Development and Evaluation of an Inhalation Aerosol of Nitroglycerin

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Abstract □ The disadvantages of sublingual tablets of nitroglycerin are discussed along with the need for a more stable, uniform, and rapidly acting dosage form. Data indicate that an inhalation aerosol product offers these advantages. Problems could be encountered in the choice of gasketing material in the aerosol valve and the type of activator. The onset time of an aerosol product when properly administered can be as short as 20 sec.

Keyphrases □ Nitroglycerin inhalation aerosol—formulated, compared to sublingual tablets in humans □ Aerosols, nitroglycerin—formulated, compared to sublingual tablets in humans

Many drugs have been used in the symptomatic treatment of angina pectoris, but the one that has most consistently been of benefit in relieving myocardial ischemia is nitroglycerin. Although it is often the drug of choice in such conditions, there has been no appreciable progress in the pharmaceutics involved in its dosage forms, and the sublingual administration of the drug by means of hypodermic tablets remains the only widely used method of administration.

The apparent disadvantages of sublingual tablets of nitroglycerin are: *(a)* volatility of nitroglycerin and resulting problems of stability, *(b)* tablet-to-tablet variation in weight and potency, *(c)* variability in absorption patterns, *(d)* friability of tablets, *(e)* diminutive size of tablets and difficulty in handling, and *(f)* difficulty in packaging. Many of these problems were discussed previously (1). It seems that a pressurized aerosol product would be a more convenient, stable, and accurate dosage form; it would also be faster acting, releasing the active ingredient instantly, and would surmount most of the pharmaceutical disadvantages inherent in the present commercial sublingual tablet. Several advantages of administering medicinals by inhalation as compared to other routes of administration were reported (2–6).

An aerosol of nitroglycerin[1], delivering 0.13-mg doses, is available and the manufacturer claims that it relieves anginal pain in less than half of the time taken by any other nitrate preparation. However, no scientific documentation regarding formulation or clinical effectiveness has been published. One report (7) commented that the claim of rapid onset of action rests on the results of a single, uncontrolled, and unpublished trial in which angina patients were given the product and then asked for their opinions. Consultants of this publication (7) further stated that rapid onset of effect is not required and that it was difficult to believe that use of an aerosol would be easier and more convenient. They concluded that there was no good evidence that inhalation of nitroglycerin aerosol is any better than sublingual administration.

A comparison of the effectiveness in angina of an aerosol and sublingual tablets of nitroglycerin was made by Sandler and Clayton (8). They reported that the active aerosol was no more effective than a placebo aerosol and that the tablets significantly improved ischemic changes in the ECG when given before exercise but not when given after it. However, their experiment was poorly designed. They compared two 0.13-mg doses of the aerosol with a single 0.5-mg tablet. In addition, they did not disclose the aerosol formulation, particularly in respect to stability of the drug. They did not mention how much drug was retained on the actuator, which could be more than 20% of the dose (9).

Therefore, it was decided to formulate a nitroglycerin inhalation aerosol and evaluate its stability, effectiveness, and onset of action compared to sublingual tablets.

EXPERIMENTAL

The nitroglycerin used in the study was recovered from a commercially available 10% blend in lactose by shaking with water. Nitroglycerin, being very slightly soluble and heavy, settled to the bottom of the flask. The drug was purified by repeated washings with water and was then filtered through a sodium chloride bed, using a büchner funnel to remove traces of water and insoluble impurities. The final filtrate was assayed and found to be 99.3% nitroglycerin.

Assay—The spectrophotometric method suggested by Bell (10) was selected to assay nitroglycerin because of its simplicity, rapidity, and reproducibility in assaying microgram quantities of the drug. This stability-indicating assay involves alkaline hydrolysis to nitrite ion followed by diazotization and a coupling reaction followed by subsequent colorimetric determination of the dye formed. Five actuations of aerosol were obtained by depressing the inverted container without actuator into a small beaker containing about 25 ml of water, and the volume was adjusted to 250 ml. An aliquot was used as the assay sample.

