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THE EFFECT OF DEVICE CHARACTERISTICS ON NITROGLYCERIN RELEASE FROM A TRANSDERMAL PATCH

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THE EFFECT OF DEVICE CHARACTERISTICS ON NITROGLYCERIN RELEASE FROM A TRANSDERMAL PATCH

by

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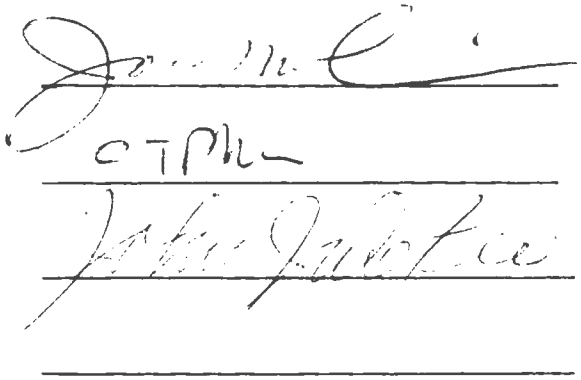

Dean of the Graduate School

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ABSTRACT

The effects of device characteristics on the release of nitroglycerin from a silicone disc were evaluated. A series of silicone discs containing nitroglycerin was prepared at varying levels of drug concentration, surface area, and firmness. A modification of the U.S.P. Dissolution Method I was used to evaluate the effects of these parameters on the in-vitro release of nitroglycerin from the discs. Reverse-phase HPLC was used for the quantitative analysis. The in-vitro dissolution method appeared reliable and reproducible in that there was good correlation (r=0.920) of dissolution profiles in both commercial transdermal products and test systems. The experimental data indicate that drug concentration and surface area have the greatest effect on the rate and extent of release of nitroglycerin over a 24 hour period. Firmness of the matrix appeared to have no significant effect on the rate or total amount of drug released from the systems.

ACKNOWLEDGEMENTS

The author wishes to express her appreciation to the faculty, staff members, and friends at the University of Rhode Island College of Pharmacy, and to the Richardson-Vicks Corporation for its support of graduate education. Special thanks are extended to Jon Ericson for his patience and technical services, and to Jack Hoblitzell for sharing his knowlege of the computer.

I am especially grateful to Dr. Joan M. Lausier for her advice and support during the course of this project, both of which I could not have succeeded without.

DEDICATION

To my father, Phillip E. Guilmette:

His wisdom, understanding, and love have guided me tnrough many difficult hours in my life. I especially thank him for having faith in my abilities, and for encouraging me to complete this project.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (continued)

OBJECTIVE

The recent addition of controlled delivery systems to the transdermal route of drug administration was expected to provide a method of drug delivery which would minimize absorption variability and maintain more constant blood levels of drug. However, studies have shown that unpredictable release can occur (Hollenberg, 1984; Weber, 1984; Reichek, 1984; Olivari, 1983; Jordan, 1985; Karim, 1983), and evidence suggests that changes in certain physical and chemical characteristics may be responsible. The effects of these factors on controlled drug release have been found to vary between systems.

The purpose of this study was to examine the individual and interactive effects of various device characteristics on the release of nitroglycerin from silicone discs. Data obtained in this study will contribute to the expanding field of drug release from transdermal delivery systems.

INTRODUCTION

1. Principles of Percutaneous Absorption

1.1 The Skin

The skin is considered the largest organ in the human body and its high accessability make it an excellent target for drug administration. In order to exert a systemic effect following application of a transdermal device, a drug must pass through one or more cell barriers or membranes. It is therefore necessary to consider the composition of this system and the transport processes involved in the movement of drug molecules across the intact membrane.

The skin includes the tissue that covers the body along with the mucous membranes and modified cutaneous structures. The average surface area of the skin of an adult male is about 20 square feet and may weigh 9 or 10 pounds. Human skin is a stratified tissue composed of three basic layers: the epidermis, the dermis, and the underlying tissue. The structure of the skin is shown in Figure 1. The functions of the skin include sensation, protection, thermoregulation and secretion. The skin forms an elastic resistant covering which protects man from the environment and prevents the passage of harmful physical, chemical and microbiological agents. (Mullins, 1980).

The epidermis is a relatively thin layer, only 0.1 to 0.2 mm in thickness. The epidermis itself is composed of four layers: the stratum corneum, stratum lucidum, stratum granulosum, and the stratum germinativum. The stratum corneum forms the outermost layer of the epidermis. It is well established in the literature that the stratum

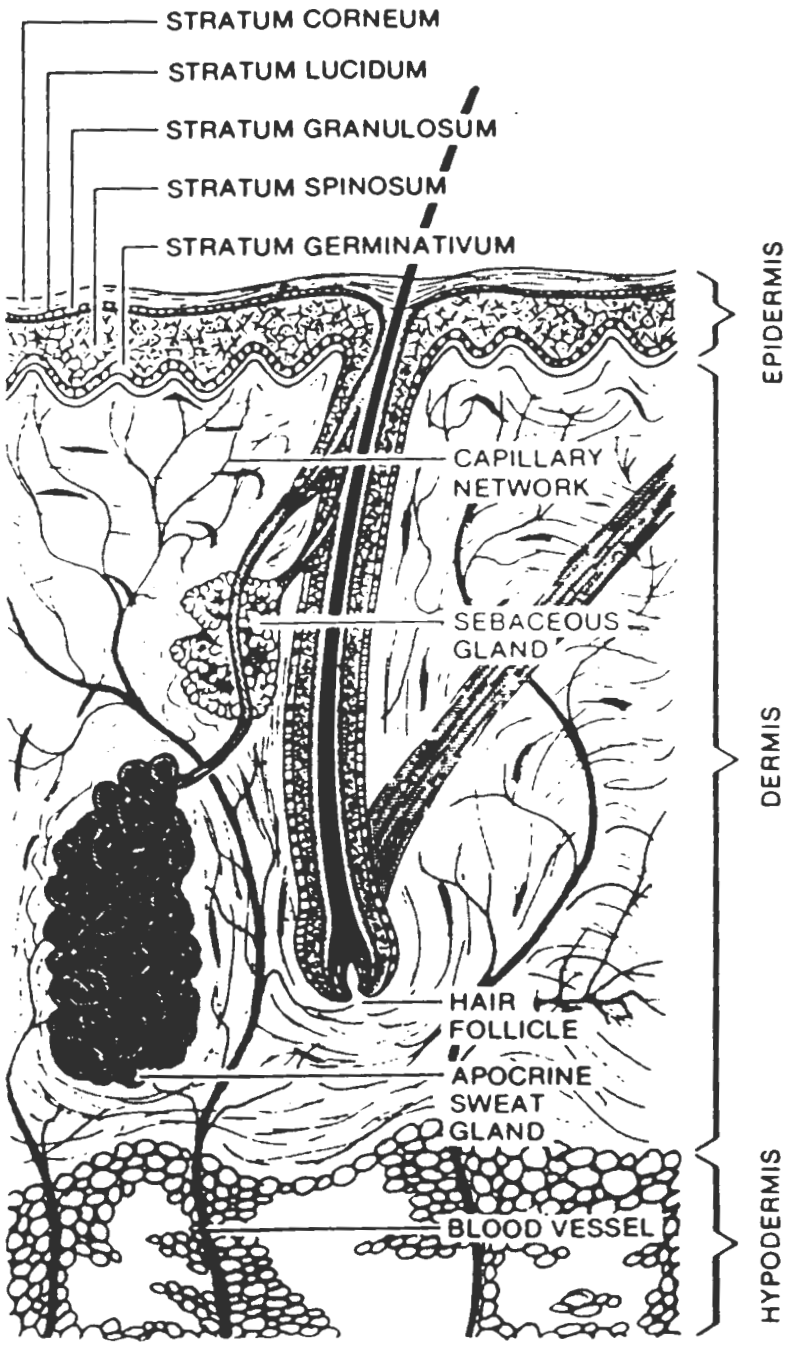


Figure 1. Cross-section of the skin. (Handbook of Nonprescription Drugs, 5th ed., p. 300, American Pharmaceutical Assoc., Washington, D.C., 1977)

corneum is the principal barrier to the permeation of drugs through the intact skin (Shaw, 1983). It is composed of 40 percent protein, 40 percent water, and the balance is lipids. This layer is 10-15 um thick, is comprised of dead, dry cells, and it exhibits regional differences in thickness over the body (Michaels, et al, 1975). The cells of the stratum corneum are stacked in overlapping "columns" and are more ordered in the areas of thinner epidermis. Aggregates of keratin inside the cells of the stratum corneum are cross-linked in a characteristic folded configuration (alpha keratin), rendering the fibers elastic. When stretched, the keratin chain is drawn into a more linear form (beta keratin).

The outermost surface of the stratum corneum is covered in a film made up of emulsified lipids, which act as a seal to bind the cells together. The surface has an acid pH, ranging from 4 to 6.5, depending on the area of the body. The lipid layer between the cells can be considered a bilayer, in that both polar and nonpolar sections are found. The components of this layer have been shown to be directly responsible for retarding penetration of many molecules into the body (Michaels, et al, 1975).

The protein component of the stratum corneum is hydrophilic in nature and can be hydrated to swell the skin using the hydroxyl and amino groups on the molecules. The protein can also be altered by heat or chemicals, such as surfactants, to result in the conversion from alpha to beta configuration. The end result is greatly enhanced permeability of the skin.

The skin as a body organ is constantly undergoing changes. The degree of hydration, thickness, and composition will vary as a function

of age, race, and environmental conditions. Epidermal cell growth begins in the basal layer of the stratum germinativum. Cells generated in the basal area move toward the skin surface, changing in shape as they move. This process of cell turnover requires up to two months (Cooper, 1985).

In addition to passage through the stratum corneum cells, molecules may also be transported through the follicular orifices and sweat gland ducts which scatter the surface of the skin. These provide alternate pathways for absorption although their significance is questionable. The contribution of the sweat glands and hair follicles has been investigated by several workers. According to Scheuplein (1972), substances can be absorbed via the appendages by a diffusion process. He calculated that the route via the glands is important for a brief period immediately after applying the test substance. Since these appendages account for only 0.1 to 1.0 percent of the total skin surface area, the transepidermal route must be the principal route for most materials. (Idson, 1975).

