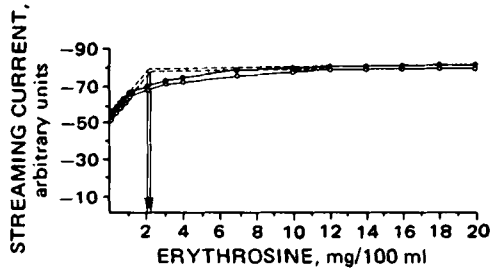

Figure 8—*Solution (●) and dispersion (○) streaming current curves for the microcrystalline cellulose–erythrosine system.*

solution curve. Typically, the dispersion curve would be below the solution curve. The reasons for this behavior are not clear, but a number of aspects of the adsorption behavior of II by III might be contributing factors. There was a drop in the aqueous dispersion pH at higher II concentrations, as discussed previously. The amount of adsorption was quite high. In measurements with the detector, the streaming current values for dispersions represent the net effect of the shearing of the double layers formed on the boot and piston surfaces as well as the double layers surrounding the dispersed particles. The contribution to the measured streaming current values from the double layers surrounding the dispersed particles may be substantially higher for dispersions with high adsorption capacity. Furthermore, higher dye concentrations were used for streaming current measurements than for adsorption isotherm measurements. At these concentrations, the dye would be expected to be in the dimer and trimer forms, with a corresponding effect on the nature of the double layer.

For the III–I system, the dispersion curve was only slightly different from the solution curve (Fig. 7). The adsorption capacity calculated from the streaming current data was also substantially lower than that estimated from the value of k_2, the Langmuir constant for the system. Where there was no adsorption, the solution and dispersion curves were similar, as shown in Fig. 8 for the IV–I system.

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Microencapsulation of Bitolterol for Controlled Release and Its Effect on Bronchodilator and Heart Rate Activities in Dogs

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Abstract □ Spheronized cores produced by extrusion and marumerization were microencapsulated with ethylcellulose by organic phase separation to produce beads exhibiting controlled-release characteristics. *In vitro* dissolution studies indicated that the drug was released as a first-order model and that the release rates were proportional to the amount of film on the bead. The bronchodilator activity in the anesthetized dog and the heart rate effect in the unanesthetized trained dog were evaluated. Microencapsulated beads were prepared which produced controlled release as assayed by bronchodilation. The heart rate increases induced by the controlled-release formulations were gradual in onset, and the total increase in heart rate over a 6-hr period was less than that associated with the plain drug powder.

Keyphrases □ Bitolterol—microencapsulation for controlled release, effects on bronchodilator and heart rate activities, dogs □ Microencapsulation—bitolterol, controlled-release characteristics assessed, effects on bronchodilator and heart rate activities, dogs □ Dosage forms—controlled-release bitolterol formulations prepared by microencapsulation, effects on bronchodilator and heart rate activities, dogs

The properties of bitolterol, an ester of *N-tert*-butylarterenol, as a long-acting bronchodilator with reduced cardiac effects, were recently reported (1, 2). Additional improvement in the duration of action and in the reduction of side effects was sought by preparing material exhibiting a controlled release.

Microencapsulation has been applied successfully to many areas of pharmaceutical interest. Microencapsulation as a unit operation was described and reviewed (3), as was the patent literature (4). An informative history of microencapsulation and its uses was presented (5). Most pharmaceutical work has been with irregularly shaped granules of powders or dispersed liquids, crystals, or sized granulations (6–16). This study concerns regular spherical cores in the 500–1000-μm range, encapsulated by organic phase separation.

Spherical particles were prepared (17) using an extrusion technique followed by spheronization in a marumerizer. Briefly, the drug and suitable excipients are moistened thoroughly with granulating agents and water until a damp mass is formed. The material is then passed through an extruder to produce strands of uniform diameter. The extruded material is introduced into the chamber

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Table I—Marumerized Core Formulations by Percent Composition

Ingredient	Core A	Core B	Core C
Bitolterol methanesulfonate	5.00	12.08	12.08
Lactose USP, regular, hydrous	57.00	50.38	47.92
Microcrystalline cellulose	19.00	18.67	20.00
Starch USP	16.00	15.87	17.00
Starch as 15% paste	3.00	3.00	3.00
Total	100.00	100.00	100.00
Batch size, g	3000	6500	12,000

of a marumerizer where the strands are broken into short rods and rolled on a spinning friction plate to form spheres.

The effect of the several process variables on the resulting product, such as the rotational speed of the friction plate and the residence time in the spheronizing chamber, was studied (18); in addition, an evaluation was made (19) of the effects of granulation moisture content, extruder screen size, and extruder speed. These studies found that particular physical qualities must be developed during processing to produce uniform solid spheres: the material must be soft enough to extrude, cohesive enough to produce strands, sufficiently friable that the strands break into short rods when placed in the spheronization chamber, and plastic enough that the short rods are converted to regular spheres when rolled.

Preliminary experiments were conducted to develop formulations of bitolterol that could be extruded and spheronized to produce uniform cores. The cores were subsequently microencapsulated with ethylcellulose to prepare coated beads. The dissolution characteristics of several products were evaluated in 0.1 *N* HCl, and relationships between the calculated film thicknesses and corresponding release rates were developed. The bronchodilator activity in the anesthetized dog and the heart rate effects in the unanesthetized trained dog were evaluated for representative products according to an established method reported for isoproterenol (20, 21).

