizole	Glyceryl Tristearate	Glyceryl Trilaurate	Lipase
A	$^{23}/_{35}$	63.50	86.35	40.63	9.52
B	$^{23}/_{35}$	66.67	90.67	42.66	—
C	$^{35}/_{50}$	63.50	86.35	40.63	9.52
D	$^{35}/_{50}$	66.67	90.67	42.66	—

EXPERIMENTAL

Manufacture of Spray-Congealed Granules—The composition of lipase–lipid–drug granules made by spray congealing was as follows:

glyceryl trilaurate[1]	16.00 g.
glyceryl tristearate[2]	34.00 g.
sulfamethizole NF[3]	25.00 g.
crude lipase[4] (5% w/w of substrate and drug)	
calcium carbonate USP[5] (0, 2, 5, and 8% w/w)	
glyceryl monostearate[1] (0, 1, 2, 5, 7, and 10% w/w)	

The hot mixture of lipid materials, sulfamethizole (7.4 μ), lipase powder, and any additive (if used) was prepared in a glass beaker immersed in a boiling water bath. The slurry was then poured into a separator previously heated to 100° by a heating pad. The mixture was spray congealed in a laboratory spray dryer[6] at room temperature by running through a centrifugal wheel atomizer (5 cm. in diameter) preheated to 110° for 3 hr. in an oven. Compressed air at a pressure of 6 kg./cm.^{2} rotated the atomizer at about 35,000 r.p.m., and the rate of atomization by centrifugal disk atomizer was approximately 100 g./min. The moisture content of spray-congealed granules determined on a moisture balance[7] ranged between 0.3 and 0.8% w/w. The granules were sized into $^{23}/_{35}$ and $^{35}/_{50}$ mesh for dissolution-rate studies. The composition of the granule formulations studied is given in Table I.

Spray congealing was used in the formulation of lipase–lipid–drug granules to minimize the possible thermal inactivation of lipase. Bullock (3) and Wilkinson *et al.* (4) reported that various spray-dried enzymes can be prepared without loss of activity using a 120° air current. A lipase powder with 4.4% moisture was stable in the oily suspension when heated to 100° for 1 hr. (3). The inactivation of lipase depends not only on the moisture content of the powder but also on the freedom with which the moisture can escape. During the preparation of lipid–lipase–drug melt, the suspension was stirred to facilitate the escape of moisture. The moisture content of the lipid–lipase–drug granules prepared was below 0.9%. The lipase in lipid melt was stable since the final temperature of the melt was 98° after the 65 min. of heating required for its preparation.

Preparation of Tablets—The ingredients and the quantities used in the manufacture of the tablets are listed in Tables II and III. A $^{23}/_{50}$-mesh portion of granules was used to compress a 200-mg. tablet on a hand-operated tablet press[8]. Double-layered tablets, containing 152 mg. of drug–lipid and 48 mg. of initial-release granules, were also compressed. The initial-release granules were composed of 33.33% drug, 40% lactose, and 13.33% each of starch and sucrose. The amount of sulfamethizole in a 200-mg. tablet ranged between 57.97 and 66.67 mg., depending on different amounts of various additives. The weight of individual tablets was checked, and tablets were checked with a Stokes hardness tester to ensure that the hardness was between 6 and 7 kg.

[1] C. P. Hall Co.
[2] Sterotex HM, The Capital City Products Co.
[3] C. M. Bundy Co.
[4] Reheis Chemical Co.
[5] Fisher Scientific Co.
[6] Nerco Niro, Nichols Engineering and Research Corp.
[7] Ohaus, model 6000.
[8] Erweka tablet press, Type EKO.