1.2 Percutaneous Absorption

Due to the nature of the stratum corneum, the process of percutaneous absorption is a passive one rather than an active one. "The absence of metabolic processes in the 'dead' keratinizing layers precludes any role for active transport" (Malkinson, 1977). This process occurs in two steps. Diffusion through the barrier layer is the first phase, and is affected only by physical factors as determined by ambient conditions. The second phase is the clearance from the dermis and this depends on blood flow and other factors inherent in the

dermal constituents (Idson, 1975). There is a delay period after the drug is placed on the surface of the skin, during which the membrane becomes charged with the penetrant, and then a longer second period during which the chemical penetrates the skin and is removed to the circulation. It is this second period that is governed by Fick's laws of diffusion (Michaels, 1975; Barry, et al, 1985). According to Fick's first law, the drug must dissolve in its vehicle and diffuse to the skin surface. Then, according to the principles of diffusion, the drug is transported across the skin. Fick's law states,

$$dQ = DA\ dc/dx\ dt\ ,$$

where the amount, dQ, of a substance diffusing in time, dt, across an area, A, is directly proportional to the change in concentration, dc, over the distance traveled, dx. "D" is the diffusion coefficient and dc/dx is the concentration gradient.

It has been shown that removal of the stratum corneum by successive "stripping" with cellophane tape can significantly increase the skin's permeability. (Grasso, 1972). This process increases transepidermal water loss and can result in a 60-fold increase in permeability (Chien, 1984).

Properties inherent in the dosage form may also affect the rate of diffusion of a substance through the skin. Both the chemical and physical properties of the test material must be considered. Ideally, the drug should be potent (i.e., be effective in low concentrations) and should have a good hydrophilic/ lipophilic balance (generally 1 or greater) (Chien, 1982). Substances will be more rapidly released from

vehicles having a low affinity for the penetrant. Pertinent factors here are related to the solubility of the penetrant in the vehicle, the rate of diffusion of the penetrant in the vehicle, and the rate of release of the penetrant from its base (Malkinson, 1977). The pH, melting point, and molecular weight of a compound all contribute to the degree of solubility of a drug in the skin (Shaw, 1983; Kligman 1984; Grasso, 1972). In the formulation of a vehicle for a topical drug application, many factors must be considered. Drug stability, specific product use, site of application and product type must be combined in a dosage form which readily releases the drug when it comes in contact with the skin. For a given concentration of a drug in a certain vehicle, the thermodynamic activity coefficient of the drug in the vehicle may vary from one vehicle to the next (Higuchi, 1960; Cooper, 1984; Horhota, 1979). Penetration is greatest when the drug is present at its maximal solubility concentration.

1.3 Factors affecting percutaneous absorption

The permeation of molecules through the skin may be influenced by physiological, physicochemical and thermodynamic factors. Physiologic factors include the age and condition of the skin and the degree of hydration. The condition of the skin, namely intactness, is one of the most important factors affecting penetration. If the stratum corneum is damaged, water loss is increased and permeability is enhanced. Increased hydration of the skin appears to "open" the compact substance of the stratum corneum and result in increased permeation of many substances. Hydration of the skin can be enhanced by occlusive dressings, and may result in 4-5-fold increases in permeability (Idson,

1975). Physical properties of the vehicle can improve the degree of occlusion produced, leading to hydration of the stratum corneum. The most occlusive vehicles are greases, oils and collodion.

Permeability of the skin appears to decrease with increasing age. This may be due in part to the greater percentage of water in the body of an infant as compared to the adult (Behl, 1984). In general, the skin of young children is sensitive to chemical irritation and harsh environmental conditions. As they mature, the cells develop resistance to many substances.

The effect of the site of application was noted in a study by Michaels, et al (1975), where it was shown that the permeation of scopolamine was greatest in regions where the stratum corneum is the thinnest. The post-auricular area possesses the thinnest layer, whereas the plantar region has the thickest (Idson, 1975).

Temperature of the skin has also been shown to affect percutaneous absorption. Under normal in-vivo conditions, substances penetrate the skin within a very narrow temperature range. For lipid soluble substances, increasing the skin temperature lowers the viscosity of the tissue lipids, thus reducing the activation energies for diffusion. The dual effects of temperature and humidity have also been shown to increase percutaneous absorption (Idson, 1975; Grasso, 1972).

2.0 Penetration Enhancers

A variety of substances are known to enhance the transport of drugs across the skin. Substances included in the formulation of cosmetics, soaps, and detergents may also produce profound local changes which can affect the permeability of the skin to other compounds. In order for

these penetration enhancers to be effective, they must modify the stratum corneum in some way. Surfactants increase penetration due to their ability to denature protein (keratin), thus "opening up" the skin structure and increasing its permeability. Other agents improve skin wettability, decrease surface tension or increase solute diffusivity (Keith, 1983; Hwang, 1983; Chien, 1982).

Few agents have been studied as intensively as dimethylsulfoxide (DMSO). In-vitro comparative studies showed DMSO to be superior to other solvents in both enhancing penetration and in favoring dermal retention of a drug (Grasso,1972). In-vivo, DMSO has been found to accelerate the penetration of many substances, such as corticosteroids, salicylic acid, dyes, and antibiotics (Sekura, 1972; Kligman, 1984; Idson,1975). For materials absorbed from the surface, a 50 percent concentration of DMSO can enhance the permeability of dermal connective tissue, apparently through a depolymerizing effect on hyaluronic acid (Malkinson, 1977). Despite the extensive studies, the exact mechanism of action is still not clear. Other possible mechanisms include partial extraction of lipids and protein configurational changes resulting from replacement of water by the vehicle.

Despite its potential in this respect, DMSO can also be quite toxic, causing refractive lens opacities, erythema, edema, and itching, and is noted also for the unpleasant taste and odor associated with its breakdown products (Sekura, 1972). Other, less toxic, agents have also been used as accelerants of drug transport. Surface-active agents (surfactants), especially the anionic surfactants, appear to alter the permeability of the skin to water. Propylene glycol and polyethylene glycol 400 have been known to both increase and decrease permeation of

substances. Concentrations of 50 percent or greater usually disturb the barrier and increase the loss of water from the skin (Idson, 1985). Calcium thioglycolate, a commercial depilating agent, was shown to improve the transdermal absorption of theophylline in rats, giving a 40-fold increase in plasma concentration over that of the control (Kushida, 1984).

Recently, another agent, AZONE (Nelson Research and Dev. Co.,Irvine, CA) was shown to enhance the absorption of triamcinolone acetonide, and was effective in a concentration of only 1 percent. This substance has been shown to be more effective than DMSO, and so has been receiving increasing attention in the past year (Chow, 1984; Vaidyanathan, 1985). Chemically, 1-dodecylazacycloheptan-2-one, AZONE is a structural analogue of pyrrolidone and methyldecyl sulfoxide. The range of concentrations of AZONE necessary for enhanced penetration is from 0.1 to 5 percent, indicating different partition coefficients for different drugs (Idson, 1985).

3.0 Transdermal Drug Delivery

The concept of using topical products to elicit a systemic response is not a new one. As early as 1900, systemic effects were reported following cutaneous application of belladonna, mercury, pilocarpine and cod liver oil (Grasso, 1972). Toxic, and even fatal, effects have also been reported from substances applied topically. Well known compounds such as salicylic acid, organophosphates, and carbon tetrachloride can all produce toxic results. However, the topical use of therapeutic agents has many advantages over traditional routes of drug administration. For example, the topical administration of

nitroglycerin in ointment formulations can minimize both the problems of extensive liver metabolism and short duration of activity.

Over the last several years, considerable interest has developed in the area of controlled release of drug substances through the skin. Controlled or sustained-release systems are being used or tested to deliver an enormous range of drugs, including drugs for the treatment of glaucoma, angina, motion sickness, narcotics addiction, and hypertension. "A well-designed controlled-release delivery system can significantly reduce the frequency of dosing and also maintain a more steady drug concentration in the blood and target tissue cells" (Chien,1982). Transdermal patches allow a drug to enter the body at an approximately uniform rate over an extended period of time. A controlled-release transdermal delivery system can also lower the total daily dosage of a drug, decrease the incidence and severity of side effects, and avoid the variable absorption characteristics of other routes of administration.

After the development of Ciba-Geigy's scopolamine-releasing transdermal delivery system, which releases drug at a continuous rate for 72 hours to control motion sickness, three nitroglycerin transdermal systems were developed independently by three pharmaceutical companies for once-daily medication of angina pectoris.

The first was Transderm-Nitro^{R}, developed by Alza and marketed by Ciba-Geigy. It consists of an outer protective layer of aluminized plastic, a drug reservoir containing a nitroglycerin suspension, enclosed in a microporous ethylene- vinyl acetate copolymer diffusion membrane, and a hypoallergenic adhesive layer. The Key Pharmaceuticals product, Nitro-Dur^{R}, is a matrix diffusion-controlled delivery system in

which nitroglycerin/ lactose triturate is homogeneously dispersed in a hydrophilic gel matrix. Finally, Searle's Nitrodisc^{R} consists of a solid, silicone-based polymer and is called a "microsealed" delivery system, since nitroglycerin is uniformly dispersed throughout the matrix and diffuses out at a controlled rate (Chien, 1984; Black, 1982).

Before transdermal patches, nitroglycerin was available in the form of sublingual tablets, sustained- release capsules, and ointments. The sublingual tablets allow rapid entry of the drug into the bloodstream but remain effective for less than 30 minutes. The time-released capsules are claimed to work for 8 to 12 hours. The ointments are applied to the skin once or twice a day, and are messy to use. It is difficult to get a uniform application each time a patient uses it. For all three marketed transdermal products, the devices contain sufficient nitroglycerin to maintain delivery of drug for 24 hours. The rate of delivery to the systemic circulation is determined by several factors, including the surface area covered and the characteristics of the skin itself (Fara, 1983). These transdermal systems permit minimal absorption variability while maintaining a constant drug level in the blood (Karim, 1983).