EXPERIMENTAL

Materials—Bitolterol[1] (α-[(*tert*-butylamino)methyl]-3,4-dihydroxybenzyl alcohol 3,4-di-*p*-toluate methanesulfonate) was pharmaceutical grade with a mean particle size of 12 μm. The core formulations were compounded of commonly used excipients including microcrystalline cellulose USP[2], lactose USP[3] (regular, hydrous), and starch USP[4]. The encapsulating medium was composed of ethylcellulose USP[5] (100 cps), polyethylene[6] (pharmaceutical grade), and cyclohexane[7] (practical grade). The dissolutions were conducted in 0.1 *N* HCl[8] (analytical grade).

Core Preparation—The formulations for the core materials are presented in Table I. Powders were passed through an oscillator[9] fitted with a 30-mesh screen and blended in a twin-shell dry blender[10]. The material was transferred to a planetary mixer[11] and granulated with starch paste; sufficient additional water was incorporated to make a very damp mass.

The material was then extruded[12] through 1.0-mm screens and spheronized in a marumerizer[13] operating at 400 ± 50 rpm. The spheronized cores were dried in a hot air oven at 40° until the moisture was no more than 2% as determined by a moisture balance[14]. After drying, the cores were classified to remove particles smaller than 40 mesh (420 μm) or larger than 16 mesh (1190 μm)[15]. About 95% yields were obtained.

Microencapsulation—Compositions of the microencapsulation schemes are shown in Table II. In a typical laboratory batch, ethylcellulose and polyethylene were dissolved in hot cyclohexane in a three-necked, round-bottom flask equipped with a thermometer, stirrer, and reflux condenser. The prepared cores were introduced into the system and suspended by rapid agitation while the temperature was brought to reflux (about 78°) for 5 min. Heating was then discontinued, and the system was allowed to cool; stirring was continued until 40° was reached.

During this cooling period, the ethylcellulose phase separated and coated the suspended particles. The polyethylene was present to limit ethylcellulose solubility in the solvent, thus causing the film to be formed at a higher temperature where its viscosity was low enough to allow each particle to be uniformly coated. The encapsulated beads were washed with three portions of cold cyclohexane to remove residual ethylcellulose and polyethylene. The product was then allowed to air dry at ambient temperature.

Initial studies were made with material encapsulated in small batches in this laboratory using Core A. Later, encapsulations were scaled up[16] to test the feasibility of producing larger lots; a more concentrated core formula, representing 10% bitolterol as the base (120.8 mg of methanesulfonate salt = 100.0 mg of bitolterol base), was used.

Dissolution Studies—A modification of the USP rotating-basket method (22) was used to examine the dissolution patterns of each core and encapsulated bead preparation. A quantity of cores or beads containing 15 mg of bitolterol was placed in the basket and immersed in 250 ml of 0.1 *N* HCl at 37°, with the basket rotating at 100 rpm. Samples, 5 ml, were withdrawn every 15 min for the 1st hr, every 30 min during the 2nd hr, and hourly thereafter. Sample replacement was made with 5 ml of 0.1 *N* HCl to maintain a constant volume.

After appropriate dilutions were made, samples were read at 247 nm using a UV-grating spectrophotometer[17]. A separate analysis for total drug content was made by crushing a sample of beads in a mortar and pestle, extracting the drug with 0.1 *N* HCl, and reading the solution as before.

Ethylcellulose Assay—Ethylcellulose present in the microencapsulated bead products was assayed by a residue weight method. Approximately 5 g of microencapsulated beads was finely powdered in a mortar and weighed into a 250-ml flask to which 100 ml of chloroform[18] was added. The mixture was shaken for 30 min and filtered. Fifty milliliters of filtrate was evaporated to dryness and a constant weight in a 105° oven. A 3-ml sample of the remaining filtrate was assayed for bitolterol to correct for extracted drug present in the ethylcellulose residue.

Particle-Size Analysis—A sample of beads was suspended in a drop of immersion oil[19] on a microscope slide and examined through an image-splitting eyepiece at a magnification of 35×. The horizontal diameters of at least 500 particles from each product were measured and classified into appropriate 50-μm intervals from which the volume–surface distributions were constructed.

Determination of Film Thickness—No direct method of measuring the thickness of the applied films around the hard cores proved satisfactory. The beads shattered or were dislodged from the embedding matrix by all microtome techniques attempted. For purposes of batch characterization, it was possible to estimate the average thickness of the film for the ideal case (assuming perfect sphericity and film uniformity) by calculation. There is good indication (23) that the thickness of an applied film is fairly constant over a broad range of particle sizes simultaneously microencapsulated and is independent of the core diameters within that range.

The film content of many systems can be determined by a direct analysis of the encapsulated beads, either by assaying for the film itself

[1] Winthrop Laboratories, New York, N.Y.
[2] Avicel PH 101, American Viscose Division, FMC Corp., Newark, Del.
[3] Foremost Foods Co., San Francisco, Calif.
[4] Hubinger Co., Keokuk, Iowa.
[5] Hercules Inc., Wilmington, Del.
[6] Epolene Wax, C-10, Eastman Chemical Products, Kingsport, Tenn.
[7] Matheson, Coleman & Bell, Norwood, Ohio.
[8] J. T. Baker Chemical Co., Phillipsburg, N.J.
[9] Model 43B, F. J. Stokes Corp., Philadelphia, Pa.
[10] Patterson-Kelly Co., East Stroudsburg, Pa.
[11] Model A-200, Hobart Manufacturing Co., Troy, Ohio.
[12] Model EXDCS-100, Elanco Products Co., Indianapolis, Ind.
[13] Model Q-400, Elanco Products Co., Indianapolis, Ind.
[14] Model 6000, Ohaus Scale Corp., Union, N.J.
[15] Model 24-444 Dynoscreen separator-classifier, Master Machines, Houston, Tex.
[16] Capsular Products Division, National Cash Register Corp., Dayton, Ohio.
[17] Model DB-G, Beckman Instruments, Fullerton, Calif.
[18] Ashland Chemical Co., Columbus, Ohio.
[19] Type B, R. P. Cargille Labs Inc., Cedar Grove, N.J.

