Stabilized Compressed Nitroglycerin Tablets

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Abstract □ A stabilized compressed nitroglycerin tablet, containing microcrystalline cellulose NF and povidone NF as stabilizing agents, was formulated. Bioavailability and physical tests were conducted on the compressed tablet and two leading brands of molded tablets. There was no difference in the mean pulse rate between a molded tablet formulation and the compressed tablet in a crossover study using 10 human volunteers and three tablet strengths, 0.3, 0.4, and 0.6 mg. The compressed tablet was most uniform when tested according to the USP method for weight variation and content uniformity. Various tests for nitroglycerin loss due to volatility, *i.e.*, open dish test, thermogravimetric analysis, and a simulated in-use test, all indicated that the stabilized compressed tablet was substantially more stable than partially stabilized or unstabilized molded tablets.

Keyphrases □ Nitroglycerin—bioavailability and stability, stabilized compressed tablet compared to commercial products □ Bioavailability—nitroglycerin, stabilized compressed tablet compared to commercial products □ Stability—nitroglycerin, stabilized compressed tablet compared to commercial products □ Dosage forms—stabilized compressed nitroglycerin tablets prepared, bioavailability and stability compared to commercial products □ Tablets—stabilized compressed nitroglycerin tablets prepared, bioavailability and stability compared to commercial products □ Vasodilators, coronary—nitroglycerin, bioavailability and stability, stabilized compressed tablet compared to commercial products

Nitroglycerin, in spite of its association with a bygone era, is well recognized today as a safe and effective drug for treatment of angina pectoris. Perhaps its historical significance is that it is one of the few tablets still being manufactured as a tablet triturate. Other types of tablet triturates such as dispensing tablets and hypodermic tablets are rapidly becoming less important than they were. The drugs previously used in the hypodermic tablet form are now available as single- or multiple-dose injectable products or have been replaced by newer synthetic drugs, and the dispensing tablet form is needed less frequently because fewer prescriptions are now compounded.

However, the particular attribute of nitroglycerin tablets to disintegrate rapidly has only been obtainable thus far by manufacturing them as tablet triturates. In spite of some inherent problems in the physical stability aspects of nitroglycerin, the molded tablet method has not been satisfactorily replaced by the modern method of tablet manufacture, *i.e.*, compression.

BACKGROUND

Molded tablet manufacture has several definite disadvantages, especially for nitroglycerin, a liquid substance at room temperature whose vapor pressure is small but large enough to cause loss from conventional tablets, particularly if they are improperly stored. Molded nitroglycerin tablets are usually manufactured by mixing a lactose–nitroglycerin trituration and sucrose with a hydroalcoholic solution. After thorough wetting and mixing, the damp mass is forced into a perforated plate to form individual tablets. This process is now generally done by an automatic tablet triturate machine.

After a short time in the mold, the tablets are ejected by pegs onto a conveyor belt. The conveyor belt usually carries the tablets through a drying tunnel, which substantially removes the residual water and alcohol. The high solubility of nitroglycerin in ethanol (about 1 in 4) causes nitroglycerin to migrate to the outer tablet surface during the drying step. This concentration of nitroglycerin near the tablet surface enhances its ability to escape into the surrounding air. A study of the preparation of warfarin sodium tablets by wet granulation demonstrated unsatisfactory tablet content uniformity due to the migration of the drug during drying (1). However, the use of alginic acid in the formulation inhibited migration and gave satisfactory content uniformity.

Recent formulation improvements to inhibit nitroglycerin migration in molded tablets have increased the stability of the products of the two leading manufacturers. In Product A[1] nitroglycerin tablets, polyethylene glycol 400 is added to deter nitroglycerin migration and volatility (2). In Product B[2] nitroglycerin tablets, a small amount of stabilizer[3] (3) is added to improve stability.

The loss of nitroglycerin from tablets due to volatilization and the factors affecting such a loss were reported previously (4–8). The specific stabilizing effect of microcrystalline cellulose was first reported by Richman *et al.* (4). While older literature alluded to a loss of potency in nitroglycerin tablet formulations, the scope of the problem was not realized until a satisfactory single-tablet assay method was developed (9). In this procedure, the interference of lactose with the diazotization reaction is eliminated by using strontium hydroxide as the alkalizing agent. By this means, individual tablets were assayed accurately. A further adaptation of this procedure to the automated analyzer permitted the assay of large numbers of individual tablets, 30/hr (7), thus giving researchers the methodology for studying various factors affecting nitroglycerin stability.

Reports of nitroglycerin instability have involved several main areas concerned with packaging and storage aspects of the tablets: (*a*) the particular container employed, glass or plastic; (*b*) the type of closure used, including the cap-lining material; and (*c*) the stuffing material used in the containers.