Chien, et al (1983) performed skin permeation studies on the abdominal skin of the hairless mouse in order to examine and compare the controlled skin permeation kinetics of nitroglycerin delivered by these three transdermal delivery systems. Results indicated that the release rate profiles adhere fairly well to zero-order kinetics, although different release rates were found between the three systems. The total dose of nitroglycerin delivered at 24 hours by each system was close in magnitude although different technologies were used to manufacture them.

Other companies are also working on transdermal nitroglycerin systems (Sanders, 1985). Hercon division of Health-Chem Corp. is awaiting FDA approval for its Nitroderm^{R} system. This system consists of polyvinyl chloride copolymers and terpolymers and has a release-controlling membrane. LecTec Corp. of Minnesota is working on a semisolid, hydrophilic gel system containing a polymeric adhesive to improve its strength and tackiness. Nelson Research and Development Co. of Irvine, California is working on its TranZone^{R} transdermal patches, which consist of a drug dispersed in various commercially available polymeric matrices along with the company's penetration enhancer, AZONE. Boehringer Ingelheim recently introduced its Catapres-TTS^{R} (transdermal therapeutic system), which contains the antihypertensive clonidine. This patch effectively delivers drug for a period of seven days (Weber, et al, 1984).

4.0 Nitroglycerin

Nitroglycerin has been used for over a century in the treatment of angina pectoris. Millions of patients throughout the world have placed nitroglycerin tablets under the tongue and have experienced rapid relief from the pain of angina.

Nitroglycerin was first synthesized in 1846 by A. Sobrero, by mixing cold concentrated sulfuric and nitric acids with glycerin. The first reported use in medicine was in 1847 by Hering and Davis in Philadelphia (Krantz, 1975).

Chemically, nitroglycerin is glyceryl trinitrate, or trinitroglycerin,

$$CH_2\text{-}O\text{-}NO_2$$

$$CH\text{-}O\text{-}NO_2$$

$$CH_2\text{-}O\text{-}NO_2$$

The organic nitrates are dilators of arterial and venous smooth muscle. They reduce the requirement of the myocardium for oxygen by their effects on the systemic circulation. The sublingual route of administration of nitroglycerin is the most appropriate to relieve acute angina attacks and for immediate prophylaxis. The sustained- release formulations of nitroglycerin, namely the ointment, capsule, and transdermal formulations have prolonged pharmacologic activity. It has been postulated that the oral forms of nitroglycerin are ineffective because of their high hepatic degradation rates. However, studies have shown the oral forms to have prolonged pharmacologic activity (Needleman & Johnson, 1980).

Organic nitrates, like other esters, undergo hydrolysis. This reaction occurs more rapidly in alkaline than acid conditions (McNiff,1980). The incubation of nitroglycerin in 4N sodium hydroxide for fifteen minutes at 37 degrees Centigrade produced almost complete denitration, whereas treatment in 4N hydrochloric acid for six hours produced only a 28 percent decrease (Johnson, 1975).

Pure nitroglycerin is highly explosive and is used in the manufacture of dynamite. Nitroglycerin for use in experimentation can be safely handled when adsorbed onto a suitable diluent such as lactose

(Johnson, 1975). Nitroglycerin is heat labile and volatile (McNiff, 1980). It is soluble in water to the degree of 1.2 to 1.8 grams per liter. Cacace (1979) found nitroglycerin to be stable in aqueous solution for six months under refrigeration. Incubation at 37 degrees Centigrade for six hours in water produced no detectable denitration (Johnson, 1975).

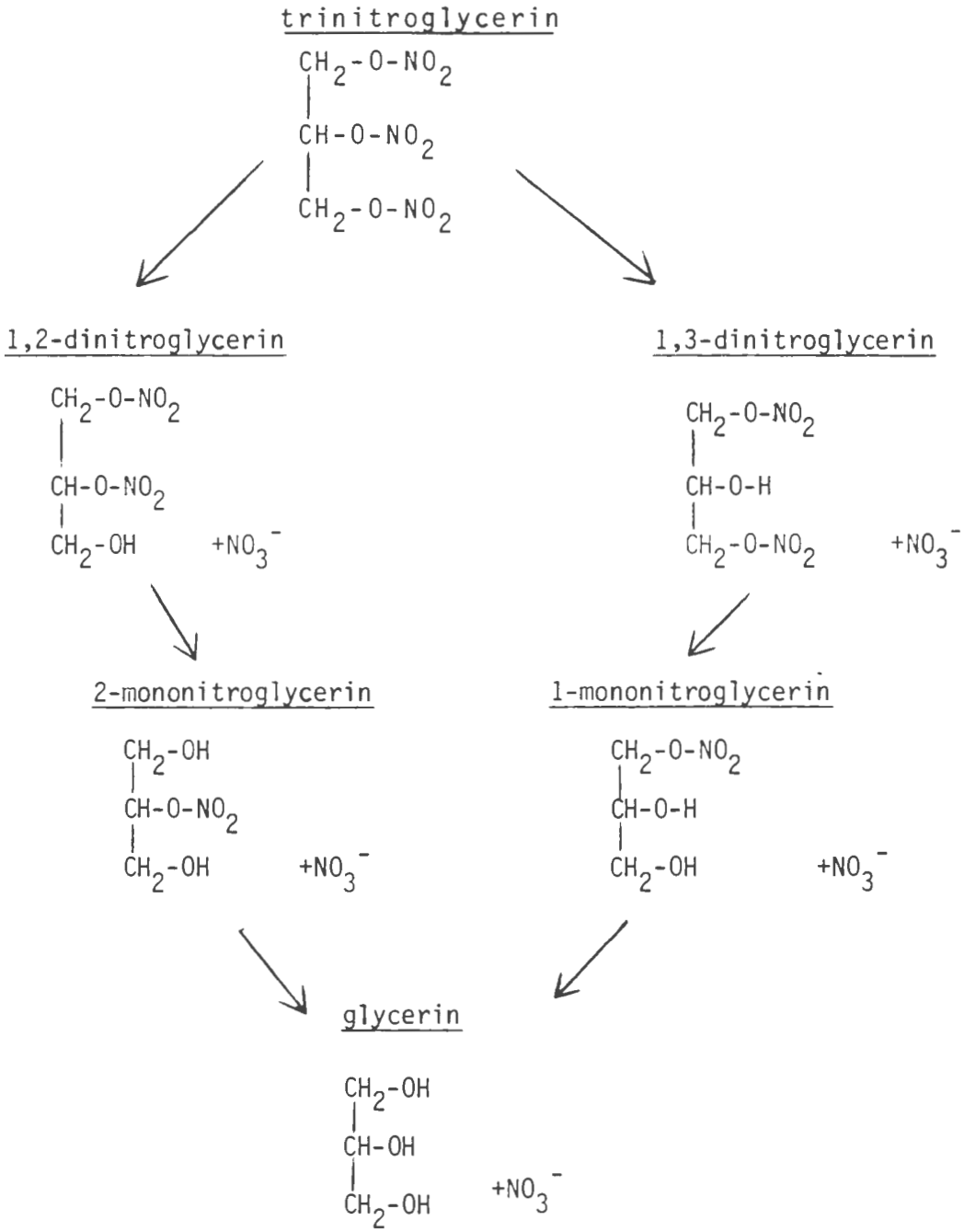
Identification of nitroglycerin and its breakdown products (Figure 2) has been done by spectrophotometric (McNiff, 1980), colorimetric (Bell, 1964), and chromatographic techniques. Spectrophotometric methods are time- consuming and complex and do not indicate stability, or differentiate nitroglycerin from its degradation products (Baaske, et al, 1979). Gas chromatography (GLC), although it requires a time-consuming extraction step, has been used for its sensitivity. Quantitative data are possible even though nitroglycerin is thermally unstable (Wu, 1981).

Several HPLC methods have been reported for the analysis of nitroglycerin and its in-vitro degradation products (Crouthamel & Dorsch, 1979; Baaske, et al, 1979; Wu, 1981; Baaske, et al, 1983; Gelber, 1980; Olsen & Scroggins, 1984). The HPLC methods are faster than GLC, and can be stability- indicating as well (Baaske, et al, 1979).

5.0 Methods for in-vitro release studies

Many studies have been published, which carry out permeability measurements on cadaver skin and animal skin (Keith, 1983). The most widely reported in-vitro method for evaluating percutaneous absorption of drugs uses a membrane mounted between two fluid- filled chambers

Figure 2. Breakdown of trinitroglycerin



(Akhter & Barry, 1985). The device, a diffusion cell, allows drug permeation to be monitored from the side exposed to the stratum corneum (donor) through to the dermal side (receptor) (Bronough, 1985). The Franz diffusion cell (figure 3) simulates clinical conditions by maintaining the receptor compartment at 37 degrees Centigrade while allowing the donor compartment to be exposed to ambient temperatures and humidities. The receptor compartment typically contains a propylene glycol or buffer solution to solubilize the drug and to simulate sink conditions. Hairless mouse skin or human cadaver epidermis may be used as the test membrane (Chien, 1983; Swarbrick, 1984; Behl, 1984; Cavey, 1985). Silicone elastomers such as polydimethylsiloxane have also been used successfully as transport membranes (Hsieh, 1985; Flynn & Roseman, 1971). In diffusion cell studies, permeation of drug across the membrane is affected by the composition of the donor solution. For example, the addition of fatty acids or alcohols to a solution of salicylic acid solution greatly increases the permeability of this drug across excised human abdominal epidermis.