Table II—Quantities, in Milligrams, of Ingredients in a 200-mg. Single-Layered Tablet Made with $^{23}/_{50}$-Mesh Spray-Congealed Drug–Lipid Granules

Formulation	Sulfamethizole	Glyceryl Tristearate	Glyceryl Trilaurate	Lipase	Calcium Carbonate	Glyceryl Monostearate
E	63.50	86.35	40.63	9.52	—	—
F	66.67	90.67	42.66	—	—	—
G	62.30	84.73	39.88	9.35	3.74	—
H	65.36	88.89	41.83	—	3.92	—
I	60.61	82.42	38.79	9.09	9.09	—
J	63.49	86.35	40.64	—	9.52	—
K	59.00	80.23	37.76	8.85	14.16	—
L	61.73	83.95	39.51	—	14.81	—
M	62.89	85.54	40.25	9.43	—	1.89
N	66.01	89.77	42.24	—	—	1.98
O	62.31	84.73	39.87	9.35	—	3.74
P	65.36	88.89	41.83	—	—	3.92
Q	60.61	82.42	38.79	9.09	—	9.09
R	63.49	86.35	40.64	—	—	9.52
S	59.52	80.95	38.10	8.93	—	12.50
T	62.31	84.73	39.88	—	—	13.08
U	57.97	78.84	37.10	8.70	—	17.39
V	60.61	82.42	38.79	—	—	18.18
W	61.16	83.18	39.15	9.17	3.67	3.67
X	64.10	87.18	41.02	—	3.85	3.85

Dissolution Studies—The rotating-bottle method, described by Souder and Ellenbogen (5), was used for testing the *in vitro* release of the drug. An accurately weighed amount of 200 mg. of drug–lipid granules or one 200-mg. tablet was placed in each of six bottles containing 75 ml. of dissolution medium. The bottles were rotated at 40 r.p.m. in a water bath maintained at 37 ± 1°. The dissolution media used were simulated gastric fluid (6) (pH 1.2) for the first 1.5 hr. and then simulated intestinal fluid (pH 8.3) (7) for up to 12.5 hr. One bottle was removed from the bath at 0.5-, 1.5-, 3-, 6-, 10-, and, occasionally, 12.5-hr. intervals, and a portion of the fluid was filtered.

A 0.5-ml. portion of filtrate was analyzed using a modified Bratton–Marshall (8) assay procedure for sulfonamides. The percent transmittance of the resulting solution was determined on a colorimeter[9] at 545 nm. and compared to a standard curve. Since tablets contained different amounts of drug, the release was calculated on the basis of percent drug released from the dosage form. Duplicate dissolution tests were conducted on two formulations prepared separately using the same formula, and an average percent drug release is used for reporting the results. The variation between the two release values from all the formulations containing lipase was less than ±9.2%, whereas the variation from formulations without lipase ranged up to ±10.2%. A formulation blank without lipase was tested simultaneously to account for the release due to leaching and surface dissolution in addition to the main release caused by the lipolytic digestion of substrate.

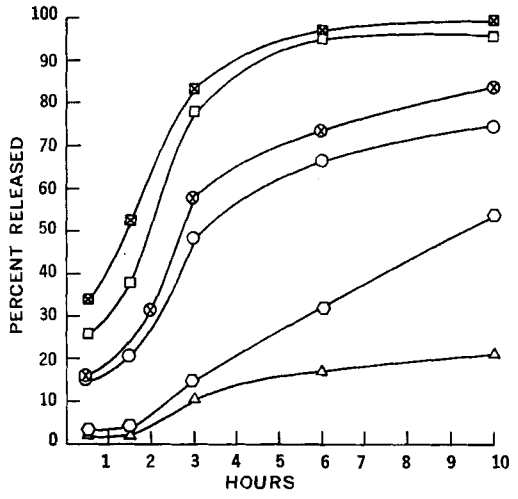

Figure 1—*Percent of sulfamethizole released from granules and tablets during* in vitro *dissolution. Key:* □, *Granules A;* ○, *Granules B;* ⊠, *Granules C;* ⊗, *Granules D;* ○, *Tablet E; and* △, *Tablet F.*

[9] Spectronic 20, Bausch & Lomb.