Nitroglycerin tablets packaged in polyethylene blisters with aluminum foil backing contained only 0–10% of the labeled amount (10). However, nitroglycerin tablets were relatively stable when packaged in glass or high density polyethylene vials with screw caps but not in polystyrene, polyvinyl chloride, or polypropylene vials (5). The effect of fillers on the retention of nitroglycerin in tablets of two leading manufacturers also was reported (6). Rayon packing absorbed less nitroglycerin from commercial packages than did absorbent cotton, absorbent gauze, lamb's wool, and nonabsorbent cotton. The stabilized brand also absorbed less nitroglycerin than the nonstabilized brand.

Further studies on packaging components were carried out by Fusari (7), who noted that stuffing materials (cotton and rayon), as well as the material used in cap liners, affected the average tablet assays as well as the potency ranges. This report was the first to give data acquired by means of the automated analytic adaptation of the Bell (9) assay method. Therefore, a wide variation in the content of nitroglycerin in tablets was reported for many combinations of packaging components and storage conditions.

A typical lot of fresh 0.4-mg nitroglycerin tablets reported in this study (7) had an initial content relative standard deviation in the area of 3–6%. These tablets were aged 1–14 months at room temperature; after reassay, 12 of 32 lots fell below the minimum 75% limit of the USP XVIII. During this time the relative standard deviation of nitroglycerin content increased and ranged from 6 to 17%. It was surprising to learn of these wide variations, which occurred even in glass bottles of 100 tablets with metal screw-on caps. A later report (8) compared two nitroglycerin tablet formulations, the previous conventional formulation and a partially stabilized one, demonstrating an improvement in intertablet dose variation for the stabilized form.

[1] Parke-Davis & Co.
[2] Eli Lilly Co.
[3] Determined in these laboratories to be povidone.

Investigations in these laboratories led to the development of a compressed nitroglycerin tablet formulation that matches the traditional excellent disintegration and bioactivity of molded tablets and has significantly improved stability aspects compared to the two leading brands, particularly under stress conditions.

EXPERIMENTAL

Tablet Manufacture—Compressed tablets[4] were made by a modified direct compression process. Two stabilizers were used in the formulation, microcrystalline cellulose[5] (NF XIII) and povidone[6] (NF XIII). Microcrystalline cellulose was mixed with anhydrous lactose (USP XVIII), starch (USP XVIII), and colorant and was granulated with a solution made by dissolving povidone in nitroglycerin spirit diluted with ethanol. The granulation was dried in a forced air oven at 30–35° and milled; a small amount of tablet lubricant, calcium stearate, was blended into the granulation.

Tablets were compressed on a high-speed rotary tablet machine, producing 2000–2500 tablets/min using 3.97-mm ($\frac{5}{32}$-in.) standard concave tools. Normal quality control procedures during compression included frequent weighing of groups of tablets as well as individual tablets. Compression weight was determined by obtaining an assay of the granulation and calculating the theoretical weight required to produce the labeled potency along with a small excess. Generally, compression weight was about 29.5–30.5 mg/tablet. An in-process control of tablet disintegration was periodically made; other processing controls of the finished tablets included tablet breaking strength and friability[7].

Granulation Assay—*Reagents*—Glacial acetic acid, stronger ammonia water, and phenoldisulfonic acid TS (USP XVIII) were used.

Potassium Nitrate Standard Solution—Transfer about 65 mg of potassium nitrate standard, accurately weighed, to a 200-ml volumetric flask containing 1.0 ml of distilled water, mix to dissolve completely, dilute to volume with glacial acetic acid, and mix.

Sample Solution—Transfer an accurately weighed amount of granulation, equivalent to 1 mg of nitroglycerin, to a glass-stoppered 50-ml conical flask. Pipet 5 ml of acetic acid into the flask, stopper, and shake mechanically with a wrist-action shaker for 1 hr. Filter.

Procedure—Pipet 1 ml of sample, standard, and glacial acetic acid into separate 25-ml volumetric flasks. Add to each 2.0 ml of phenoldisulfonic acid TS, mix, and let stand 15 min. Dilute with about 8 ml of distilled water, cool to room temperature, and cautiously add 10 ml of stronger ammonia water (watch for spattering, keep solution cool); then dilute to volume with distilled water.

Concomitantly determine the absorbances of the sample and the standard at 410 nm in 1-cm cells in a suitable spectrophotometer. Calculate according to:

$$\% \text{ nitroglycerin} = \frac{A_u}{A_s} \times \frac{5C}{2SW} \times 0.749 \qquad \text{(Eq. 1)}$$

where A_u and A_s are the absorbances of the sample and the standard, respectively; 0.749 is the factor for nitrate to nitroglycerin; C is the weight, in milligrams, of potassium nitrate taken; and SW is the sample weight, in milligrams.