Another method for testing transdermal products is dissolution testing. Dissolution testing is the measurement of the rate and extent of dissolution of a drug substance in an immiscible liquid in an in-vitro test system. It is usually performed on oral dosage forms such as tablets and capsules as a quality control measure. All aspects of the test system and the sampling times are standardized by a compendial or regulatory agency. However, the physical characteristics of the drug substance, the dosage form, and other variables are controlled only by the manufacturer, and may differ significantly for one drug. The subject of in-vitro/ in-vivo correlation is quite complex. Due to these

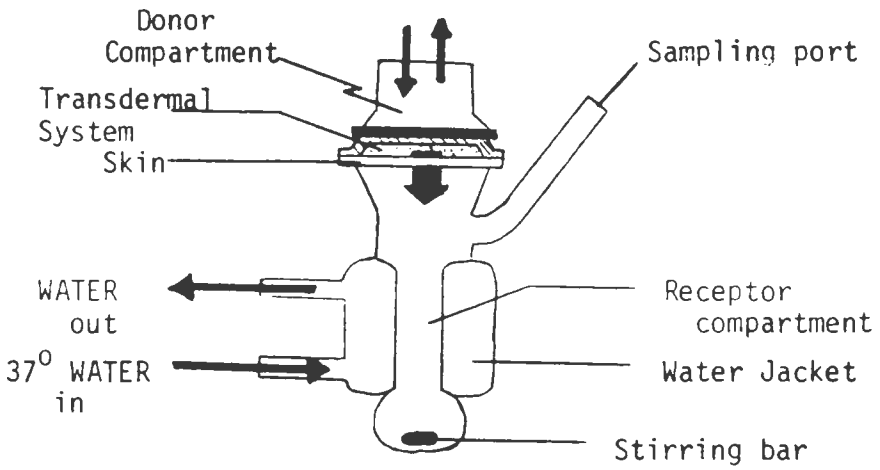


Figure 3 . Schematic illustration of Franz diffusion cell apparatus.

complexities, dissolution testing has been used only as a guide to the formulator in the early stages of drug product design, and "...as a quality control device to ensure process and batch-to- batch consistency for a particular formulation of a particular manufacturer" (Cooper, 1984). At the present time, there are no official criteria for the regulation of dissolution testing of transdermal products, because of the wide range of products available. This method is used by a number of companies who manufacture transdermals for assessing the release characteristics of their devices. A distinct advantage of this method is that it eliminates the step of the transport of drug across a membrane (a difficult factor to control) and instead allows evaluation of the release characteristics of the device.

EXPERIMENTAL

1.0 MATERIALS AND SUPPLIES

Citric acid (granular anhydrous), Pfizer Chemical Division, lot 633100-H2041

Ethanol 95% , USP

Gel-silicone Q7-2218 Parts A and B, Dow Corning Corp., Midland, MI, lot HH05401

1-Heptanesulfonic acid HPLC grade, Fisher Scientific, lot 732799

Hydrogen peroxide 30%, Fisher Scientific, lot 745518

Methanol HPLC grade- Fisher Scientific, A-452

Nitroglycerin 10%/lactose- Gyma Laboratories, Garden City, NY ,lot 09-9/82

Propylene glycol, USP, J.T. Baker Chemical Co., lot 025395

Scopolamine hydrobromide, USP, Amend Drug & Chemical Company, Irvington, NJ, lot G20293K11

Sodium hydroxide 0.1 N

Tetrahydrofuran

Transpore Tape (one-inch wide), 3M Corporation

Tridil^{R} 5 mg/ml- American Critical Care ,lot 3CA180

2.0 INSTRUMENTATION

Blue-M oven

Brookfield viscometer, model RVT (serial no. 51543)

Flat-bottom kettles, 500 ml

Linear chart recorder

Metal shafts for rotating basket

Micro-stir bars, 3 mm x 10 mm, Fisher Scientific Co.

Reciprocal shaker

Vankel Dissolution Apparatus

Water-driven stirrers, GFS Chemical Company

Waters Auto Sampler, WISP 710-B

Waters C-18 u-Bondapak column (P/N 27324 S/N)

Waters Associates Solvent Delivery System, model 6000A

Waters Associates variable wavelenth detector, M-480

METHODS

1. Preparation of Silicone Discs containing Nitroglycerin

Gel-silicone discs containing nitroglycerin were prepared by a polymerization method. The discs were made using gel-silicone Q7-2218 Parts A and B (polydimethylsiloxane and a platinum catalyst) and nitroglycerin 10%/lactose triturate. Formulations were produced by varying one parameter for each batch in a 3x3x3 factorial design at three treatment levels for each parameter. The parameters evaluated were concentration, firmness and surface area. Final concentrations of 10, 20 and 30 mg/cm^3 were prepared.

Variations in firmness were attained by changing the ratio of silicone to catalyst at three treatment levels, 1:1.5, 1:2.0, and 1:2.5. The silicone/catalyst mixture was preheated at 60 degrees Celsius for 15 to 18 minutes to cause polymerization and produce a viscosity of approximately 1000 centipoise. The viscosity of the mixture was tested using the following procedure. Three formulations were prepared of the three ratios of polymer:catalyst. 50 ml of each were placed into plastic beakers and labeled for time and ratio. The samples were placed in a 60 degree Centigrade oven and removed at the appropriate time to be tested. A Brookfield viscometer was used with spindle sizes 3 and 4. The spindle was lowered into the sample to about 0.5 cm from the bottom of the beaker. It was rotated for one minute before the reading was taken. The average of three consecutive readings taken at every other rotation were taken and converted to centipoise values.

After preheating the silicone/catalyst mixture for the appropriate length of time, the nitroglycerin/lactose was slowly stirred in manually. The polymer mixture was poured into a 10" x 7" stainless steel pan, covered with aluminum foil and set on a flat surface for 24 hours to cure. Discs of three sizes were then cut from each of nine formulations, using aluminum cutters of 1.00, 1.25 and 1.50 inch diameters, for a total of twenty-seven batches. Each disc was weighed and the thickness was calculated based on surface area and weight.

2. Degradation of Standard Nitroglycerin

In order to observe the effects of degradation on nitroglycerin, the standard, Tridil^{R} , was subjected to acid, base, peroxide, heat, and a combination of heat and peroxide. Six test tubes were prepared containing 10.0 ml of a stock solution (40 mcg/ml). Tube number one was the control. To the second, third and fourth tubes, 10 drops of 1% citric acid, 0.1 N sodium hydroxide, or 30% hydrogen peroxide, respectively, were added. The fifth tube was placed in a 90 degree Centigrade oven for 60 minutes. The sixth tube contained 10 drops of 30% hydrogen peroxide and was heated at 90 degrees Centigrade for 60 minutes. After 60 minutes, each test tube was analyzed by HPLC under the aforementioned chromatographic conditions.

3. Potency of 10% Nitroglycerin/lactose triturate

The relative potency of the 10% nitroglycerin/lactose triturate was tested against Tridil^{R} 5.0 mg/ml injectable nitroglycerin. The United States Pharmacopoeia requires that injectable drugs contain within (+)

or (-) five percent of the labeled content. Due to this regulation, and the explosive nature of pure nitroglycerin, the use of an injectable nitroglycerin solution as a standard was deemed acceptable for our purposes.

Four solutions (100, 135, 150, and 200 mcg/ml) were prepared using 10% methanol in water to solubilize the nitroglycerin/lactose triturate. The solutions were vortexed for two minutes, left to stand for ten minutes, and then vortexed for one minute longer. HPLC was used to quantitate the samples.

4. Dissolution

Drug release studies were performed using conventional dissolution equipment. To assess in-vitro drug release from the vehicle, the dissolution apparatus (Figure 4) was set up following U.S.P. Dissolution Method I with some modifications. Flat-bottom kettles were substituted for round-bottom kettles. Stirring of the dissolution-media was provided by a magnetic stir-bar placed in the bottom of the kettle, driven by a magnetic stirrer located in the water bath beneath the kettle. Stirring was at approximately 80 rpm. A patch was attached to the bottom of a 250 ml Erlenmyer flask using Transpore Tape (3M Corp.). A hole in the tape slightly larger than the disc but smaller than the patch exposed the disc to the dissolution media. The mouth of the flask was fitted over the end of a rotating-basket shaft and lowered into the dissolution media, such that the bottom of the flask touched the media and was centered to the kettle. The dissolution media was 250 ml of distilled water. A gap of approximately 0.5 cm between the edge of the

IN-VITRO DISSOLUTION APPARATUS

A= WATER BATH
B=SPINDLE DRIVE UNIT
C=250 ml ERLENMEYER
D=NITROGLYCERIN DISC
E=500 ml FLAT-BOTTOM KETTLE
F=DISSOLUTION MEDIA
G=MICRO-STIR BAR
H=WATER-DRIVEN STIRRER
I=8-oz. JARS (SUPPORTS)
J=THERMOMETER
K=HEATING UNIT
L= SHAFT

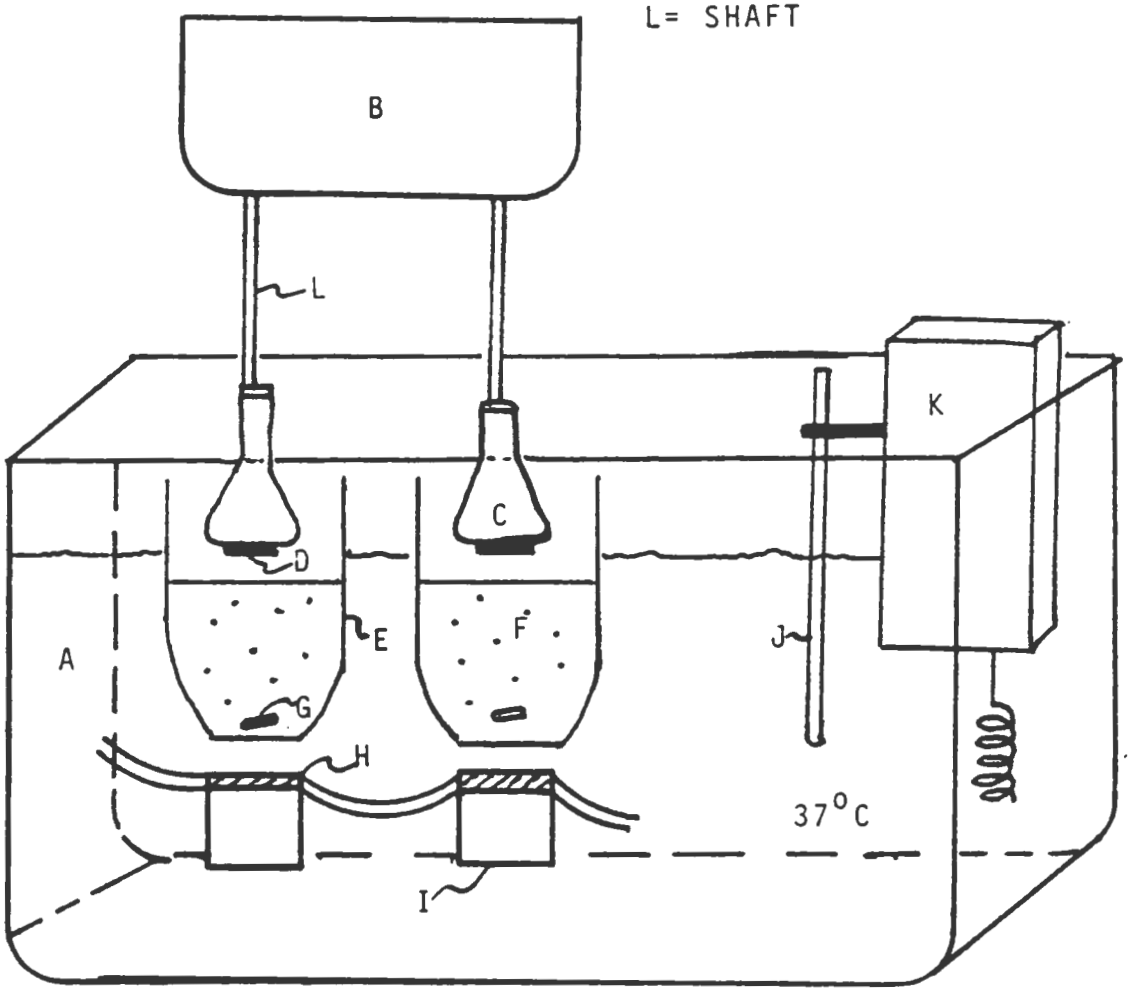


Figure 4. In-vitro dissolution apparatus.