Determination of Percent Hydrolysis—During the dissolution-rate studies, a determination of lipid hydrolysis was made in simulated intestinal fluid (after the tablets were in the simulated gastric fluid for 1.5 hr.), using a modification of the Lazo-Wasem method (9) to establish a correlation between the amount of drug released and the percent hydrolysis. Determinations for preparations with and without lipase were carried out simultaneously to account for the free fatty acids present in the lipid substrate used in formulation. A 25-ml. portion of fluid was measured from the sample bottle at each interval. To this portion, 1 ml. of concentrated hydrochloric acid was added to destroy any lipase, if present. The solution was shaken with 50 ml. of benzene for 30 min. to extract fatty acids into the benzene layer. The flasks containing the mixtures were stored in a refrigerator to facilitate the separation of two layers. A 25-ml. portion of the upper benzene layer was titrated with 0.055 *N* NaOH solution in methanol, and percent hydrolysis was calculated as follows:

$$\text{percent hydrolysis} = \frac{\text{meq. substrate hydrolyzed} \times 100}{\text{meq. substrate used}} \quad \text{(Eq. 1)}$$

RESULTS AND DISCUSSION

A timed-release formulation was prepared by spray congealing lipase–lipid–drug combinations and compressing the granules into tablets. Lipase causes the controlled digestion and erosion of the substrate and subsequent release of the embedded drug particles.

To determine the effect of granule size on the rate of drug release, granules of $^{23}/_{35}$ and $^{35}/_{50}$ mesh were tested. Figure 1 shows that there was essentially no difference in the rate of release between Granules A and C and Granules B and D, except during the first 3 hr. where faster dissolution occurred in both formulations. The more rapid drug release in the first 3 hr. was due to the effect of leaching and surface dissolution of partially exposed and some free drug on the granules. The difference in the amount of drug released from formulations with and without lipase ranged approximately from 10 to 30% in both $^{23}/_{35}$- and $^{35}/_{50}$-mesh granules. The difference, which is relatively larger after 1.5 hr., is indicative of the release caused by the lipolytic digestion of substrate in alkaline pH. Further studies with granules were discontinued because the release from granules with lipase was only about 62% higher than the release from granules without lipase at the point of biggest difference; the release was 153% higher from tablets with lipase as compared to tablets without lipase. The difference in drug release from granules was probably due more to the effect of surface dissolution, while for tablets it was due more to the digestive effect of the lipase on the substrate.

Table III—Quantities, in Milligrams, of Ingredients in a 200-mg. Double-Layered Tablet Made with Spray-Congealed Drug–Lipid Granules and Initial-Release Granules

Formulation	Drug–Lipid Granules					Initial-Release Granules			
	Sulfamethizole	Glyceryl Tristearate	Glyceryl Trilaurate	Lipase	Glyceryl Monostearate	Sulfamethizole	Lactose	Starch	Sucrose
Y	47.35	64.40	30.31	7.10	2.84	16.00	19.20	6.40	6.40
YF	49.67	67.56	31.79	—	2.98	16.00	19.20	6.40	6.40
Z	46.06	62.64	29.48	6.91	6.91	16.00	19.20	6.40	6.40
ZF	48.25	65.63	30.88	—	7.24	16.00	19.20	6.40	6.40

Figure 1 shows that about 90% of the drug was released within 4 hr. from Granules A and C containing lipase. However, when the granules were compressed into tablets, the release was extended more than 10 hr. Figure 1 also shows the percent of sulfamethizole released from Tablets E and F made from $^{23}/_{50}$-mesh granules with and without lipase. The slow rate of drug release from tablets is due to the compaction, decreased porosity, and reduced surface area caused by the tableting of granules. A difference of about 33% drug release between Tablets E and F at 10-hr. periods demonstrates that lipolytic action resulting in the digestion of substrate in the tablet causes increased drug release.

It was reported in the literature that calcium ions accelerate the action of lipase on triglycerides (10). Wills (11) reported that calcium helps to stabilize the activity of lipase. Experiments were carried out to determine the effect of calcium ions on lipase activity in terms of drug release. As seen in Fig. 2, no increase in drug release was observed in acidic medium up to 1.5 hr. because lipase is not activated until it reaches alkaline pH. An increase in the percentage of sulfamethizole released from Tablets G, I, and K containing lipase and various amounts of calcium carbonate was noted in simulated intestinal fluid as compared to the release from Tablet E, whereas no increase was observed from Tablets H, J, and L containing various amounts of calcium carbonate but no lipase. Since the percent drug release from Tablets H, J, and L containing various concentrations of calcium carbonate was approximately the same, only one curve was drawn in Fig. 2 to represent the drug release from all of the blank formulations. Since the amount of sulfamethizole released after 1.5 hr. was primarily related to the rate of hydrolysis, the marked increase in the drug release demonstrated that calcium ions exert an accelerating effect on the rate of hydrolysis. This finding is in agreement with the results of Brewton (1) and Wills (11).