Semiautomatic Content Uniformity Assay—*Equipment*—The automated system[8] consisted of a sampler[9], a proportioning pump[10], a water bath[11], and a colorimeter[12]. A strip-chart recorder[13] also was utilized.

Reagents—For the 1% strontium hydroxide reagent, add about 20 g of strontium hydroxide to a 2000-ml volumetric flask and then add about 1800 ml of distilled water. Heat to hasten solution. When saturated, cool and dilute to volume. This solution should be prepared the day before use to allow the precipitate of strontium carbonate to settle out.

For the 0.3% procaine hydrochloride reagent, dissolve about 3 g of procaine hydrochloride in 1 liter of distilled water. For the 2.5 *N* HCl reagent, dilute 210 ml of hydrochloric acid cautiously to 1 liter with distilled water. For the 0.025% *N*-(1-naphthyl)ethylenediamine dihydrochloride reagent, dissolve 250 mg of *N*-(1-naphthyl)ethylenediamine dihydrochloride in 1 liter of distilled water.

Standard Preparation—Pipet 5 ml of nitroglycerin spirit (assayed by the procedure described under *Standardization of Nitroglycerin Spirit*) into a 100-ml volumetric flask. Then add 50 ml of 95% ethanol, dilute to volume with water, and mix. Pipet 10 ml of this solution into a second 100-ml volumetric flask, dilute to volume with water, and mix well. Dilute either 7.0 ml (0.6-mg tablet) or 5.0 ml (0.4-mg tablet) to exactly 100.0 ml with water or 7.0 ml (0.3-mg tablet) to exactly 200.0 ml.

Sample Preparation—Transfer one tablet to a 25-ml flask and add about 15 ml of distilled water. Swirl to mix, dilute to volume with distilled water, stopper, and shake vigorously.

Procedure—Assemble the apparatus as shown diagrammatically in Fig. 1. Turn on the recorder and the colorimeter and allow them to stabilize for at least 30 min. Charge the reagent lines. Place 2-ml sample cups in the sampler, fill with the appropriate solution, and arrange so that there are five standards initially followed by 10 samples and a standard. Each subsequent 10 samples are bracketed with a standard preparation.

Assay in the automated system, employing a 20/hr cam with a 1:4 sample to wash ratio. Set the recorder at 50 mv with a chart speed of 0.51 cm (0.2 in.)/min. Use a 550-nm filter, a No. 2 aperture, and a 15-mm flowcell.

Standardization of Nitroglycerin Spirit—Pipet 5 ml of nitroglycerin spirit, shown to be "one-spot" material by TLC, into a 100-ml volumetric flask. Then add 50 ml of 95% ethanol and dilute to volume with water. Pipet 10 ml of this dilution to a 100-ml volumetric flask and dilute to volume with acetic acid. Mix well.

Standard Preparation—Dissolve about 129 mg of potassium nitrate, accurately weighed, in 1.0 ml of water and dilute to 200.0 ml with acetic acid. Mix well.

Procedure—Pipet 1.0 ml of the standard, sample, and acetic acid to separate 100-ml volumetric flasks. Add to each 2 ml of phenoldisulfonic acid, mix well, and let stand 5 min. Dilute to about 80 ml with water, add 10 ml of ammonium hydroxide, mix, and cool to room temperature. Dilute to volume with water and mix.

Determine the absorbances at a wavelength of maximum absorbance at about 410 nm *versus* the acetic acid blank in 1-cm cells in a suitable spectrophotometer. Calculate according to:

$$\% \text{ nitroglycerin in spirit (w/v)} = 0.749 \frac{A_u}{A_s} \times C \qquad \text{(Eq. 2)}$$

where A_u and A_s are the absorbances of the sample and standard, respectively; C is the weight, in milligrams, of potassium nitrate taken; and 0.749 is a factor relating nitroglycerin to potassium nitrate.

Weight Uniformity and Content Variation—All tablets were subjected to the weight variation test given in USP XVIII (p. 951) and a content uniformity test by the automated procedure. Three lots of each of the three potencies, 0.3, 0.4, and 0.6 mg, were tested.

Disintegration, Dissolution, and *In Vitro* Availability—Three lots of each of three strengths of nitroglycerin tablets, Products A, B, and C, were tested for disintegration according to USP XVIII (p. 934) for sublingual tablets. In this test, water is the disintegration fluid and no disks are used. Six tablets of each batch were individually tested. Experiments were performed to compare the membrane permeability of nitroglycerin from solution and from all three products. The experimental apparatus was a 5-ml dialysis chamber[14]. The permeability of 5 ml of nitroglycerin solution, 0.5 mg/ml, and the same solution containing 1 mg/ml of povidone was tested. In addition, four 0.6-mg tablets of each formulation were added to 5 ml of distilled water in the dialysis chamber. The dialysis cell contained the same volume of water on the other side, and a cellulose membrane[15] was used to separate the two sides. The apparatus was mechanically agitated at room temperature, and the nitroglycerin content of both chambers was determined after 5 hr (9).