Erlenmyer flask and the dissolution kettle was made through which samples were taken.

Single samples (3 ml) were taken with replacement of media during a 24-hour time period, according to the following time schedule: 0.5, 1, 2, 4, 6, 8, 12, 18, and 24 hours. Samples were refrigerated until the time of analysis.

5. Assay

Reverse-phase high-performance liquid chromatography (HPLC) was used for the quantitative analysis of nitroglycerin release. The system consisted of a Waters Associates pump, model 6000A; Waters detector, model 480; Waters Intelligent Sampling Processor (W.I.S.P.), model 710B, for automatic and precise sample injection; and a Waters C-18 u-Bondapack column. The chromatographic peaks were recorded using a Linear chart recorder (10 mV).

Approximately one liter of mobile phase was prepared fresh daily by mixing 600 ml of methanol with 400 ml distilled water. The mobile phase was filtered through a 0.5 um filter prior to use. Chromatographic conditions included: the wavelength of 214 nm, the flow rate of 1.5 ml/minute, the chart speed of 15 cm/hour, and an injection volume of 80 mcl. The detector sensitivity was set at 0.5 (AUFS 0.5). A stock nitroglycerin solution was prepared for each time the assay was performed. The solution of 100 mcg/ml was prepared by diluting 1.00 ml of Tridil^{R} 5 mg/ml to 50.0 ml with distilled water in a 50 ml volumetric flask. The standard curve was produced by injecting increasing volumes of the stock solution, two injections each of 5, 20, 40, 60, 80, and 100 mcl. This method of producing the standard curve

was validated by comparison to the usual dilution method. Standard solutions of 5, 20, 40, 60, 80, and 100 mcl were prepared and 80 mcl of each solution was injected. Both methods were found to produce the same curve (r=0.985).

6. Instron

The relative firmness of the discs was quantitated using the Instron hardness tester, model 1122. Two discs from each batch of medium-sized (1.25 inch diameter) silicone discs containing nitroglycerin were tested. The samples were placed on weighing paper on the platform beneath the stainless steel probe. The crosshead and chart speeds were set at 50 and 100, respectively. Return limits were calculated for each sample in order to produce 50 percent deformation. At least four trials for each sample were performed, rotating the sample after each trial in order to test a new spot. The average values were calculated and converted to compression force in kilograms.

7. Extractions

In order to evaluate the total drug content per disc, an extraction procedure was done using two discs from each of the twenty-seven batches of silicone discs containing nitroglycerin. Using a metal spatula, the discs were cut into 5x5, 6x6, or 7x7 pieces, respectively for the 1.00 inch, 1.25 inch, and 1.50 inch diameters. Each disc was placed in a 500 ml volumetric flask with 200 ml of extracting fluid. The extracting fluid was composed of 80 percent ethanol and 20 percent tetrahydrofuran. Six flasks at a time were placed on a reciprocal shaker and shaken for

two hours. A single sample was removed from each flask and filtered before assaying by HPLC.

8. Penetration Enhancer Study

A formulation was chosen from the twenty-seven batches of silicone discs containing nitroglycerin which showed relatively slow release characteristics. This formulation was composed of the high concentration of nitroglycerin (30 mg/ml), the 1.25 inch diameter, and the medium firmness (silicone to catalyst ratio of 1 to 2). Two batches of this formulation were prepared, one containing 10 percent and the other 20 percent dimethylsulfoxide (DMSO). The polymer components were mixed and preheated, as before. The nitroglycerin/lactose triturate was stirred in, and finally the DMSO was added. Due to the small batch size, these formulations were injected into 1.25 inch molds rather than poured into sheets and cut. The volume injected was 2.5 ml, in order to maintain an average thickness of 0.3 cm. These were covered with aluminum foil and allowed to cure for 24 hours.

9. Comparative studies

The results of the nitroglycerin study were expected to indicate which parameters have the greatest impact on drug release from this system. The degree to which these effects can be applied to transdermal systems in general was tested by evaluating their contribution to the release of scopolamine from the same system. Scopolamine was chosen for this portion of the study since it is reported to possess percutaneous absorption properties (Chandrasekaran, et al, 1984), and since it is

already marketed as a transdermal drug delivery system (Transderm-Scop, Ciba-Geigy).

9.1 Preparation of Scopolamine Base

Scopolamine free base was used rather than the hydrobromide salt since its physical characteristics (better lipid solubility) make it more suitable for transdermal delivery. The free base was prepared by dissolving 1.00 gram of scopolamine hydrobromide in 20 ml of methanol and 40 ml of chloroform. This was reacted with 20 ml of a 1 percent sodium bicarbonate solution (pH= 8.0) in a separatory funnel. This mixture was shaken and vented several times to complete the reaction. The methanol phase was separated off, leaving the scopolamine base dissolved in chloroform. The chloroform was allowed to evaporate by air, leaving the clear, viscous liquid scopolamine base. Several "batches" of the base were prepared and combined. The purity of the free base was verified by HPLC prior to use, using the hydrobromide salt as a reference.

9.2 Preparation of Scopolamine discs and release studies

Silicone discs containing scopolamine were prepared using two different concentrations of drug, 1% and 2%. The ratio of silicone to catalyst was 1 to 2, and the diameters chosen were 1.00 and 1.25 inches. The polymer/ catalyst mixture was preheated at 60 degrees Centigrade for 12 to 15 minutes and the drug was carefully stirred in. The polymer/ drug mixture was poured into pans and allowed to cure for 24 hours

before cutting the discs. The finished discs were weighed and the thickness was calculated.

Release studies were performed using the same dissolution procedure as the nitroglycerin studies. Three discs each from the total of four batches were run. Samples were taken at 0.5, 1, 2, 4, 6, 8, 12, and 24 hours and refrigerated until analysis.

9.3 Scopolamine Assay

The release of scopolamine was analyzed using the same HPLC system as in the nitroglycerin assay. The mobile phase consisted of 70 percent water and 30 percent acetonitrile with 10 ml of PIC (Paired Ionic Chromatography) reagent B-7 added per liter. This reagent was prepared by dissolving 11.0125 grams of 1-heptane sulfonic acid in 50 ml distilled water in a 100 ml volumetric flask. To this was added 0.4 ml glacial acetic acid using an automatic pipette. The solution was brought to volume with distilled water and ten mls of PIC B-7 were added to a liter of mobile phase for a final concentration of 0.005 M.

The chromatographic conditions included a flow rate of 1.5 ml per minute, detector sensitivity 0.02 AUFS, an injection volume of 30 ul, and the wavelength of 254 nm.

RESULTS

1. Manufacture of Discs

Silicone discs containing nitroglycerin were manufactured using a polymerization process. The first step was to initiate the polymerization of the silicone gel. By varying the ratio of the silicone (Part A) to the catalyst (Part B), a range of firmness levels was achieved. A:B ratios of 1:1.5, 1:2.0, and 1:2.5 were found to produce acceptable levels of firmness in the finished product.

The silicone precursor used may be cured by a variety of temperatures and curing times. For our purposes, the mixture was preheated at 60 degrees Centigrade, until an appropriate viscosity was attained before adding the drug. Since nitroglycerin is a heat labile substance, the polymer was allowed to cool before the addition of the nitroglycerin to minimize loss of the active. Without this preheating step, and in the case where the polymer was insufficiently preheated, the nitroglycerin/lactose settled to the bottom of the beaker within a few minutes. The effect of various preheating times on the viscosity of the silicone mixture was investigated in order to determine an appropriate mixing schedule to produce uniform dispersion of the drug in the matrix. As seen in Tables 1 and 2, rapid increases in viscosity were seen after about 1000 centipoise. The time required for preheating the silicone varied slightly with the three levels of polymer:catalyst used, but an average of about 15 minutes was sufficient to exceed 1000 centipoise. The polymer with the highest ratio of catalyst took the longest time to preheat. This was perhaps due to the large excess of liquid catalyst in the mixture, which would mask the gelling effect for

Table 1. Preliminary viscosity studies (time 0 to 18 minutes)

Ratio A:B= 1:1.5		Ratio A:B= 1:2.0		Ratio A:B= 1.2.5	
time (min.)	cps	time (min.)	cps	time (min.)	cps
0	650	0	600	0	575
12	750	12	700	12	607
14	1000	15	933	15	850
16	2000	17	1400	18	1567
18	3800	21	(off)	20	(off)

Table 2. Preliminary viscosity studies (taking 16 minute sample and resuming viscosity testing).