Formulation—After numerous experiments, the following formulation was selected for evaluation:

nitroglycerin	1.0% w/w
propylene glycol	3.0% w/w
ethanol (absolute)	15.0% w/w
dichlorodifluoromethane [propellant]	16.0% w/w
dichlorotetrafluoroethane [propellant]	65.0% w/w

The nitroglycerin and propylene glycol were dissolved in ethanol. The solution and propellant blend were chilled in an acetone–dry ice bath to approximately −45° and filled by weight into prechilled, 20-ml plastic-coated glass vials onto which inverted 50-μl meter valves with emptying cups were crimped. The pressure of the product was approximately 27 psig. Dose reproducibility was excellent (Table I).

[1] Cardamist, Nicholas Laboratories, Ltd., England.

Table I—Dose Reproducibility

Dose	Nitroglycerin
1	0.521
2	0.515
3	0.524
4	0.535
5	0.517
6	0.521
7	0.517
8	0.524
9	0.536
10	0.518
Mean	0.523
SD	0.0072

Since actuators are expected to retain a part of each dose, the amount remaining on the actuator must be determined and should be taken into account in the final formulation. A plastic actuator (11), 40 mm long and with a diameter of 21 mm, was used; it delivers the product at a right angle to the container. Based on a series of seven actuations, it was found that 0.13 ± 0.01 mg of nitroglycerin was retained on the actuator during each administration. This amounted to almost 25% of the average dose (0.523 mg) delivered from the meter valve and should be considered when trying to evaluate dose response.

Particle-Size Measurement—The samples for particle-size measurements were obtained by actuating the product through a camera shutter adjusted for 0.03-sec opening time and collecting the sprays from a distance of 15 cm on glass slides warmed to 37°. This was done to get the samples with as little superimposition of particles as possible. The slides were warmed to get the conditions as close to that obtained during actual use of the product.

Magnified pictures of the sample slides were taken[2], and a combination of ocular and objective lenses was used to yield 240× magnification. Illumination was provided with a heat-absorbing filter glass. The particles in the photomicrographs were classified by comparison with a standard micrometer scale picture taken at the same magnification. The measurements were made under a low power of a stereoscopic microscope[3]. About a thousand particles from eight different samples were classified in three groups. About 95% of the particles was smaller than 5 μm in diameter, about 4% fell between 5 and 10 μm, and less than 0.5% was above 10 μm in diameter.

Particle-size measurement of a solution-type aerosol product is not an absolute procedure. The three major problems encountered in the measurements of particle size of the experimental aerosol products were: *(a)* time and temperature, *(b)* sampling procedures, and *(c)* limitation of the microscope. Due to the volatile nature of the formulation, the particles decreased in size during the first minutes after spraying. This was overcome to a great extent by spraying onto a 37° surface, which caused immediate volatilization of drops and resulted in particles made up of nitroglycerin dissolved in propylene glycol. The different forces at which the particles hit the slide gave variation in degrees of flattening. Furthermore, the use of a light microscope and camera setup limited visible particles to above 1 μm. It is theorized that many particles below this size were actually produced by the aerosol.

Packaging and Stability—Three sets of test product containers were prepared. The first set contained the usual single rubber gasket to help create a seal between the rim of the glass container and the valve ferrule. The second set had three rubber gaskets placed in the product in addition to one normally used in the valve. The third set had three polyethylene gaskets placed in the product in addition to the one polyethylene gasket used with the valve. Four units each from the first two sets of containers were stored at room temperature (28 ± 1°) and at 53 ± 0.5° in a hot air oven[4]. Two units from the third set were stored at room temperature (28 ±1°).

Duplicate assays on a group of five doses from each container were carried out periodically during the stability test. It was observed, particularly with the containers stored at elevated temperature, that the assay results were showing an increase in nitroglycerin content when assayed after a long interval. This was no doubt due to the loss of propellant from the emptying cup of the valve, thereby concentrating nitroglycerin in the cup.

Table II—Experimental Design

Time, hr	Group	Treatment
Initial	1	A
	2	B
	3	C
48	1	B
	2	C
	3	A
96	1	C
	2	A
	3	B

Methods of Pharmacological Evaluation—An experiment was designed to evaluate various pharmacological effects of nitroglycerin delivered by the inhalation aerosol dosage forms and to compare these effects with those obtained from an equivalent amount of the drug in the form of sublingual tablets (nitroglycerin tablets USP[5], 0.4 mg).