Comparative Bioactivity of Nitroglycerin Tablet Formulations—All three nitroglycerin tablet preparations were tested sub-

[4] Product C, Warner-Chilcott Division of Warner-Lambert Co. (patent pending).
[5] Avicel PH-102, American Viscose Division, FMC Corp.
[6] Plasdone K-29-32, General Aniline and Film Corp.
[7] Breaking strength was determined on a Heberlein model 2E tester with a light pendulum replacement for the heavy pendulum and a new scale with intervals of 0.1 kg. Friability was determined with a standard Roche-type friabilator made of Plexiglas.
[8] Autoanalyzer, Technicon, Inc., Tarrytown, N.Y.
[9] Sampler II.
[10] Proportioning Pump II.
[11] Adjustable heating bath, Technicon.
[12] Colorimeter I.
[13] Mosely 7100 B.
[14] Bellco No. 3221.
[15] Technicon type C.

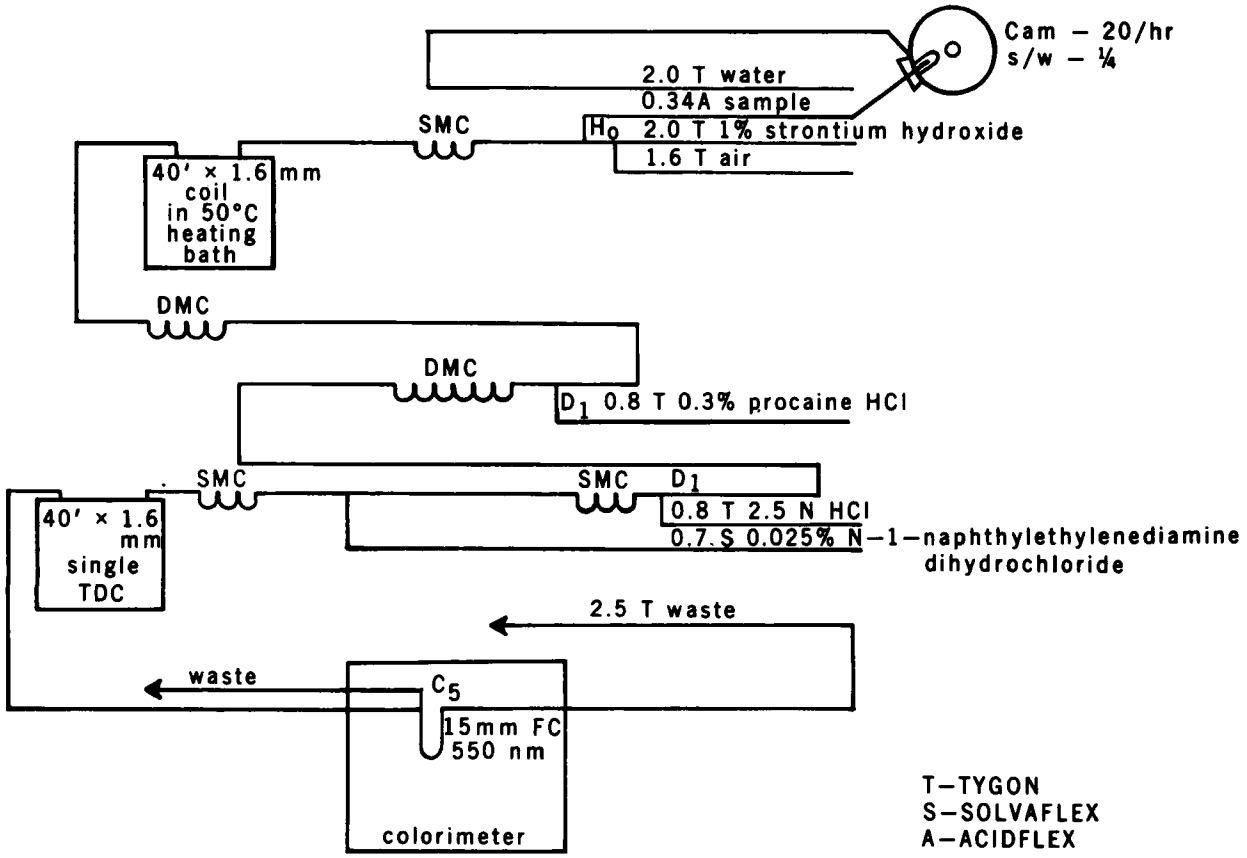

Figure 1—*Flow diagram for content uniformity assay.*

lingually in three dogs at two potency levels, 0.3 and 0.6 mg. Animals weighing 13–16 kg were anesthetized with pentobarbital sodium, 30 mg/kg iv, and aortic blood pressure and heart rate were measured. Then tablets were placed under the tongue and dissolved using a few drops of saline solution. The doses given were 22–88 μg/kg. Aortic blood pressure and pulse rate were measured again.