Table II—Microencapsulation Schemes and Analytical Data for Experimental Products

	Product												
	A	A-1	A-2	B	B-1	B-2	B-3	C	C-1	C-2	C-3	C-4	C-5
Core A, g	—	332	280	—	—	—	—	—	—	—	—	—	—
Core B, g	—	—	—	—	150	250	300	—	—	—	—	—	—
Core C, g	—	—	—	—	—	—	—	—	2100	2000	1600	2,000	2,000
Ethylcellulose, g	—	10	10	—	5	10	10	—	70	80	80	100	100
Polyethylene, g	—	29	29	—	20	20	20	—	140	160	160	200	200
Cyclohexane, g	—	2000	2000	—	1000	1000	1000	—	7000	8000	8000	10,000	10,000
Bitolterol content, %	4.9	4.8	4.7	12.4	11.8	11.9	11.6	12.0	12.0	12.0	11.8	11.8	11.7
Film content, %	—	2.6	3.3	—	3.2	4.0	3.1	—	3.2	3.6	4.2	4.6	5.0
Density, g/cm^3	1.37	—	—	1.38	1.31	1.23	—	1.32	—	—	—	—	—
Mean volume-surface diameter, μm	740	782	774	824	861	900	1020	1026	1075	1037	1035	995	1099
SD, μm	200	200	200	225	225	250	225	175	250	225	225	250	225

or for a decrease in the specific activity of the drug species. Where chemical analysis is not possible due to the complexity of the film system or to a low level of drug content, the film may be determined by stripping a weighed sample of beads with a suitable solvent, in which case the film fraction, F, is given by:

$$F = \frac{M_b - M_c}{M_b} \qquad \text{(Eq. 1)}$$

where M_b is the mass of the encapsulated beads and M_c is the mass after stripping, and with suitable corrections for extractables in the core, equivalent to the mass of the cores. Since the number of particles of diameter d per unit weight is equal to $6/\pi d^3\rho$, where ρ is the density of the particles, and the surface area of one particle is πd^2, the total surface area for a sample of cores is:

$$S = \frac{6}{\pi d^3 \rho} \pi d^2 M = \frac{6M_c}{d_{vs}\rho_c} \qquad \text{(Eq. 2)}$$

where d_{vs} represents the mean volume–surface diameter of the sample and ρ_c is the density of the cores. For thin films, the surface of the cores may be considered planar of area S. The film thickness is then given by:

$$h = \frac{\text{volume of film}}{\text{surface of cores}} = \frac{F(M_b)/\rho_f}{6(M_c)/d_{vs}\rho_c} = \frac{Fd_{vs}(M_b)\rho_c}{6(M_c)\rho_f} \qquad \text{(Eq. 3)}$$

where ρ_f is the density of the film material. In the limit as $F \rightarrow 0$, the ratio M_b/M_c approaches unity and Eq. 3 simplifies to:

$$h = \frac{Fd_{vs}\rho_c}{6\rho_f} \qquad \text{(Eq. 4)}$$

It is useful to compare the more exact expression presented previously (23), which, when expressed in the above terms, is:

$$h = \frac{Fd_{vs}\rho_c}{6[(1-F)\rho_f + F\rho_c]} \qquad \text{(Eq. 5)}$$

where again for very small F the denominator becomes $6\rho_f$ and is otherwise equivalent to Eq. 4. For film fractions of 10% or less, the thicknesses estimated by Eqs. 4 and 5 differ by less than 2%. Over the range of film fractions considered, the thicknesses estimated by the two equations differed by less than 1% and were considered directly proportional to the amount of film present. Densities were determined gravimetrically by displacement in n-hexane[20], the ethylcellulose film fraction was determined by residue weight as described, the mean volume–surface diameter was found by microscopic particle-size analysis, and the film thickness was estimated from Eq. 4.

Bronchodilator Activity—The bronchodilator activity of selected formulations was compared to plain bitolterol powder in the intact, anesthetized (pentobarbital sodium, 30 mg/kg iv)[21] open-chest dog maintained under artificial respiration using a constant-volume respirator[22] attached to a tracheal cannula. The chest cavity was opened by sternotomy and kept retracted. The opening was covered loosely with a moist gauze. A specially designed, nonrebreathing Leucite valve was attached to the cannula, regulating the inflow and outflow of air.

Bronchoconstriction was induced by intravenous injections of histamine diphosphate[23] (20–50 μg/kg). The changes in airway pressure were measured by a pressure transducer[24] and recorded on a polygraph[25]. The degree of bronchoconstriction was determined by measuring with a planimeter the area of the airway pressure recordings above the baseline for a 5-min period after the histamine injection. A dose of histamine was chosen that would double the airway pressure. An initial dose of 20 μg/kg was given. If the airway pressure were not approximately doubled by this dose of histamine, a higher dose (30–50 μg/kg) was administered 30 min later. The response to this second dose was then used as the control, against which the bronchodilator effects of the microencapsulated products and the plain powder were measured. Bronchodilation was expressed as the percent inhibition of the control histamine-induced bronchoconstriction.