Six healthy male subjects, ranging in age from 22 to 36 years (average 28 years), with normal systolic blood pressure ranging from 111 to 145 mm of mercury (average 135 mm) and with weights ranging from 57.2 to 73.5 kg (126 to 162 lb) [average 64.9 kg (143 lb)], were selected. To minimize the time, subject, dosage form, and tolerance effects, the subjects were divided into three groups of two, which were administered either the active tablet (A), active aerosol (B), or placebo aerosol (C) in a crossover random order. The subjects were not informed whether the medication given to them was active or a placebo. A 48-hr interval was allowed between each test. The exact layout of the test is shown in Table II.

Preliminary tests with placebo tablets proved to be completely negative and were not included in the study due to limitations of subjects. These preliminary tests involved the establishment and evaluation of the ECG and plethysmographic procedures. During this time, several subjects were given both active and placebo tablets. No change in either ECG or plethysmographic readings was noted upon administration of any placebo tablet.

All subjects were asked to adhere strictly to the following restrictions on their daily routine during the experiment:

1. No alcohol or drugs 24 hr prior to test.
2. No food, coffee, tea, or smoking for 3 hr prior to test.
3. No strenuous exercise on the day of test.

The experiments were carried out with the subjects in the supine position. To obtain somewhat higher response to the drug, the head of the bed was elevated by about 25°. The subjects were allowed to rest for 20 min after fixing the electrodes for ECG measurements, the plethysmographic cuff on the right arm, and the sphygmomanometer cuff on the left arm. Preliminary studies demonstrated that 20 min was sufficient to equilibrate the plethysmographic cuff with the volumetric pressure transducer[6] and to bring the blood pressure to its normal resting level. The subjects were given instructions as to the purpose of the study and were told to signify subjective responses such as headache, pounding, or flushing by gently raising the index finger of the left hand. Before, during, and after the administration of the drug, the subjects were asked to remain still, relaxed, and quiet. It was absolutely necessary to refrain from any movement to achieve dependable results.

During preliminary experiments, problems of improper self-administration of drug by the subjects were encountered, particularly with the aerosol products. The major portion of the spray was found to be striking the walls of the oral cavity instead of reaching the lungs. This gave a delayed onset of action at about 1-2 min. Moreover, the movement of the body during self-admin-

[2] A Polaroid Land Camera was mounted on an American Optical Series 682 shutter apparatus which, in turn, was mounted over a standard student model microscope (Bausch and Lomb Optical Co., Rochester, N.Y.).
[3] American Optical Spencer Cycloptic Series 58.
[4] Model A, Precision Scientific Co., Chicago, Ill.
[5] Lot 2 EG 7A, Eli Lilly & Co., Indianapolis, Ind.
[6] Model PT 5A, Grass Instruments, Quincy, Mass.

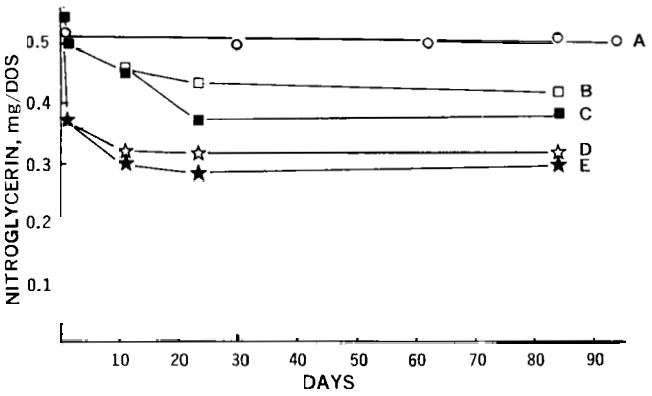

Figure 1—*Effect of time, temperature, and gasket material on the stability of nitroglycerin in aerosol dosage forms. Each point represents an average of four replicates. Key: ○, three plastic gaskets and sealing gasket at 28°; □, rubber sealing gasket only at 28°; ■, rubber sealing gasket only at 53°; ☆, three rubber gaskets and sealing gasket at 28°; and ★, three rubber gaskets and sealing gasket at 53°.*

istration created disturbances in the recording of plethysmograph. Although there would appear to be no problem of educating patients to proper use during self-administration, it was decided to administer the test drugs by a trained individual. The tablets were inserted sublingually. The aerosol preparations were administered during oral inhalation.