Products B and C and a placebo were tested in a group of 10 healthy subjects, eight males and two females, 21–47 years of age. Three tablet potencies were studied: 0.3, 0.4, and 0.6 mg. A placebo and each nitroglycerin potency of both manufacturers were studied in every subject. Prior to taking the test compound, each subject sat and rested for a minimum of 5 min and then counted his or her own pulse for 1 min every other minute for 8 min (four readings).

Beginning 1 min after taking the test drug or placebo, each subject counted his or her own pulse rate for 1 min every other minute for the next 16 min (eight readings) and then every 5 min for the next 15 min (three readings). If the pulse rate had not returned to the predrug level by this time, readings were continued every 5 min until it did.

Volatility Studies—A rapid screening method was developed to compare the effectiveness of potential stabilizing agents. Thermogravimetric analysis was found to be an excellent means of determining nitroglycerin volatility from tablets under conditions of constant temperature and controlled atmosphere. Two tablets were placed on the analyzer[16] at 80° under a nitrogen flow of 20 ml/min, and the weight loss was determined. After 90 min of treatment, a good approximation of the extent of nitroglycerin loss could be made from the curve developed (weight loss *versus* time).

Products A, B (unstabilized), and C, all 0.6 mg, were individually subjected to thermogravimetric analysis. A comparison was made among them by noting the rate of nitroglycerin loss. Furthermore, these tablets were individually assayed after exposure to verify that

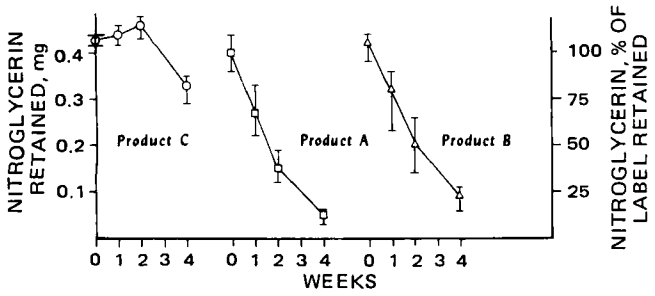

Figure 2—*Stability of nitroglycerin tablets (0.4-mg label) stored in open dishes at room temperature: average assay value and range of 10 individual tablets. Key: □, Product A; △, Product B; and ○, Product C.*

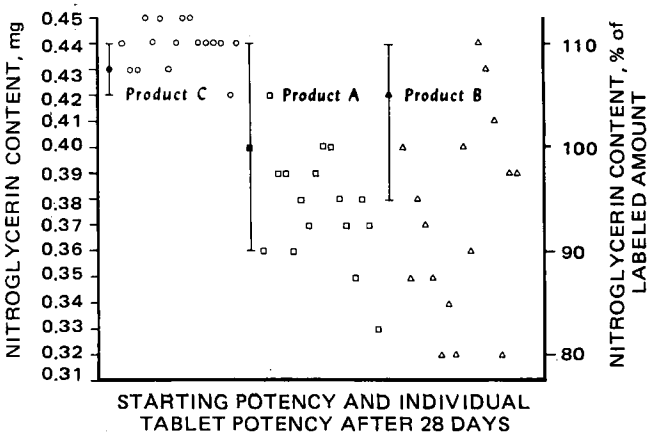

Figure 3—*Results obtained by testing the 16 individual tablets (0.4-mg label) remaining after a 28-day in-use test. Each bottle was opened three times a day for 30 sec, and one tablet was removed at each opening. Key: ●, ■, ▲, average assay and range of various tablet formulations at the start; and ○, □, △, individual tablet assays for Products C, A, and B, respectively, after 28 days of usage.*

[16] Du Pont model 950 in conjunction with model 900 console.

Table I—Comparison of Weight Uniformity of Nitroglycerin Tablets[a]

Product	Strength, mg	Lot	Tablet Weight, mg		RSD, %
			Average	Range	
Product A	0.3	PC142	34.5	32.4–36.5	3.8
	0.4	PM216	33.9	33.0–35.3	1.8
	0.6	PA165	35.5	34.2–36.5	2.7
Product B	0.3	7JSO2C	35.8	34.2–37.8	3.3
	0.4	6WF29B	35.4	34.1–38.5	4.4
	0.6	6TT84B	34.2	31.0–37.5	5.6
Product C	0.3	4900	29.5	29.2–29.8	0.7
	0.4	4902	30.8	30.4–31.4	1.2
	0.6	4906	28.9	28.2–29.7	1.8

[a] USP XVIII, p. 951.