In vitro studies showed that glyceryl monostearate accelerates lipolysis by emulsifying the insoluble fats and oils in alkaline pH, thus exposing a larger surface area for lipase action (12). Experiments were carried out to determine the effectiveness of glyceryl monostearate as a lipase accelerator by studying the rate of drug release. Figure 3 shows that the release from Tablets M, O, and Q, containing 1, 2, and 5% glyceryl monostearate with lipase, respectively, increased in simulated intestinal fluid; the release from Tablets S and U, containing 7 and 10% glyceryl monostearate, respectively, decreased as compared to the release observed from Tablet E containing lipase but no glyceryl monostearate. The decrease in drug release at 7 and 10% concentrations of glyceryl monostearate may be due to the micellar solution entrapment of the drug. No increase in drug release was noted in the formulations containing lipase and various concentrations of glyceryl monostearate in acidic medium up to 1.5 hr. With 1–10% concentrations of glyceryl monostearate in Tablets N, P, R, T, and V without lipase, no increase was observed in the amount of drug released. Since the percent drug release from Tablets N, P, R, T, and V containing various concentrations of glyceryl monostearate was approximately the same, only one curve was drawn in Fig. 3 to represent the drug release from all of the blank formulations.

The possibility existed that differences in the amount of drug release from formulations with and without lipase might be due to some nonenzymatic effect of lipase *per se, i.e.*, solubility. However, heat-inactivated lipase and albumin substituted for lipase gave a release similar to the formulations containing no lipase.

Since the drug is released mainly through the hydrolysis of the substrate by lipase in addition to the release by surface dissolution and leaching, it was desirable to know some empirical relationship between the percent hydrolysis and the corresponding drug release. Figure 4 shows that the percent drug release was dependent upon the percent hydrolysis of the substrate in tablets.

Since the drug was not being released in sufficient quantity in the first 1.5 hr., an initial-release portion of the drug was incorporated by employing a double-layered tablet using a sugar and starch granulation. Figure 5 presents the drug release from formulations made from drug–lipid granules containing 2% glyceryl monostearate and initial release granules with 25% of the total drug in a tablet. In both Tablets Y and YF, with and without lipase, respectively, a release of about 28% was observed in the first 1.5 hr. due to the drug in the initial-release granules. After 10 hr. of the digestion period, 99.1% of the drug was released from Tablet Y containing lipase as compared to

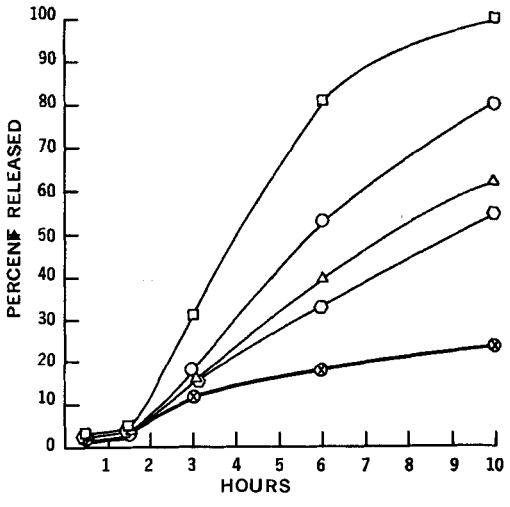

Figure 2—*Percent of sulfamethizole released from tablets containing various concentrations of calcium carbonate during* in vitro *dissolution. Key:* ○, *Tablet E;* △, *Tablet G, 2%;* ○, *Tablet I, 5%;* □, *Tablet K, 8%; and* ⊗, *Tablets H, J, and L, 2, 5, and 8%.*