Measurement of Experimental Parameters—The heart rate was continuously recorded by ECG measurement on lead II using a polygraph[7]. A chart speed of 15 cm/min was used during the experiment. Recordings, at a chart speed of 50 cm/min, for each individual before the start of the experiment were made to check the normal nature of the ECG. Heart rate was calculated from the recording between 1 and 3 min before the administration of the drug and for 2 min immediately after the onset of action of the drug, and the difference was noted. Onset time was obtained both from the measurement of change in arm volume and from subjective response.

To measure change in arm volume, the right arm of the subject was elevated approximately 7 cm above the level of the heart so the venous pressure was zero (12, 13). A sphygmomanometer cuff was placed around the right upper arm and was connected to a water-filled buret to measure the amount of pressure inside the cuff; the pressure in the cuff was kept at about 2.5 cm of water. The connection with the buret was cut off, and the cuff with the pressure of 2.5 cm of water was then attached to the pressure transducer. The pressure transducer was connected to the polygraph for a continuous recording of a change in cuff pressure induced by a change in the volume of the arm. At least 20 min was allowed for equilibration before the administration of the drug. The recording was continued for 20 min after drug administration. The onset time was determined by taking the difference between the time of drug administration and the time at onset point, which was the point of intersection of the baseline and the slope due to the effect of the drug.

Fall in systolic pressure was measured as a parameter for evaluation of the test product. The blood pressure was determined by ausculatory measurements made at the brachial artery of the left arm using a mercury sphygmomanometer. Measurements were made initially and 5 min before, 5 min after, and 20 min after drug administration. The difference in systolic blood pressure between the readings taken at 5 min before and 5 min after drug administration was recorded.

RESULTS AND DISCUSSION

The stability of the nitroglycerin aerosol dosage form was investigated by employing both room temperature and accelerated aging tests, using rubber as well as plastic gaskets. Solutions containing the polyethylene plastic gaskets showed no decrease in potency for nitroglycerin on aging for more than 90 days at room temperature, as can be seen in plot A of Fig. 1. However, plots B and C, illustrating data collected from the test containers utilizing one regular rubber gasket, show a significant decrease in drug content for about a month. As would be expected, the solution at the higher temperature (plot C) showed a greater loss in potency. Plots D and E represent data from solution in which three rubber gaskets were placed in addition to the one normally found in the valve. The results are similar but more dramatic than in those containers containing only the single gasket.

[7] Model 50, Grass Instruments, Quincy, Mass.

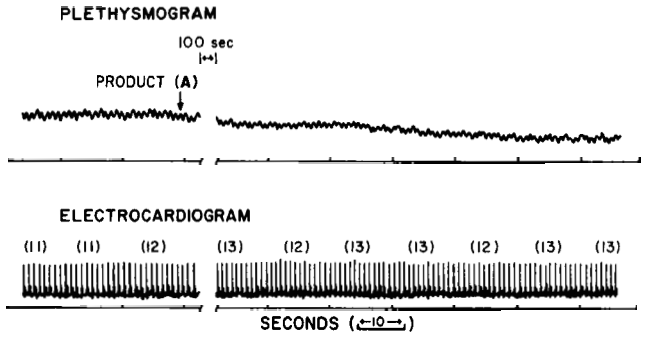

Figure 2—*Plethysmogram and ECG of a healthy male subject before, during, and after the administration of a sublingual tablet containing 0.4 mg nitroglycerin (chart speed = 15 cm/min).*

The slight increase in nitroglycerin content in the samples stored at elevated temperature was most probably due to leakage of propellant over 3 months, which caused the drug solution to become more concentrated. The fact that the decrease in potency reached a minimum and then leveled off indicates that the loss may result from a surface effect which exhibits itself rapidly but is self-limiting. However, the drop in potency from an initial reading of 0.54 mg to a reading of 0.27 mg in the case of plot E represents a 50% loss. Even the single gasket sample at room temperature exhibited a drop from 0.54 to 0.43 mg, or almost a 20% loss. In containers with a lower concentration of nitroglycerin, as in the study of Sandler and Clayton (8), this loss could be even more dramatic and could account, along with other things, for the failure to obtain significant results.