Table II—Comparison of Content Uniformity of Nitroglycerin Tablets, Label Claim 0.4 mg

Product	Lot	Nitroglycerin Content			Number of Tablets Outside 15% Specification
		Average[a], mg	Range, mg	RSD, %	
Product A	PM216	0.40	0.36–0.44	6.72	0
	RC147	0.38	0.32–0.42	8.61	1
	PL265	0.36	0.30–0.43	10.42	2
	RB489	0.35	0.32–0.38	5.83	2
Product B	6W29B	0.42	0.38–0.44	4.71	0
	7HF65B	0.37	0.30–0.42	9.87	2
	7KJ73B	0.37	0.30–0.46	13.40	3
	7LH21B	0.43	0.34–0.55	14.05	0
Product C	4901	0.42	0.41–0.43	1.83	0
	4902	0.44	0.43–0.46	1.89	0

[a] Represents average of 10 randomly selected tablets.

the loss apparent from the weight *versus* time curve was in agreement.

Additional volatility experiments were conducted by exposing the tablets (all containing stabilizers) from the same manufacturers and of the same potencies in open dishes in the laboratory at 24–26°. Individual tablet assays were run at 1, 2, and 4 weeks.

A simulated in-use study was also carried out in which one tablet was removed from a commercial bottle three times every day for 28 days. The exposure time was 30 sec at each opening. The remaining 16 tablets were assayed individually.

RESULTS

Tables I and II give the weight variation and content uniformity of batches of tablets of various potencies. Generally, the weight variation for molded tablets was higher than for compressed tablets, and the difference was statistically significant at the 99.5% probability level when tested by Bartlett's χ-square statistic (11). Content variabilities of molded tablet formulations were also higher than with compressed tablets; this difference was also statistically significant at the 99.5% level by the Bartlett test.

Tablet disintegration of the three brands is shown in Table III. The data indicate that, although all brands disintegrated rapidly, Products B and C ranged from 3 to 7 sec while Product A ranged from 10 to 17 sec. These differences in disintegration times do not necessarily indicate a difference in rapidity of drug release or onset of pharmacological activity.

The results of the last comparative *in vitro* availability test, membrane permeability, are shown in Table IV. Nitroglycerin alone and nitroglycerin in the presence of povidone were both 31% dialyzed after 5 hr. Product A and C tablets dialyzed to the same extent as the solutions, while Product B tablets were somewhat slower.

Testing of various brands of nitroglycerin tablets in dogs indicated that the onset of activity, *i.e.*, decrease in mean aortic blood pressure and increase in heart rate, occurred within 1 min for each product at each dose level. Peak pharmacological responses occurred within 3 min after dosing regardless of the dose level. A decrease in mean aortic blood pressure, pulse pressure, and/or pulse contour and an increase in heart rate occurred with each product. There was a dose–response relationship. The higher dose had a longer lasting effect on blood pressure whereas the heart rate returned to normal in 10–20 min for all doses.

Table III—Disintegration of Nitroglycerin Tablets

Individual Tablet	Disintegration Time,[a] sec								
	0.3-mg Tablets			0.4-mg Tablets			0.6-mg Tablets		
	Product C	Product A (Lot PC142)	Product B (Lot 7BH46B)	Product C	Product A (Lot PM216)	Product B (Lot 6WF29B)	Product C	Product A (Lot MA153)	Product B (Lot 6UT66A)
1	4	15	7	6	15	5	6	10	7
2	5	17	6	7	16	5	6	17	4
3	3	15	3	7	18	5	6	12	5
4	6	16	7	7	16	5	5	13	3
5	5	12	7	7	14	4	6	10	4
6	6	14	5	7	17	6	6	14	4
Average of six	5	15[b]	6	7	16[b]	5	6	13[b]	5

[a] According to USP XVIII method for sublingual tablets, p. 934. [b] These results agree with those data reported in Product A advertising literature.

Table IV—Permeability of Nitroglycerin from Solutions and Tablet Dosage Forms[a]

Sample	Permeation, %
Nitroglycerin solution, 0.5 mg/ml	31
Nitroglycerin solution, 0.5 mg/ml, and 1.0 mg/ml of povidone	31
Product A tablets[b] (Lot PA165)	32
Product B tablets[c] (Lot 6UT66A)	20
Product C tablets	31

[a] Five-hour study; see text for details. Four tablets in 5 ml of distilled water were used. [b] Product contains a stabilizer. [c] Product contains no stabilizer.