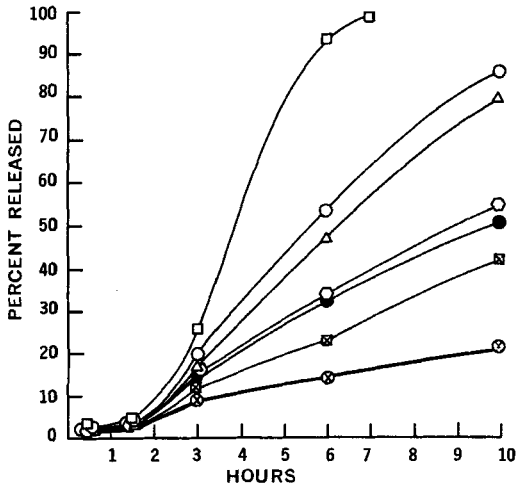

Figure 3—*Percent of sulfamethizole released from tablets containing various concentrations of glyceryl monostearate during* in vitro *dissolution. Key:* ○, *Tablet E;* □, *Tablet M, 1%;* ○, *Tablet O, 2%;* △, *Tablet Q, 5%;* ●, *Tablet S, 7%;* ⊠, *Tablet U, 10%; and* ⊗, *Tablets N, P, R, T, and V, 1, 2, 5, 7, and 10%.*

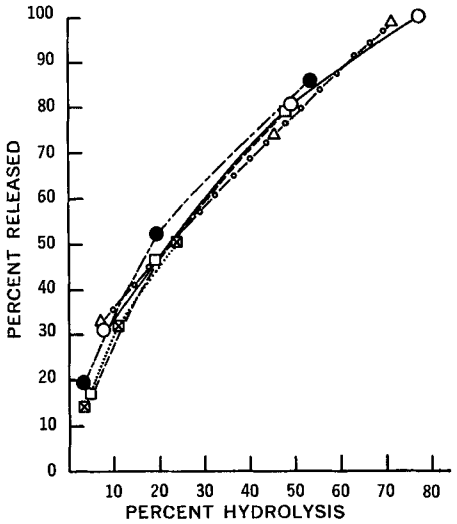

Figure 4—*Correlation of percent hydrolysis of substrate in tablets with percent of sulfamethizole released during* in vitro *dissolution at 3-, 6-, and 10-hr. periods. Key:* ○, *Tablet K, 8% calcium carbonate;* ●, *Tablet O, 2% glyceryl monostearate;* □, *Tablet Q, 5% glyceryl monostearate;* ⊠, *Tablet S, 7% glyceryl monostearate; and* △, *Tablet W, 2% calcium carbonate and 2% glyceryl monostearate.*

46.1% from Tablet YF without lipase. In previous experiments with different amounts of glyceryl monostearate, it was found that higher concentrations of glyceryl monostearate delayed the drug release. Based on this finding, the amount of glyceryl monostearate was increased to 5% in drug–lipid granules to extend the release to a 12-hr. period. The amount of drug released from Tablets Z and ZF, with and without lipase, respectively, was 98.9 and 47.4%, respectively, after a 12.5-hr. digestion period (Fig. 5).

From the present studies carried out on sulfamethizole–lipid–lipase preparations, it appears that adjustment of the lipase–lipid ratio and other lipase accelerators could be used to prepare timed-release dosage forms with desired release patterns.

SUMMARY AND CONCLUSIONS

1. *In vitro* dissolution rates of some timed-release, spray-congealed formulations of sulfamethizole, pancreatic lipase, lipid substrate, and certain lipase accelerators such as calcium carbonate and glyceryl monostearate were tested.

2. The release of the drug from the granules of $^{23}/_{35}$ and $^{35}/_{50}$ mesh was essentially the same except during the first 3 hr. where faster dissolution was observed. The granules were compressed into tablets so that the release was extended more than 10 hr.

3. The addition of various concentrations (2, 5, and 8%) of calcium carbonate to the drug–lipid granules progressively increased the drug release from tablets containing lipase, whereas no increase was noticed from tablets containing the same amount of calcium carbonate but no lipase.

4. Lower concentrations (1, 2, and 5%) of glyceryl monostearate together with lipase increased the drug release, while concentrations of 7 and 10% decreased the drug release as compared to the release from the tablets containing lipase only. The drug release from tablets containing various amounts of glyceryl monostearate without lipase was essentially the same as the release from the tablets without lipase (blank formulations).