Ratio A:B=1:1.5		Ratio A:B= 1:2.0		Ratio A:B=1:2.5	
time (min)	cps	time (min.)	cps	time (min.)	cps
17.5	2300	17	1100	17	1000
18	2450	19	1250	18	1100
18.5	2600	20	1400	19	1300
19	2800	21	1500	21	1750
20	2950	23	1650	23	2000
21	3100	25	1750	26	2500
22	4350	28	2000	28	2950
23	4850	30	2200	30	3600
24	7500			31	3800
26	9500+			33	4750
				34	5100
				35	5900

a short period. This effect can also be seen in mixtures prior to heating; the 1:2.5 ratio had the lowest initial viscosity. Additional heating past the 1000 centipoise level produced a system too viscous to allow uniform dispersion of the nitroglycerin/ lactose triturate.

After dispersion of the nitroglycerin adsorbed powder, the polymer mixture was poured into pans and allowed to cure in sheet form at room temperature for 24 hours. To ensure uniform thickness of the polymer sheet while curing, it was extremely important that the surface on which the pans were set to cure be level. Individual discs were cut from the sheets and weighed. The mean weight per batch was calculated (Table 3), and overall average weights by size were determined to be 1.5561 ± 0.1432 grams (1.00 inch diameter), 2.3771 ± 0.2525 grams (1.25 inch diameter), and 3.4086 ± 0.3875 grams (1.50 inch diameter). Differences in thickness of gel between the two ends of a pan appeared to be most responsible for the variation in individual weights of silicone discs. 75 percent of the discs that were used in the release studies varied in weight less than 5 percent. The remaining discs were within 10 percent of mean weights.

The actual thickness of the finished discs was not physically measured, but was calculated for each disc based on the weight and known surface area of the disc. It was found that 10.0 ml of uncured silicone weighed 10.0 grams, therefore the conversion factor of 1.0 gram/cm^3 was used in the volume and thickness calculations. Table 4 contains the values of surface area, volume, and theoretical drug load of the discs produced at the three diameters. The average thickness of the discs was 0.30 cm, with a range of 0.25 cm to 0.35 cm for most of the 27 batches.

Table 3. Overall Weight Variation of Nitroglycerin Discs (by batch)
(*a=conc., b=firmness,c= S.A. ; 1=low or small, 2=med., 3= highest)

Batch #	Description* a	b	c	$\overline{\text{Weight}}$ ± S.D. (gm) n=10-14	Range (gm) in weight	Range (cm) in thickness	Mean thickness (cm)
1	1	1	1	1.5380+ 0.1797	1.2169-1.7659	0.24-0.35	0.30
2	1	1	2	2.2760+ 0.2704	1.9088-2.5648	0.24-0.32	0.30
3	1	1	3	3.3742+ 0.5810	2.7031-4.2232	0.24-0.37	0.30
4	2	1	1	1.5179+ 0.0782	1.4192-1.7005	0.28-0.34	0.30
5	2	1	2	2.3845+ 0.1790	2.2062-2.7336	0.28-0.34	0.30
6	2	1	3	3.3882+ 0.2882	3.0240-4.0887	0.27-0.36	0.30
7	3	1	1	1.5707+ 0.1350	1.2973-1.7715	0.26-0.35	0.31
8	3	1	2	2.3790+ 0.2051	2.0569-2.5782	0.25-0.36	0.30
9	3	1	3	3.4716+ 0.2775	3.1238-4.0441	0.26-0.36	0.31
10	1	2	1	1.4464+ 0.1073	1.3235-1.6390	0.26-0.32	0.29
11	1	2	2	2.4143+ 0.2431	2.0377-2.8732	0.25-0.36	0.30
12	1	2	3	3.3250+ 0.4747	2.6549-4.1052	0.25-0.36	0.29
13	2	2	1	1.6209+ 0.2196	1.2832-2.0664	0.28-0.36	0.32
14	2	2	2	2.3562+ 0.3480	1.6212-2.9040	0.25-0.36	0.30
15	2	2	3	3.4482+ 0.6710	2.2511-4.5368	0.26-0.36	0.30
16	3	2	1	1.6030+ 0.1578	1.3970-1.8858	0.28-0.37	0.31
17	3	2	2	2.4031+ 0.3100	1.8210-2.8693	0.27-0.36	0.30
18	3	2	3	3.4261+ 0.3289	2.8749-3.9024	0.25-0.34	0.30
19	1	3	1	1.5227+ 0.1627	1.2983-1.8114	0.26-0.36	0.30
20	1	3	2	2.2938+ 0.3323	1.9629-2.8348	0.26-0.36	0.29
21	1	3	3	3.3976+ 0.3962	2.8779-4.0910	0.25-0.36	0.30
22	2	3	1	1.5092+ 0.1511	1.3688-1.8626	0.27-0.37	0.30
23	2	3	2	2.4761+ 0.2512	2.1170-2.7444	0.27-0.35	0.31
24	2	3	3	3.4188+ 0.3176	2.9519-3.8268	0.26-0.34	0.30
25	3	3	1	1.5966+ 0.0972	1.4980-1.8169	0.30-0.36	0.32
26	3	3	2	2.3754+ 0.1331	2.2394-2.6425	0.28-0.33	0.30
27	3	3	3	3.4258+ 0.1517	3.1895-3.6966	0.28-0.32	0.30

Table 4. Theoretical* volume and total drug content of silicone discs.

Disc diameter in.	cm	Surface area cm^2	Volume cm^3	Conc. 1 (10 mg/cm^3)	Conc. 2 (20 mg/cm^3)	Conc. 3 (30 mg/cm^3)
1.00	2.54	5.07	1.52	15.20	30.40	45.60
1.25	3.18	7.94	2.38	23.80	47.60	71.40
1.50	3.81	11.40	3.42	34.20	68.4	102.60

*Based on average thickness of 0.30 cm

The relative firmness of the discs was quantified using the Instron hardness tester. This instrument produced reliable values for comparison, using a standard procedure. The compression force in kilograms required to produce a 50 percent deformation of the discs was measured at several points on two discs from each medium size (1.25 inch diameter) batch (Table 5). Compression force as a function of firmness (Figure 5) and of concentration (Figure 6) were plotted. In general, as the total amount of catalyst was increased, the compression force also increased. Likewise, increasing the drug concentration produced an increase in the compression force, indicating a firmer disc (30 mg/cm^3 > 20 mg/cm^3 > 10 mg/cm^3). In Figure 5 the discs having the highest concentrations of drug showed the highest compression rates and increased in a linear fashion as the catalyst ratio increased ($r= 0.997$). The 10 and 20 mg/cm^3 discs produced increased compressions relative to concentration and catalyst levels, however there appeared to be a leveling effect from the catalyst ($r= 0.983$ and 0.939 for the 20 and 10 mg/cm^3 concentrations).

When compression force was plotted as a function of concentration, the results were similar in that higher ratios of catalyst and higher concentrations produced higher compressional forces. The slopes increased as a function of catalyst level and the linearity was better than generally seen in Figure 5 ($R= 0.998$, 0.978, 0.962). In any case, we can conclude that higher catalyst levels and concentrations of suspended material produce firmer discs. The significance of this finding was tested by evaluating the effect of firmness of release of drug from the discs.

Table 5. Instron Studies: Compression force* as a measure of firmness.

Concentration	Firmness		
	Low (1: 1.5)	Medium (1:2.0)	Firmest (1:2.5)
10 mg/cm^3	0.60	1.05	1.15
20 mg/cm^3	0.88	1.27	1.47
30 mg/cm^3	1.23	1.75	2.41

* force (kg) required to produce 50% deformation

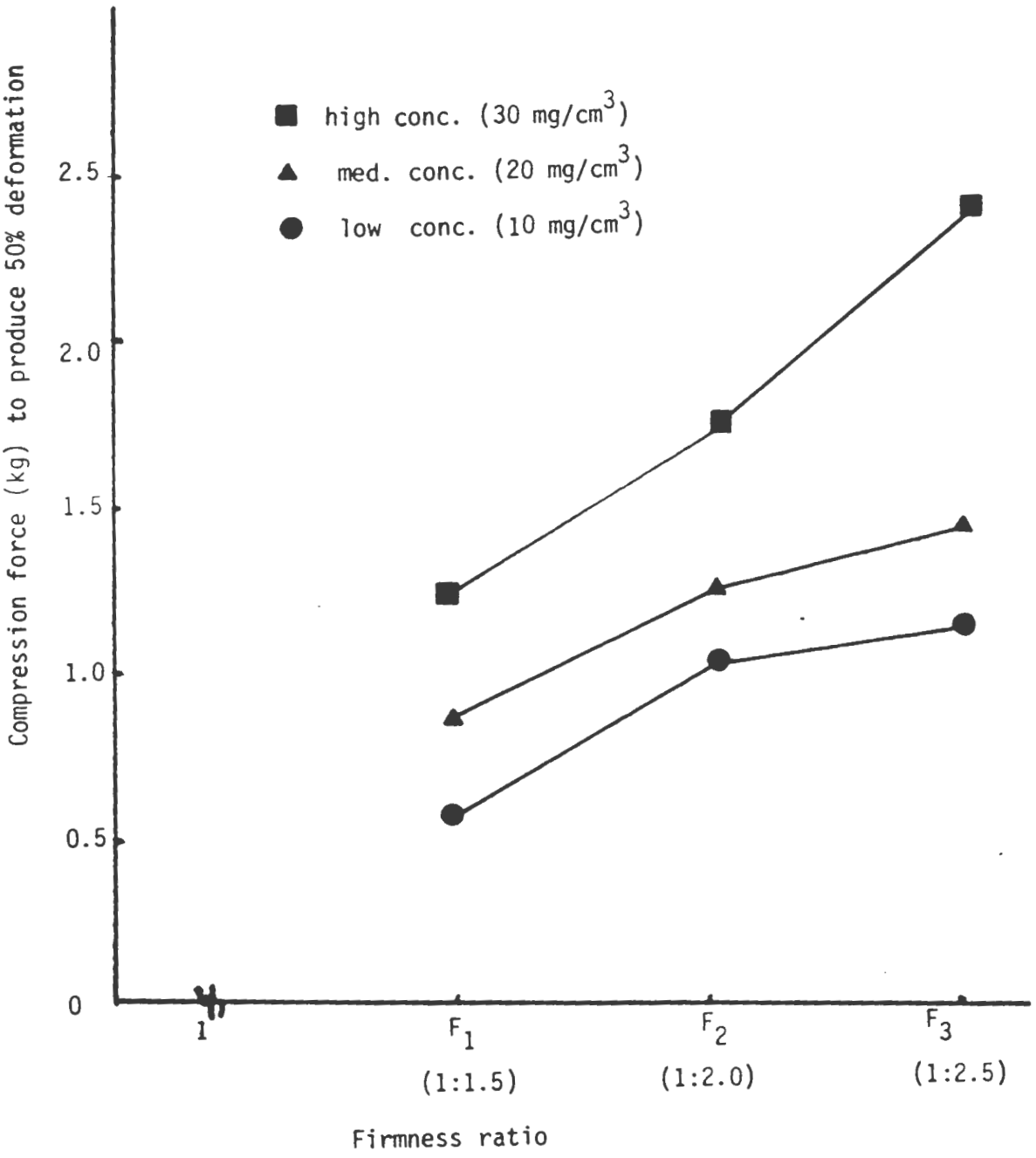


Figure 5 . Instron studies: compression force as a function of firmness ratio.