The results obtained by testing three potencies of two drug products in human volunteers are given in Table V. Each dose of nitroglycerin caused a statistically significant increase in pulse rate whereas the placebo tablet had virtually no effect on pulse rate. The Product B nitroglycerin dose did not differ significantly from the same Product C dose with respect to the magnitude, the time of onset, the time of peak activity, or the duration of pulse rate elevation. Neither was there any significant difference in the responses between different doses. With both formulations, the onset of action was statistically significant at 3 min, the peak activity was 3–5 min after drug administration, and the duration of action was 9–11 min.

Volatility studies employing thermogravimetric analysis and chemical assay are summarized in Table VI. The loss of nitroglycerin was most pronounced with Product B tablets (unstabilized) followed by Product A. Product C tablets, on the other hand, exhibited remarkable stability under these experimental conditions.

The results obtained from open dish exposure of 0.4-mg nitroglycerin tablets are shown in Fig. 2. Tablets were spread out in a petri dish and fully exposed to air without protection. These tablets were randomly sampled at 1, 2, and 4 weeks. This type of test readily indicates the relative stability of various nitroglycerin tablet formulations. The partially stabilized brands lost substantial amounts of nitroglycerin in 1 week, *i.e.*, 32% for Product A and 24% for Product B tablets. This downward trend for these products continued until less than 13 and 23% of the drug, respectively, were retained at the end of 4 weeks. Product C tablets remain unaffected by exposure times of up to 2 weeks, after which a loss in nitroglycerin content occurred. The vertical lines in Fig. 2 indicate the range of assay values of the individually assayed tablets. These ranges were highest for Products A and B.

Results from the simulated in-use test of nitroglycerin tablets are given in Fig. 3. After 28 days of usage, again the average content of nitroglycerin in Product C tablets remained unchanged. Product A tablets and Product B averaged 0.02 and 0.05 mg, respectively, less than the initial assay values. The ranges of the content, which are also given in Fig. 3, indicate that the range of nitroglycerin content in Product C tablets after 28 days was 0.03 mg, the same as the original tablets. The ranges for the two other brands of nitroglycerin tablets increased over the initial ranges, changing from 0.08 to 0.09 mg for Product A tablets and from 0.06 to 0.12 for Product B.

Table VI—Comparison of Nitroglycerin Volatility from Various Tablet Formulations Subjected to Thermogravimetric Analysis[a]

	Nitroglycerin Remaining, %		
	Tablet 1[b]	Tablet 2[b]	Average
Product A[c] (Lot PA165)	54 54	56 59	56
Product B[d] (Lot 6MG53A)	23 21	22 21	22
Product C	97 98	93 95	96

[a] At 80° for 90 min; chemical assay values. [b] Duplicate assay results from individual tablets. [c] Product contains a stabilizer. [d] Product contains no stabilizer.

DISCUSSION

The overall quality of pharmaceutical products available from various sources has been discussed frequently in recent years by both scientific and government groups. Considerable attention has been given to the comparison of bioavailability of drug dosage forms from various sources, but the more fundamental quality aspects of drug products and their relative stabilities probably have not been compared to the same extent.

In this study, three brands of nitroglycerin tablets were compared for conformance to tests in the USP XVIII monograph; bioavailability tests were performed, and nitroglycerin instability was determined. The weight variation of the molded tablet formulations was significantly larger than that of the compressed formulation. Thus, the method of tablet manufacture is a significant factor in the dose uniformity of nitroglycerin.

The content uniformity of nitroglycerin tablets reflects both variation in weight and variation in content *per se* due to direct volatilization and tablet to tablet migration of the drug. The two molded tablet formulations had relative content variations of about 6–14%. Some of these tablets, labeled 0.4 mg, contained as little as 0.30 mg and as much as 0.55 mg. On the other hand, the compressed tablet formulation had a relative content variation of about 2% and a content range of 0.02–0.03 mg. Four of eight lots of the molded tablets contained two or three tablets out of 10 that were more than 15% below the average content, and one lot contained one tablet below the 15% average. Where more than one tablet of 10 varies by more than 15% from the average tolerance, the USP specifies that additional testing should be carried out on 20 additional individual tablets and that none should be less than 75% or more than 135% of the labeled amount. This additional test was not required for the compressed tablet formulation since it was within the 15% limit.

Comparative drug availability testing included both *in vitro* and *in vivo* tests. Similar results were obtained for all three products tested for disintegration, permeability, and bioavailability.

Volatility studies indicated that Product C was superior in retaining nitroglycerin. Thermogravimetric analysis was initially used to screen formulations in the development phase; testing at 80° for 90 min was a good screening method. In this test, the comparative losses of nitroglycerin were 4% for Product C, 56% for Product A, and 78% for Product B without stabilizer. In the open dish test, Product C was much more stable than the other two brands.