Bitolterol powder or formulations equivalent to 240 μg/kg as the base were administered directly into the duodenal lumen through a small stab opening and washed in with 2 ml of water. The opening was then closed by suturing. The duodenum had been made accessible through a small midline incision in the abdomen. The first postmedication histamine injection was given at 10 min, with subsequent injections at 0.5 and 1 hr and hourly thereafter for 6 hr.

Intravenous injections of saline (1 ml) were given to three dogs to assay the stability of the histamine response. Under these experimental conditions, the degree of histamine-induced bronchoconstriction remained constant throughout the test.

Heart Rate Effect—In the heart rate study, six trained dogs of both sexes, 10–14 kg, were used repeatedly but not more than once a week. Food was withheld from these dogs 17–18 hr prior to the test. During the experiment, the dogs were placed in sling-stand frames designed to keep the animal still with a minimum of restraint. Heart rate was monitored every 5 min *via* a lead II ECG[26].

The microencapsulated formulations, as well as the plain powder of bitolterol in a dose of 240 μg/kg (base), were placed in a No. 4 hard gelatin

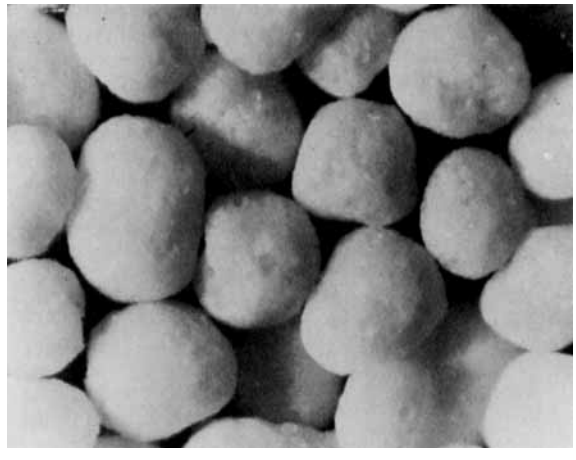

Figure 1—*Marumerized cores prior to microencapsulation containing 12.08% bitolterol mesylate; mean volume–surface diameter = 784 μm.*

[20] Ashland Chemical Co., Columbus, Ohio.
[21] Nembutal Sodium, Abbott Laboratories, North Chicago, Ill.
[22] Model 607, Harvard Apparatus Co., Dover, Mass.
[23] Dickenson & Co., Orangeburg, N.Y.
[24] Model P23B, Statham Instruments, Hatorey, Puerto Rico.
[25] Model 5, Grass Instrument Co., Quincy, Mass.
[26] Model 300 Visette, Sanborn Co., Waltham, Mass.

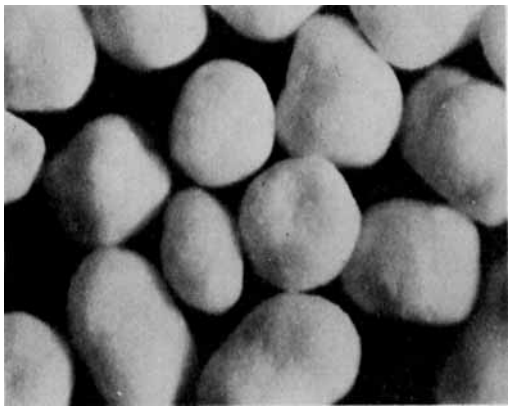

Figure 2—*Microencapsulated beads; calculated film thickness = 6.6 μm.*

capsule and administered to the animal orally, followed by 10 ml of water. Each dog was medicated at weekly intervals with either the microencapsulated formulations or the plain powder until each had received each product twice. Two dogs were used on each test day.

For the evaluation of heart rate effects, baseline pulse rates were analyzed to determine the limits of expected control values. Contrasting baseline values for the different experimental periods, days, dogs, and observation times (20-, 10-, and 0-min pretreatment) produced 95% confidence limits ranging from 83 to 117% of the mean. Consequently, on any one experimental day, the onset of a drug effect was defined as that first point in time where four successive 5-min readings were equal to or greater than 1.17 times the mean of a test animal's pretreatment readings. The end of a drug effect was defined as that first point where either four successive 5-min readings were less than the mean baseline or, in a few instances, the 300-min time period was reached.

The statistic employed in the analysis was the sum of the pulse changes from baseline over the drug effect portion of the time–response curve. The single dog not showing a drug effect was assigned a result of zero for the sum of the pulse changes.

Since all six dogs received, in different order, all possible treatments twice, in six experimental periods, the order effect was assumed to be small relative to other sources of variation.

RESULTS AND DISCUSSION

Appearance of Microencapsulated Products—The uncoated cores appeared to be regular crystalline agglomerates, generally spherical with an occasional rod- or dumbbell-shaped particle (Fig. 1). Microencapsulation with ethylcellulose produced a very glossy surface, free from gross irregularities (Fig. 2). For visualization purposes, the thin film was swelled in Fig. 3 by flooding the field of coated beads with cyclohexane. The frequently reported knobs of film at either end of some ellipsoidal beads, formed when the polymer was still mobile under conditions of high agitation and spinning, appear somewhat exaggerated in this photomicrograph due to light refraction and to the optical advantage of viewing the swelled film through a smaller cross section. The significance of these knobs regarding their possible effects on dissolution or thickness calculations has not been evaluated. The phenomenon appears to be a function of particle shape rather than size and does not affect the usefulness of the correlations obtained among calculated film thickness, release rate, and biological effect.