Evaluation for Effectiveness and Onset of Action—The changes in heart rate, arm volume, systolic blood pressure, and subjective responses such as headache, pounding, palpitation, and flushing were recorded to evaluate the effects of nitroglycerin in both the aerosol and the sublingual dosage forms.

Typical ECG's (lead II) of a healthy individual taken before, during, and after the administration of active tablet, active aerosol, and placebo aerosol on alternate days are shown in Figs. 2–4. From the graphs it can be seen that an increase in heart rate was obtained after administration of active medication, while no change was noticeable with placebo medication. Table III shows the increase in heart rate obtained from the records of six individuals with the three test products. The results clearly show the difference between the active and the placebo products. However,

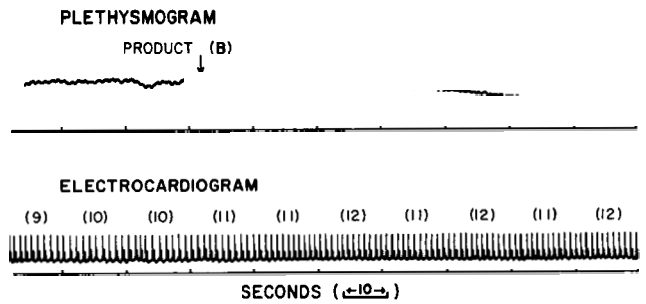

Figure 3—*Plethysmogram and ECG of a healthy male subject before, during, and after the administration of an inhalation aerosol containing 0.4 mg nitroglycerin (chart speed = 15 cm/min).*

Table III—Increase in Number of Heart Beats per Minute as Recorded by ECG in Response to Administration of Test Products[a]

Day	Test Products: Active Tablet	Active Aerosol	Placebo Aerosol	Total
1	18	12	−1	29
	12	9	−3	18
3	8	16	3	27
	14	13	−2	25
5	9	12	−1	20
	14	14	0	28
	75	76	−4	147

Analysis of Variance

	Sum of Squares	Degrees of Freedom	Mean Square	*F* Ratio	
Row means	2.33	2	1.17	0.13	$F_{0.95}(2,9) = 4.26$
Column means	702.33	2	351.17	40.04	$F_{0.95}(2,9) = 4.26$
Within groups	113.83	13	8.77		
Total	818.5	17			

[a] Three groups of two subjects on 3 different days.

Table IV—Time (Seconds) Required for Onset of Action in Response to the Administration of Test Product[a]

Day	Test Products: Active Tablet	Active Aerosol	Total
1	123	22	145
	108	72	180
3	103	19	122
	89	97	186
5	150	16	166
	68	15	83
	641	241	882

Analysis of Variance

	Sum of Squares	Degrees of Freedom	Mean Square	Mean *F* Ratio	
Row means	795.5	2	397.75	0.338	$F_{0.95}(2,6) = 5.14$
Column means	13,333.3	1	13,333.33	11.33	$F_{0.95}(1,6) = 5.99$
Within group	9,410.2	8	1,176.27		
Total	23,539	11			

[a] Three groups of two subjects on 3 different days.

a t value of −0.56 showed that there was no significant difference between the active aerosol and the active tablets.

The effects of test products on the plethysmograms of the right arm of a healthy individual are shown in Figs. 2–4. A point of onset of action can be determined by the intersection of the baseline and the slope of the plethysmograph curve due to the change in arm volume. The chart speed was fixed at 15 cm/min. It can be seen from these figures that there was no onset of action when the placebo product was administered while there was a quick onset with the active aerosol and a delayed onset with the active tablet. The data obtained regarding time required for onset of action are recorded in Table IV. No record was made for the placebo aerosol because no onset was observed with this product in any subject.

The data in Tables III and IV were subjected to analysis of variance to test for subject and time effects, if any, due to differences in results obtained on different days. The *F* values for row means were not significant at the 5% level, indicating no subject and time effects in the experiment. However, the *F* value for the column means in Table IV is significant at a 5% level, indicating that the active aerosol gave a significantly more rapid onset of action when compared with the active sublingual tablet.