Table V—Mean Pulse Change from Baseline for Seven Treatments at 11 Time Periods[a]

Treatment	Minutes after Drug										
	1	3	5	7	9	11	13	15	20	25	30
Product B											
0.3 mg	4.8	12.0	10.8	9.4	7.6	4.0	1.7	−0.4	0.5	−1.4	−0.6
0.4 mg	4.2	12.4	11.3	10.5	8.1	6.4	5.0	3.5	−0.7	0.1	0.2
0.6 mg	4.9	12.1	10.6	10.3	6.8	6.1	2.3	1.3	0.6	−0.2	1.0
Product C											
0.3 mg	2.3	10.2	11.1	9.4	6.4	4.3	2.3	2.7	0.9	1.0	1.0
0.4 mg	3.1	10.7	11.1	9.5	6.6	4.4	2.9	2.4	−0.3	0.6	1.1
0.6 mg	3.1	13.3	12.9	10.5	9.0	6.3	4.6	3.5	0.6	−0.1	0.2
Placebo	0.0	0.1	−0.1	0.4	−0.4	−0.9	−0.9	−0.5	−0.1	−1.6	−1.6

[a] Ten human subjects; see text for details.

In the 28-day simulated use test, the remaining Product C tablets had the same original average content and content range as the starting tablets. Both Product A and B tablets experienced some potency loss as well as an increasing variability.

In conclusion, the compressed tablet formulation was as active as two other widely used nitroglycerin tablet formulations. However, the uniformity and stability significantly exceeded those of the two popularly used molded tablet formulations, particularly under the stress conditions likely to be encountered in a patient usage regimen.

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Electrochemistry of Drug Action I: Electroreduction of Ferredoxins

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Abstract □ Ferredoxin serves as an electron carrier in the oxidation–reduction system in anaerobic microorganisms, transferring electrons from a low potential donor to electron-accepting biochemicals. The anaerobicidal activity of some drugs may be due to their interference with the electron transport function of ferredoxin. Two types of ferredoxins (isolated from *Clostridium pasteurianum* and spinach) were studied, and their electrochemical reduction and biochemical properties were analyzed using a sensitive ac polarographic technique. The reduction potential of both ferredoxins was linearly related to pH. The mechanisms of electron transport in ferredoxin molecules were found to be related to their sulfur–iron bonds. The dissociation of the sulfur–iron bonds resulted in the formation of a free sulfhydryl group and the interruption of the electroactivity of ferredoxin. This sulfur–iron dissociation process was found to be pH dependent. The electroreduction of ferredoxins was an energy-requiring, pH-dependent process.

Keyphrases □ Electrochemistry—of drug action, electrochemical reduction and biochemical properties of ferredoxins studied using ac polarography □ Ferredoxins—electrochemical reduction and biochemical properties studied using ac polarography □ Electroreduction—ferredoxins, studied using ac polarography, effect of pH □ Polarography, ac—study of electroreduction and biochemical properties of ferredoxins □ Oxidation–reduction systems—ferredoxins studied using ac polarography, effect of pH

Ferredoxin, a nonheme iron-containing protein, was first isolated from *Clostridium pasteurianum*, an anaerobe, by Mortenson *et al.* (1), and crystalline preparations were obtained later from several other anaerobic bacteria (2, 3).

Much research interest has been generated since regarding the biochemical significance of ferredoxins (4) and their relationship to the anaerobicidal activity of several drugs (5–7). These studies indicate that ferredoxin serves as an oxidation–reduction enzyme in anaerobes by transferring electrons from a low-potential donor, *e.g.*, pyruvic acid, to electron-accepting biochemicals, *e.g.*, pyridine nucleotides, or to H^+ to produce H_2, a terminal electron acceptor characteristic of anaerobes. The interruption of this vital pyruvate phosphoroclastic reaction may result in selective toxicity to the anaerobes.

Recently, dc polarography was used to measure the redox potentials of some antimicrobial agents; their antianaerobic activity was related to their redox potentials relative to the redox potential (−470 mv) of ferredoxin (5). Studies of the complexation of metronidazole, an agent effective against anaerobes, with cupric ion indicated that ac polarography has several advantages over dc polarography in terms of sensitivity and reproducibility of electrochemical measurements (8). These advantages should be beneficial to the mechanistic analysis of ferredoxin–drug interactions.

In this study, the electrochemical reduction of two types of ferredoxins was examined, using an ac polarographic technique, to gain a better understanding of the electrochemistry of their electron transport mechanisms.

EXPERIMENTAL

Materials—Clostridial and spinach ferredoxins are available as frozen solutions in neutral tromethamine buffer[1] and were used as obtained. Triple-distilled, instrument-grade mercury[2] was applied in a dropping mercury electrode. Freshly deionized, triple-distilled

[1] Trizma, Sigma Chemical Co., St. Louis, MO 63178

[2] Bethlehem Apparatus Co., Hellertown, Pa.