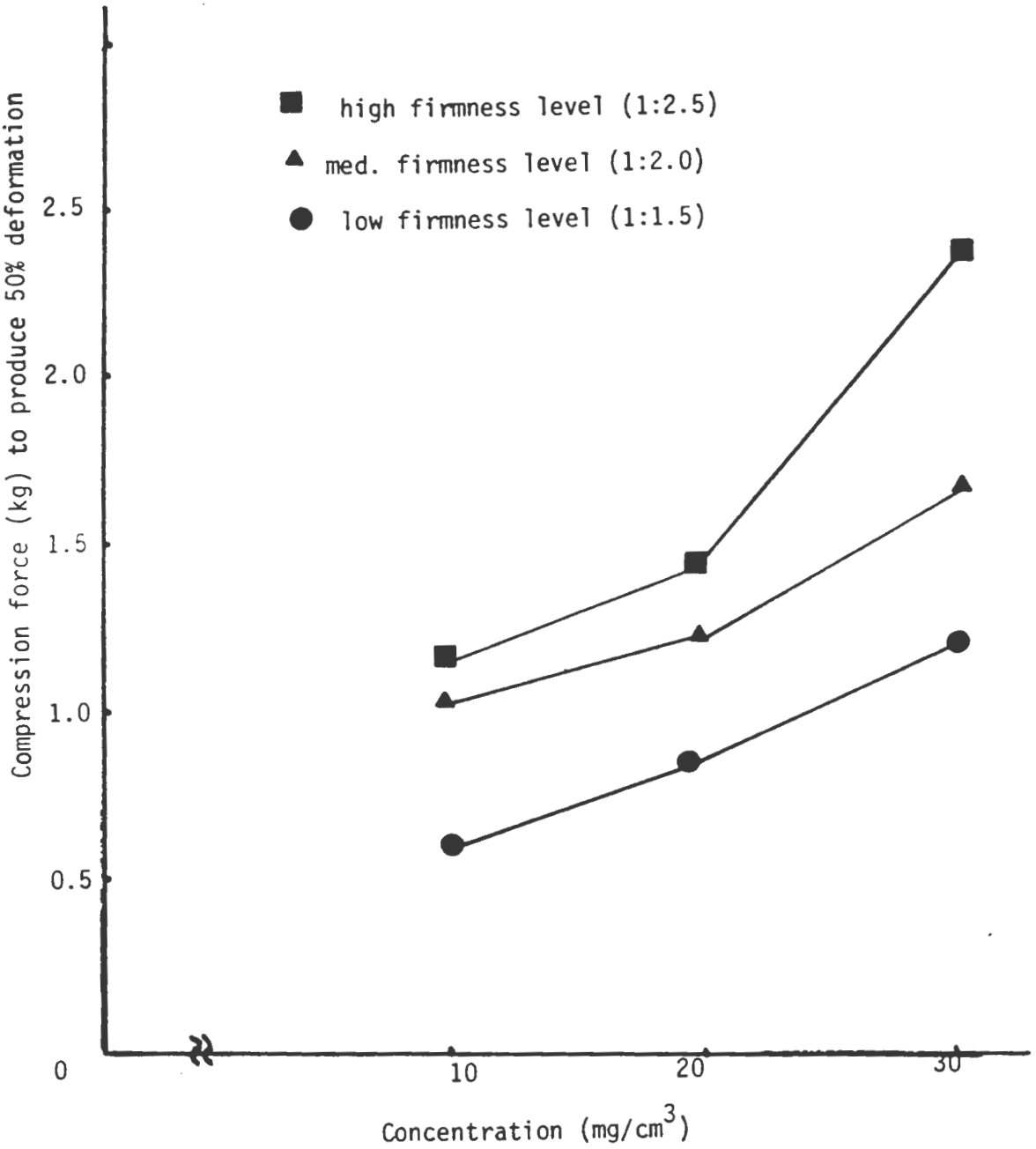


Figure 6. Instron studies: Compression force as a function of drug concentration.

2. Analytical Methods

In order to ensure a stability-indicating assay, it was necessary to observe the effect of nitroglycerin degradation on the absorption peak. A standard nitroglycerin chromatogram is shown in Figure 7. The retention time of the standard nitroglycerin was 3.90 minutes. A base-degraded sample produced the greatest change in the absorption peak, with the appearance of two additional peaks at 1.84 and 2.70 minutes (Figure 8).

Preliminary stability studies on the nitroglycerin standard solutions suggested that aqueous solutions of Tridil^{R} were stable under refrigeration for at least two weeks with no significant loss of potency. Further investigation supported this finding, although fresh standards were made daily.

Daily tests of precision of the analytical methods showed a relative standard deviation of less than one percent (0.52 $\pm$ 0.53 percent) over a four month period. Daily standard curves consistently (r= 0.999) produced average slopes of 2.2992 $\pm$ 0.221, for a relative standard deviation of 9.6 percent over a three month period.

Potency testing of the nitroglycerin/lactose triturate against the Tridil^{R} injectable solution indicated 99.0 to 100.0 percent potency of the powder before manufacture of the discs.

In order to initially assess the in-vitro release method, three commercial transdermal products were tested. The products were Key Pharmaceutical's NitroDur^{R} 10 cm^{2}, Searle's Nitrodisc^{R} and Ciba's Transderm-Nitro^{R}-5. Each of these systems is designed to release 5 mg of nitroglycerin through the skin over 24 hours. Fluxes and rates of release for the first two hours and the final 12 hours (Table 6) were

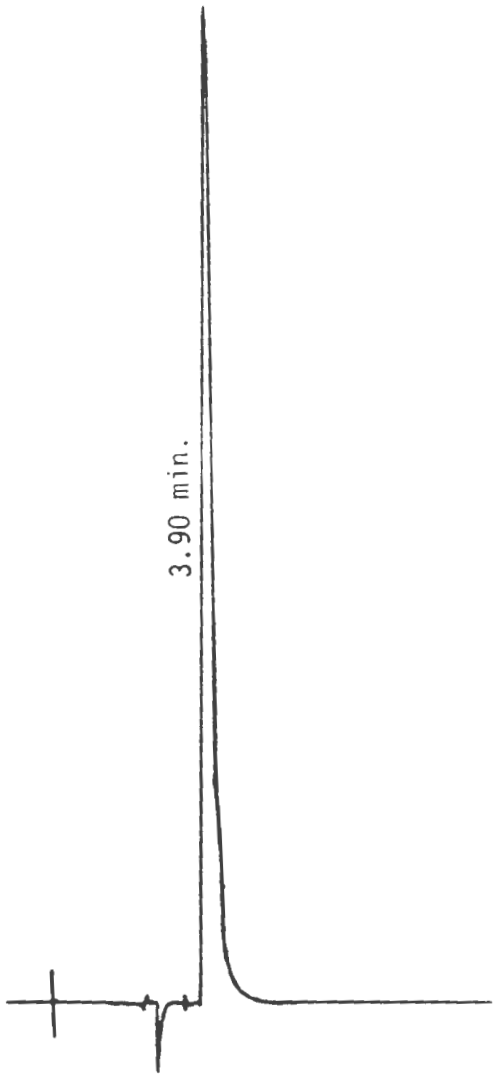


Figure 7. Standard nitroglycerin chromatogram.

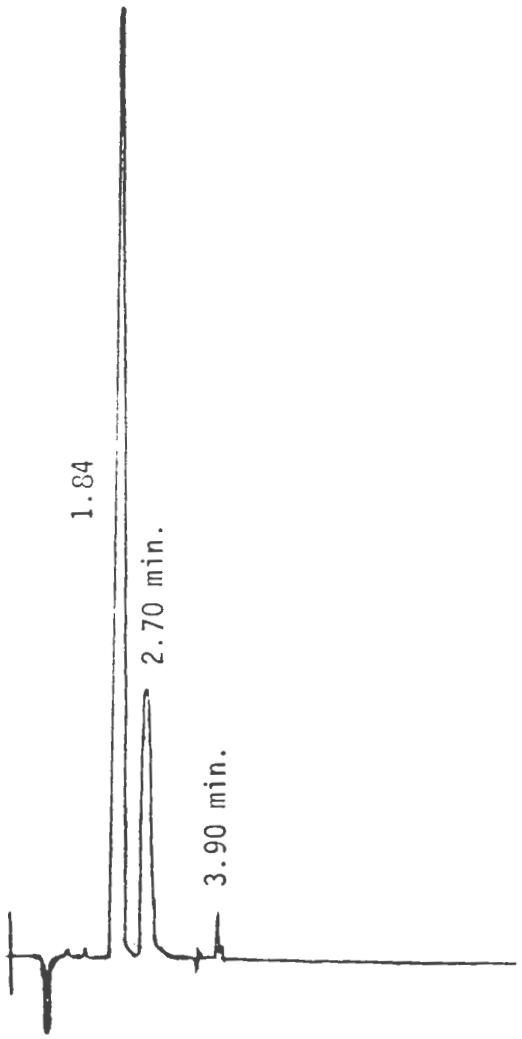


Figure 8. Base-degraded nitroglycerin chromatogram.