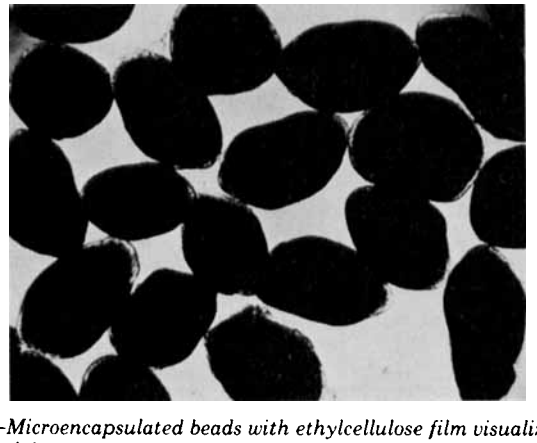

Figure 3—*Microencapsulated beads with ethylcellulose film visualized through cyclohexane perfusion.*

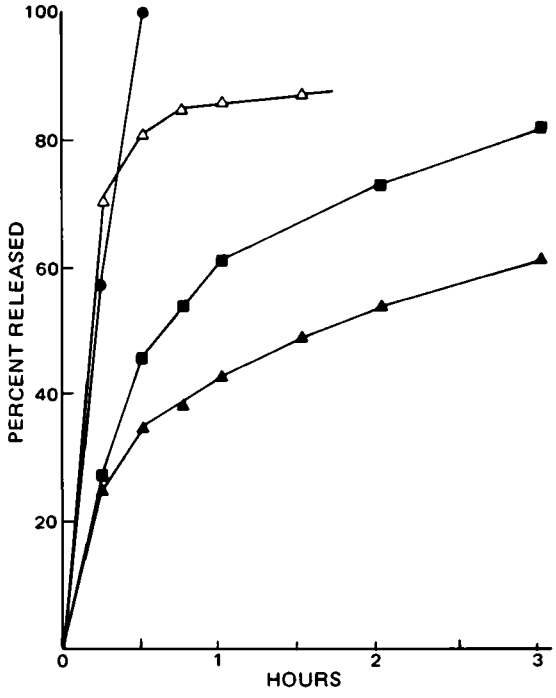

Figure 4—*Release of bitolterol in 0.1 N HCl from various experimental products. Key: ●, conventional hard gelatin capsule; △, Product A, uncoated cores; ■, Product A-1, microencapsulated beads, 2.6% film; and ▲, Product A-2, microencapsulated beads, 3.3% film.*

Dissolution Studies—Formation of the spheronized cores brought about some delay in itself, as shown in Fig. 4 where Core A and Products A-1 and A-2 are compared to the release of a conventional 5-mg capsule of bitolterol containing standard excipients of starch and lactose. The uncoated cores required about 30 min to release half of the dose *in vitro*. Encapsulation with film comprising 2.6% of the total weight of the bead resulted in a $t_{50\%}$ of 40 min. Increasing the film to 3.3% total bead weight further increased the $t_{50\%}$ to about 90 min.

During dissolution, the uncoated cores, as well as the beads, remained essentially intact after several hours of exposure to the dissolution medium in a rotating basket and did not disintegrate. Drug was presumably released by passive diffusion from a relatively unchanging surface. The dissolution of particles encapsulated with insoluble films and releasing their soluble components by diffusive mechanisms has been treated by first-order kinetics (3, 24). The microencapsulated beads prepared for this study could also be characterized by a logarithmic release model (Fig. 5). The effect of increasing the thickness of the film was to reduce the apparent first-order release rate constant. Linearity was found up to and beyond 2 hr under these conditions. The standard errors shown in Fig. 5 were calculated from four replications of the dissolution procedure and indicate the variability seen between samples.

Correlation for the semilog plots of undissolved material against time over the first 120 min was high (r = 0.97–0.99). The slopes of the independent estimates were used to calculate the dissolution rate constants and the corresponding half-lives. The regression lines were not constrained to pass through the point C = 100% at t_0, and the extrapolated t value found for the initial concentration within the bead was taken to be a measure of the penetration time, *i.e.*, the time required for the dissolution medium (0.1 N HCl) to penetrate the bead and to initiate drug release. Thicker films in general led to longer penetration times; therefore, $t_{50\%}$, the time required to release half of the bead content, was equal to the sum of the calculated half-life and the penetration time.

Table II presents the density and particle-size data used to calculate the film thickness of the encapsulating walls. Fick's law of diffusion may be written as:

$$dC/dt = -\frac{SDC}{h} \qquad \text{(Eq. 6)}$$

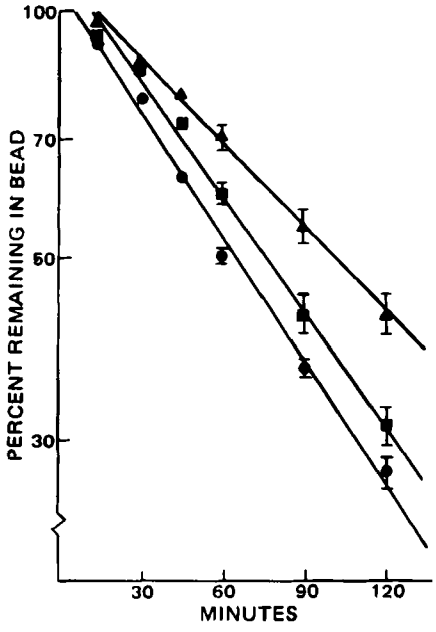

Figure 5—*Release of bitolterol in 0.1 N HCl from a series of microencapsulated beads. Key: ▲, Product B-2, film thickness = 7.3 μm; ■, Product B-3, film thickness = 6.4 μm; and ●, Product B-1, film thickness = 5.6 μm.*