The onset of action varied from patient to patient. There is a good possibility that the lengthy onset time for Patients 2 and 5 were due to improper administration, *i.e.*, failure of patient to inhale properly. Then the nitroglycerin was simply deposited on the oral mucosa and had to be absorbed, similar to the process involved in sublingual tablet administration. However, even when the slow response of these individuals is considered, the onset time for the inhalation aerosol product is significantly less than for the tablet.

A decrease in systolic blood pressure due to the administration of the test product is recorded in Table V. The data clearly indicate that the active tablet and the active aerosol caused a decrease in blood pressure while the placebo aerosol did not elicit any effect.

Subjective onset responses such as headache, pounding, palpitation, or flushing were recorded for each subject after administration of the test products. All subjects showed positive responses with active medication on the average 12 sec later than that observed in the corresponding plethysmograms. Only one subject responded positively to the placebo aerosol.

From the overall analysis of the data obtained from all experimental parameters, it can be concluded that in healthy male subjects the active aerosol is as effective as the active tablet and that the placebo aerosol is not effective when compared to either active product. The active aerosol gives a significantly more rapid onset of action.

The use of sublingual tablets of nitroglycerin in acute angina attacks has sometimes been questioned due to the delay, however short, in therapeutic effects. Most physicians feel that the pain of angina will diminish by itself within 2–3 min of onset. A dosage form that can decrease this onset time significantly as the aerosol does appears to have meaningful therapeutic advantages.

The problem of administration of an aerosol in the throes of an anginal attack certainly must be considered. This involves the re-

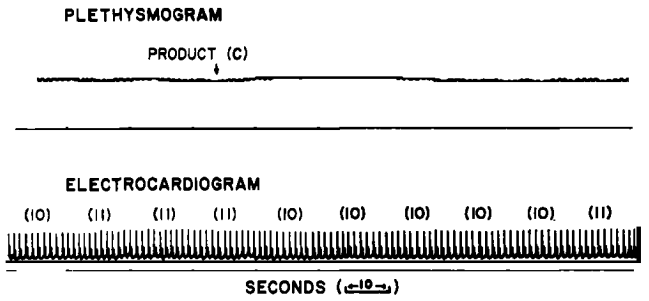

Figure 4—*Plethysmogram and ECG of a healthy male subject before, during, and after the administration of a placebo aerosol (chart speed = 15 cm/min).*

moval of the aerosol container from the pocket or pocketbook, activation of the valve, and simultaneous inhalation with valve actuation. Although these cannot be minimized, they should be surmountable with proper patient education. Even with hypodermic tablets, it is necessary for a patient to remove a screw cap from a bottle, remove a single tablet, and place the tablet beneath the tongue. It is well known that patients using nitroglycerin tablets often take more than one tablet in their anxiety to obtain relief.

The use of nitroglycerin in attacks of angina is a well-accepted treatment. Hopefully, this article will stimulate interest in the formulation of an aerosol product of nitroglycerin—if not an inhalation product, then possibly a simple sublingual one.

CONCLUSION

1. The nitroglycerin aerosol dosage form is as effective as the sublingual dosage form of the drug in healthy individuals when changes in heart rate, arm volume, and blood pressure are compared.

2. The nitroglycerin aerosol dosage form is significantly more rapid in onset of action than is the sublingual dosage form of the drug when pharmacological effects are compared in healthy male subjects.

3. The gasketing material used in aerosol meter valves offers a potential problem in formulating a stable product. Preliminary studies indicate that plastic gasketing material may be superior to traditional rubber gaskets.

4. The actuator used in inhalation products can retain significant amounts of the drug on its surface, depending upon the formulation.

5. A nitroglycerin aerosol would appear to overcome the problems of loss of potency and content uniformity associated with traditional sublingual tablets.

Table V—Decrease in Systolic Blood Pressure (Millimeters of Mercury) in Response to Administration of Test Products

Subjects	Active Tablet	Active Aerosol	Placebo Aerosol
1	5	5	−4
2	12	10	0
3	6	8	0
4	10	9	0
5	10	8	0
6	0	4	0
Average	7.1	7.3	−0.7

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