5. The percent drug release was dependent upon the percent hydrolysis after 1.5 hr., during which time the release was due more to the effect of lipase than to surface dissolution and leaching.

6. With the incorporation of 25% drug in initial-release granules and 75% drug in drug–lipid granules containing 2% glyceryl monostearate, the release was extended up to 10 hr.

7. The drug release from tablets containing 25% drug in initial release granules and 75% in drug–lipid granules with 5% glyceryl monostearate was extended up to 12.5 hr.

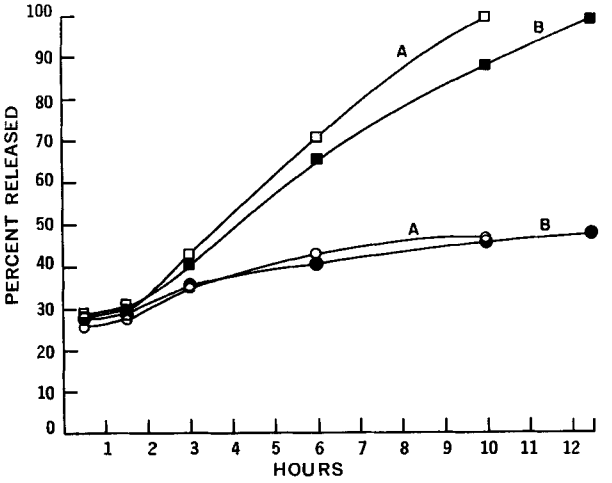

Figure 5—*Percent of sulfamethizole released from double-layered tablets made from drug–lipid granules containing: A, 2% glyceryl monostearate; and B, 5% glyceryl monostearate and initial release granules with 25% of sulfamethizole in a tablet during* in vitro *dissolution. Key:* □, *Tablet Y;* ■, *Tablet Z;* ○, *Tablet YF; and* ●, *Tablet ZF.*

8. The present studies showed that some additives such as calcium carbonate and glyceryl monostearate could affect the rate of drug release by changing the activity of lipase. By using a proper additive in appropriate concentration to control the digestion of the substrate by the lipase and by choosing the optimum lipase–lipid ratio, a timed-release system with a desired release pattern possibly could be formulated.

REFERENCES

(1) E. S. Brewton, dissertation, Graduate School, University of Mississippi, University, Miss., 1964.

(2) R. A. Lybrand, dissertation, Graduate School, University of Mississippi, University, Miss., 1966.

(3) K. Bullock, *Quart. J. Pharm. Pharmacol.*, **20,** 299(1947).

(4) J. Wilkinson, K. Bullock, and C. Cowan, *Lancet*, **242,** 281 (1942).

(5) J. C. Souder and W. C. Ellenbogen, *Drug Stand.*, **26,** 77 (1958).

(6) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 1026.

(7) M. J. Robinson, A. Bondi, Jr., and J. V. Swintosky, *J. Amer. Pharm. Ass., Sci. Ed.*, **47,** 874(1958).

(8) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128,** 537 (1939).

(9) E. A. Lazo-Wasem, *J. Pharm. Sci.*, **50,** 999(1961).

(10) P. Desnuelle, M. Naudet, and M. J. Constantin, *Biochim. Biophys. Acta*, **5,** 561(1950).

(11) E. D. Wills, *ibid.*, **40,** 481(1960).

(12) A. C. Frazer and H. G. Sammon, *J. Physiol.*, **103,** 5(1944).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 15, 1971, from the *Department of Pharmaceutics, School of Pharmacy, University of Mississippi, University, MS 38677*

Accepted for publication July 29, 1971.

Abstracted in part from a dissertation presented by K. A. Javaid to the Graduate School, University of Mississippi, in partial fulfillment of Doctor of Philosophy degree requirements.

The authors acknowledge the assistance of Dr. Donald E. Cadwallader in the preparation of this manuscript.

* Present address: School of Pharmacy, University of Georgia, Athens, GA 30601

† Dean, School of Pharmacy and Professor of Pharmaceutics. Deceased April 1970.