where C is the drug concentration inside the bead, t is time, S is the surface area of the beads, and D is the diffusion coefficient which is inversely proportional to the film thickness h and includes consideration of solute volume, film porosity, and stirring rate. Since these beads remained intact throughout dissolution, the surface area and the diffusion coefficient may be combined:

$$dC/C = -\frac{K}{h}dt \qquad \text{(Eq. 7)}$$

Integration of this expression gives:

$$\ln\frac{C}{C_0} = -\frac{K}{h}t \qquad \text{(Eq. 8)}$$

At $t = t_{1/2}$, Eq. 8 can be rearranged to:

$$h = \frac{Kt_{1/2}}{0.693} \qquad \text{(Eq. 9)}$$

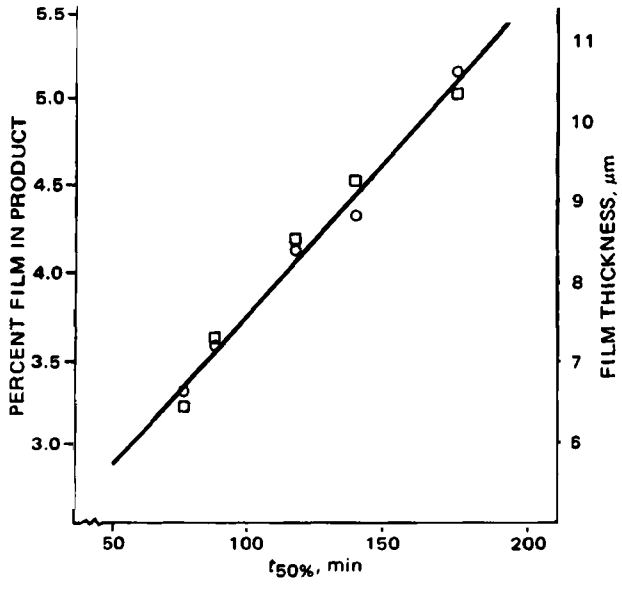

Figure 6—*Time required to release 50% of drug content ($t_{50\%}$) in 0.1 N HCl from a series of microencapsulated products as a function of percent film in sample (left ordinate) and film thickness (right ordinate). Key: □, film content; and ○, calculated film thickness.*

Table III—Heart Rate Prior to Oral Administration of Plain Drug Powder or Microencapsulated Formulations of Bitolterol

Dog	Baseline Means[a], beats/min: Plain Powder	Product B-1	Product B-2
1	53	50	55
2	66	56	62
3	65	62	63
4	54	53	60
5	60	67	58
6	67	62	58
Arithmetic mean	60.9	58.2	59.2

Analysis of Variance[b] — Source of Variation	*df*	*MS*	*F*
Dogs	5	107.69	6.2
Formulations	2	21.78	1.2
Dogs × formulations	10	30.94	1.78
Residual error	18	17.42	

[a] Arithmetic mean of 20-, 10-, and 0-min pretreatment heart rates. [b] Least significant difference at $p = 0.05$ between two treatment means = 3.6.

For large surface area and low film fraction, F was shown to be essentially proportional to the effective thickness of the film in Eq. 4. However, examination of the particle-size distributions of the products before and after microencapsulation revealed that fine particles present in the core material were removed during coating by agglomeration with larger particles. The modes of the distributions were not appreciably changed during microencapsulation, but the removal of the fines caused an upward shift in the mean volume–surface diameters. Accordingly, the d_{vs} of each microencapsulated *product* was considered to be the best measure of the size of the actual *cores* undergoing encapsulation and was used to calculate film thicknesses for the various products.

Agglomeration occurred to a greater extent in systems where the solids-to-solvent ratio was higher, with the effect that the total surface area to be covered by the film was reduced. The phase ratio (core material to film material) of Products B-1 and B-3 was the same (30:1), but Product B-3 was prepared in a system with double the solids content of B-1. The ethylcellulose assay showed virtually no difference between the amount of film present in the two products, but the mean volume–surface diameters indicated that greater agglomeration had taken place in the more concentrated system, leading to a thicker film being deposited on Product B-3.

Confirmation of the thicker film was found in the dissolution rates shown in Fig. 5, where Product B-3 was slower than B-1, although the film content of each product was about the same. Therefore, the film content is only proportional to some measure of the dissolution rate for a series of products encapsulated around identical core material from systems where agglomeration proceeds to the same extent and the mean volume–surface diameters of the microencapsulated products are similar.

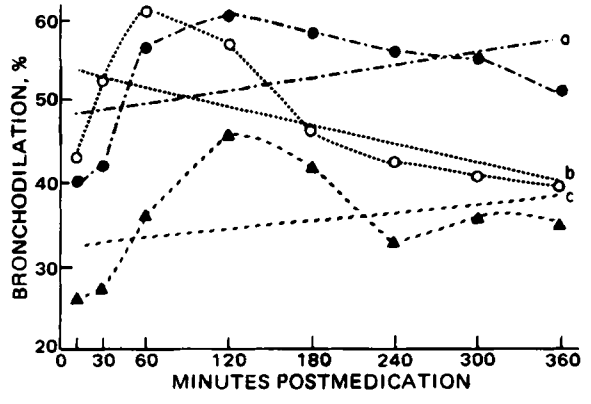

Figure 7—*Bronchodilator activity in anesthetized dogs following a dose of 240 μg of bitolterol (base)/kg administered intraduodenally. Linear effect regression lines are shown for each corresponding time–response curve. Key: ○, plain drug powder (control); ●, microencapsulated Product B-1, 3.2% film; and ▲, microencapsulated Product B-2, 4.0% film. Regression line slopes ± SE are: a, 0.024 ± 0.014; b, −0.039 ± 0.014; and c, 0.015 ± 0.014.*

Table IV—Heart Rate Effect in Unanesthetized Dogs Given 240 μg/kg Bitolterol (Base) Orally

Dog	Sum of Pulse Changes from Baseline (t_0) over Drug Effect Portion of Time–Response Curve, beats/min: Plain Powder	Product B-1	Product B-2
1	1377	1948	1300
2	1070	826	459
3	2423	369	461
4	180	1255	0
5	2940	2053	2127
6	404	266	208
Arithmetic mean	1400	1120	760

Source of Variation	Analysis of Variance[a]: *df*	*MS*	*F*
Dogs	5	3,544,999	12.0
Formulations	2	1,234,469	4.2
Dogs × formulations	10	664,720	2.2
Residual error	18	295,709	

[a] Least significant difference at $p = 0.05$ between two treatment means = 466; at $p = 0.01$, least significant difference = 566.

With allowance for the initial penetration time delays, film fraction was plotted against $t_{50\%}$ for five microencapsulated products (Core C) ranging in film content from 3.2 to 5.0% (Fig. 6). The corresponding calculated film thickness is given on the right-hand scale. Graphs of this type were useful when preparing material with specific release characteristics. The y-intercept in Fig. 6 was thought to represent the minimum amount of film (~2.0% for this series) that must be present before a sufficient number of particles was entirely coated to exhibit a depressed release rate. The significance of the intercept was somewhat obscured since the cores in these experiments were not themselves instantaneously dissolving. However, encapsulated materials carrying less than 2% film showed only slightly slower release rates than the corresponding uncoated cores.

Biological Evaluation—On the basis of *in vitro* dissolution studies, two formulations were selected for biological evaluation: Product B-1 with a $t_{50\%}$ of 65 min representing faster releasing formulations, and Product B-2, with a $t_{50\%}$ of 101 min representing the slower releasing formulations.

Bronchodilator Activity in Anesthetized Dog—The change in bronchodilator activity resulting from the administration of the two microencapsulated formulations over the 6-hr observation period was significantly different from that of plain bitolterol powder, showing delayed release properties at the early time periods and a flatter response decay at the later time periods. Linear regressions calculated for the three dosage forms are shown in Fig. 7 against the actual bronchodilation time–response curves. Both controlled-release formulations exhibited sustained activity with similar positive slopes, while the slope for the plain drug was negative. Bronchodilations ranging from 26 to 43% were obtained within 10 min after medication. A 60% peak effect was obtained at 60 min with the plain powder whereas Products B-1 and B-2 showed peak effects of 60 and 46% at 120 min, respectively.

The bronchodilation appeared prolonged for all treatments, with 35–51% effect still obtained at the 6th hr. After the 1st hr, the bronchodilation by Product B-1 was maintained at a significantly ($p = 0.05$) higher level (50–60%) than that of Product B-2 (32–46%). Bronchodilation following the administration of Product B-2 was significantly less than that from the plain powder through the 1st hr; however, neither microencapsulated formulation was significantly different from the plain powder after the 1st hr.

Heart Rate Effect in Unanesthetized (Trained) Dog—An analysis of baseline mean pulse rates showed no significant differences among the three formulations (Table III). Subsequent analysis of the posttreatment results showed a significant difference ($p = 0.01$) between the slower Product B-2 and the plain powder with respect to the mean sum of the pulse changes from baseline over the drug-effect portion of the time–response curve (Table IV). While the sum of the pulse rate changes was lower after Product B-1 relative to the powder, the difference was not statistically significant. Comparison of the mean pulse rates at 30, 60, 90, and 120 min (Fig. 8) showed significantly lower rates with both microencapsulated formulations at 30 and 60 min, significantly lower pulse rates with the slower formulation (Product B-2) than with both of the other products at 90 min, and equivalent rates for all three forms at 120 min and thereafter.

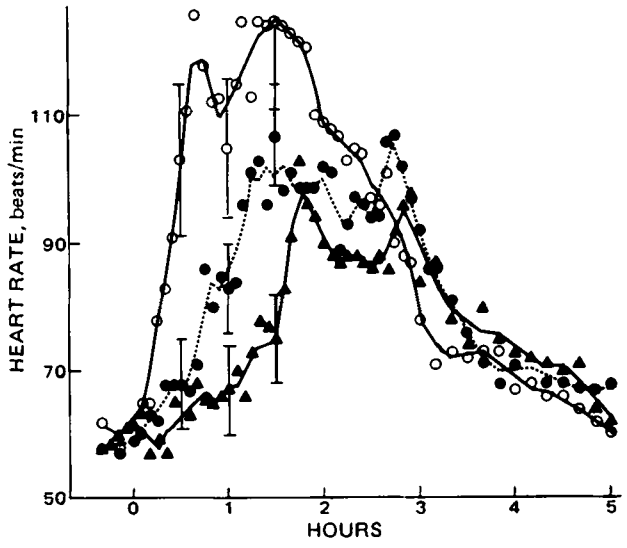

Figure 8—*Heart rate effect in unanesthetized dogs following a single dose of 240 μg of bitolterol (base)/kg administered orally; six dogs received two separate medications of each drug on different days. Key: ○, plain drug powder (control); ●, microencapsulated Product B-1, 3.2% film; and ▲, microencapsulated Product B-2, 4.0% film.*

In both anesthetized and unanesthetized dogs, the microencapsulated products demonstrated controlled-release properties, and effective bronchodilator activity was obtained with an attenuation of the concommitant cardiac stimulation.

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Rapid and Accurate Stability-Indicating Assay for Nitroglycerin

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Abstract □ A rapid high-pressure liquid chromatographic method for determining the nitroglycerin concentration in liquid dosage forms and intravenous admixture solutions is presented. A coefficient of variation of less than 1.8% was achieved over the concentration range most commonly encountered (50–500 μg/ml). A variable wavelength detector (λ = 218 nm) and a micro-alkyl phenyl column were employed. The mobile phase was acetonitrile–tetrahydrofuran–water (26:10:64). Total analysis time was 12 min.

Keyphrases □ Nitroglycerin—assay of liquid dosage forms and intravenous solutions, stability, degradation products □ Stability—nitroglycerin in intravenous solutions □ High-pressure liquid chromatography—analysis, nitroglycerin liquid dosage forms and intravenous solutions □ Cardiac vasodilators—nitroglycerin, analysis, liquid dosage forms and intravenous solutions

Intravenous nitroglycerin (glyceryl trinitrate) is commonly used for patients with acute myocardial infarction. Several methods of preparing such solutions for human patients have been reported (1–3). Recent reports indicated that intravenous nitroglycerin solutions lose potency when prepared or stored in certain containers (4–6). This loss may be due to degradation or adsorption. Thus, a rapid and accurate assay of nitroglycerin in intravenous solutions is needed.

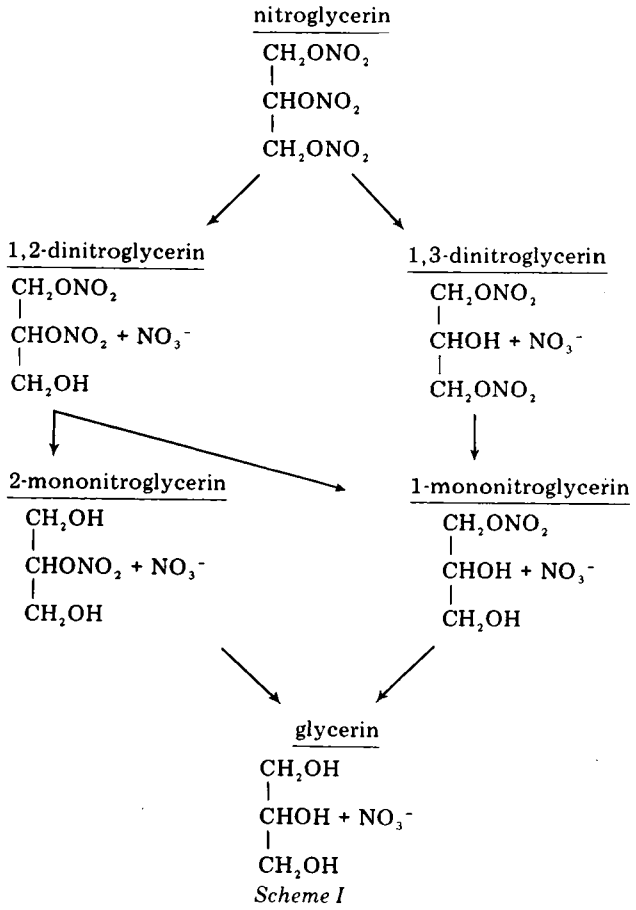
BACKGROUND

Nitroglycerin is the triester of nitric acid and glycerol. Breakdown occurs by a stepwise loss of nitrate groups (Scheme I).

Spectrophotometric methods require little instrumentation but are time consuming and complex and do not indicate stability (7–10) or differentiate nitroglycerin, mononitroglycerin, dinitroglycerin, and endogenous nitrates. TLC (11–13) is selective but not quantitative. Although polarographic methods (14, 15) offer both selectivity and sensitivity, they require sophisticated instrumentation not available in many laboratories.

A kinetic method of analysis (16) based on the transient appearance of a chromophoric intermediate during base hydrolysis offers speed and specificity but lacks sensitivity. GLC methods (17–21) give sensitivity for nitroglycerin in excess of that required. Some give the desired selectivity. However, all require a time-consuming extraction into an organic solvent.

Two normal phase high-performance liquid chromatographic methods were reported (22, 23). Both reports failed to discuss interference from excipients and breakdown products, and both methods require extraction into an organic solvent. Assay sensitivity is potentially a problem when applying these methods to intravenous nitroglycerin admixture solutions.



Scheme I

A new method is described here that allows the rapid direct measurement of nitroglycerin in intravenous admixture solutions, offers adequate sensitivity, and provides the potential for measuring breakdown products.

EXPERIMENTAL

Materials—Nitroglycerin[1] [10% in lactose or 1% (v/v) in ethanol], isosorbide dinitrate[2] (25% in lactose), and sublingual nitroglycerin tablets[3]

[1] ICI, Wilmington, Del.
[2] Napp Chemicals, Lodi, N.J.
[3] Eli Lilly and Co., Indianapolis, Ind.

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