Table 6. In-vitro Comparison Study of Trade Products

Sample (n=3)	Load (mg)	SA (cm^2)	f_1 (0-2 hr) ($mg/cm^2/hr$)	f_2 (12-24 hr) ($mg/cm^2/hr$)	Ratio $f_1 : f_2$	mg rel. per 24 hr	% rel.
Nitro-Dur R (Key)	51	10	1.07	0.14	76.43	43.50	85
Nitrodisc R (Searle)	16	8	0.28	0.003	93.33	10.9	68
Transderm-Nitro (Ciba-Geigy)	25	10	0.11	0.065	1.69	18.50	74

calculated. Overall, it was found that the rate of nitroglycerin release from the commercial products was higher during the first two hours than in the last 12 hours, with the exception of the Transderm-Nitro^{R} rate which changed dramatically during the last 12 hours (Figure 9). The Searle and Key products showed essentially no change in rate during the last 12 hours. Initial flux rates for the Key, Searle, and Ciba products were 1.07, 0.28, and 0.11 mg/cm^{2}/hr, respectively, and 0.014, 0.003, and 0.065 mg/cm^{2}/hr in the final 12 hours.

Total milligrams and percent of nitroglycerin released over 24 hours varied according to the formulation. NitroDur^{R} and Nitrodisc^{R} demonstrated decreasing rates with time due to their relatively firmer systems which retarded the migration of nitroglycerin inside the disc to the surface.

3. Disc Extractions

The results of the disc extraction study are shown in Table 7. An overall mean of 60 percent extraction was found. The effects of size and concentration on the total percent of drug extracted from the discs appeared to be insignificant.

The fact that only 60 percent of the theoretical drug load was extracted leads to several possible conclusions which include incomplete extractions, drug decomposition, standard decomposition, and physical/chemical interaction of the drug within the silicone.

The first three possibilities were examined during the method and assay validation processes. In developing the extraction process, a second extraction of each disc using fresh fluid was attempted, but no

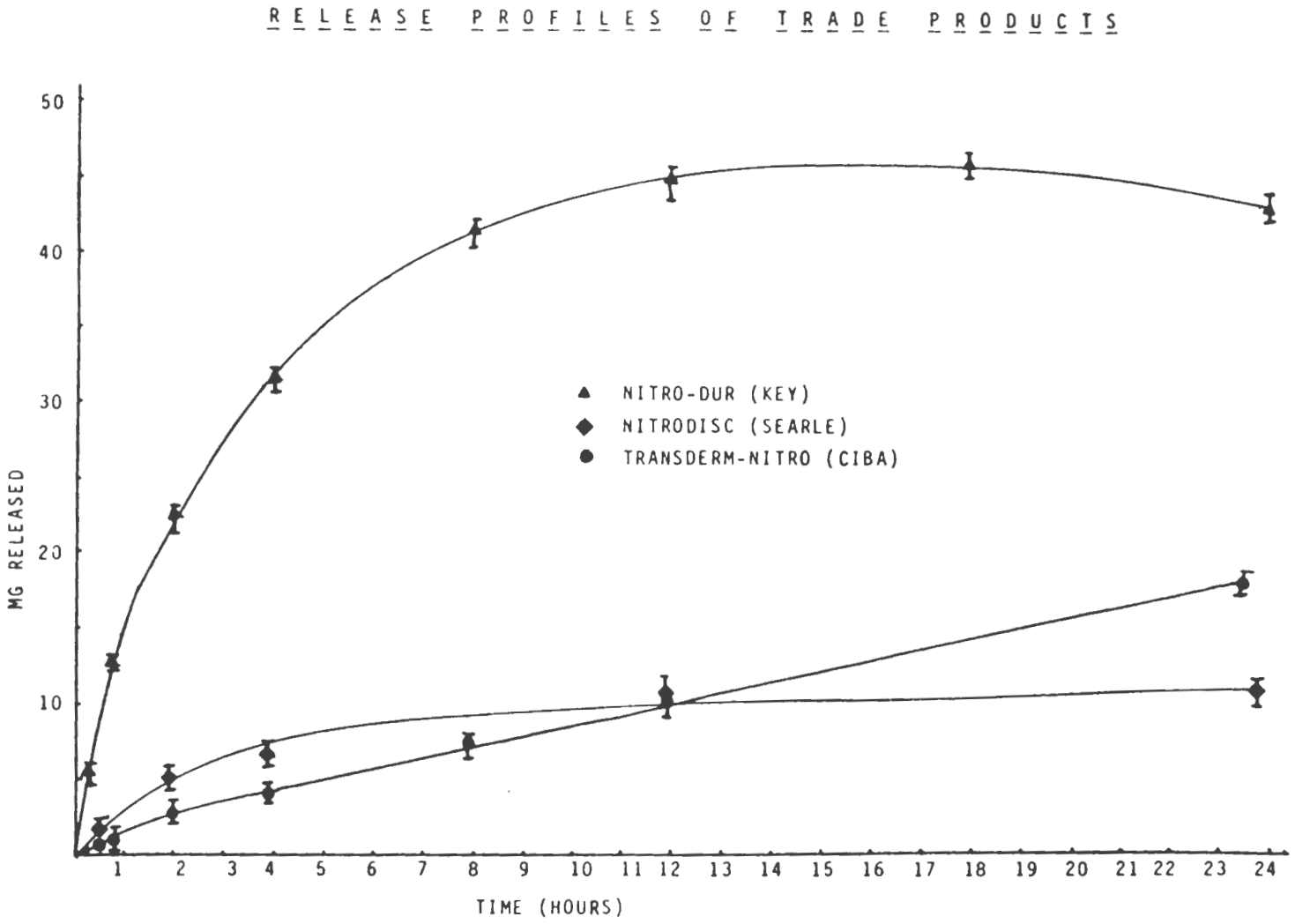


Figure 9. Release profiles of trade products: Nitro-Dur (Key), Nitrodisc (Searle), and Transderm-Nitro (Ciba).

Table 7. Release and Extraction Data from Nitroglycerin Discs (*a=conc., b=firmness, c=SA; 1= low, 2=med,3=highest)

Batch #	Descrip.* a	b	c	Amount rel. (mg) n=3	% rel. per 24 hr	mg extr.	% extr.
1	1	1	1	10.28	68.56	9.98	65.80
2	1	1	2	14.99	62.91	15.41	60.23
3	1	1	3	23.59	64.45	22.78	64.62
4	2	1	1	14.82	49.66	19.13	62.74
5	2	1	2	27.27	74.17	29.21	56.54
6	2	1	3	34.31	51.06	m	m
7	3	1	1	18.08	40.19	26.03	56.95
8	3	1	2	19.95	28.25	m	m
9	3	1	3	30.03	28.40	m	m
10	1	2	1	9.99	64.23	8.51	34.14
11	1	2	2	17.56	71.67	14.48	58.11
12	1	2	3	24.23	66.16	19.60	57.68
13	2	2	1	14.30	30.24	16.49	54.39
14	2	2	2	23.28	45.93	27.04	58.72
15	2	2	3	30.33	51.13	33.86	53.81
16	3	2	1	15.44	33.90	28.28	58.30
17	3	2	2	27.36	35.99	43.74	58.73
18	3	2	3	35.96	35.89	55.37	51.47
19	1	3	1	9.54	63.45	7.12	54.07
20	1	3	2	14.30	61.61	16.55	59.74
21	1	3	3	21.93	70.39	18.28	58.45
22	2	3	1	13.00	44.90	18.44	61.36
23	2	3	2	27.75	45.87	25.19	57.90
24	2	3	3	31.04	45.80	43.43	56.92
25	3	3	1	16.15	35.47	29.59	61.92
26	3	3	2	21.27	30.18	41.54	61.83
27	3	3	3	35.32	34.39	53.49	55.90

m= missing data

additional drug could be extracted. The initial potency testing of the nitroglycerin/ lactose triturate indicated at least 99.0 percent potency of the powder against the standard. Later assays did not indicate any significant loss of potency of the powder. Also, subsequent release studies, using a completely randomized design, showed no effect of storage time on the total percent of drug release.

Finally, the possibility of decomposition of the standard was also excluded, since fresh standard solutions were made daily. Once an ampule of the standard Tridil^{R} injectable had been opened, it was stored under refrigeration in a tightly sealed glass test tube, and was not used for more than seven days. After seven days, stability tests were performed on the standard, and indicated no loss in potency for 2-3 additional weeks.

An interesting point to be made here relates the total percent of drug extracted for each batch of discs to the 24-hour release data for those batches. In subsequent drug release studies, the greatest percent of nitroglycerin release over 24 hours (based on theoretical drug load) that could be achieved was only about 70 percent. A comparison of the percent extracted per batch to the percent released per batch indicated that there may be a limit to the potential drug release from a disc of this type. There always appeared to be some drug remaining in the disc that is a function of the driving force of the drug in the silicone. If only 60 percent of drug in any given batch could be extracted, then perhaps the active drug release was closer to 100 percent.

4. Release Studies

Subsequent drug release studies indicated that the pattern of in-vitro release of nitroglycerin from the transdermal discs very closely mimicked that of Searle's Nitrodisc^{R}. For this reason, three additional samples of Nitrodisc^{R} were run at random on separate dates among the experimental discs. This was done to test the reliability of the drug release apparatus. As seen in Figure 10, no significant differences were noted in the rate or extent of nitroglycerin release from three Nitrodisc^{R} samples. This finding is an indication of the reliability and reproducibility of this in-vitro dissolution method.

Dissolution profiles of nitroglycerin release over 24 hours from 27 batches of silicone discs are shown in Figures 11 through 19. In general, there was no leveling off of the curves. It appears that a 36 or 48 hour profile might have produced a more accurate picture of drug release from these systems. Many of the batches could possibly release drug over a period of time greater than 24 hours.

Samples of silicone discs containing nitroglycerin were tested at random, but were selected according to their weight. It was important to reduce the interfering effect of disc thickness for these studies, therefore discs were chosen which were within 5 percent of variation in thickness. However, due to the small batch size, it was occasionally necessary to select a disc which varied up to 10 percent from the mean. Approximately 75 percent of the samples were in the 5 percent range, and the remaining 25 percent of samples were in the 10 percent range.

The percent-release data for each batch were plotted as a function of the square-root of time. The amount of drug released should be proportional to the square-root of time (Banaker, 1984; Pirotte &

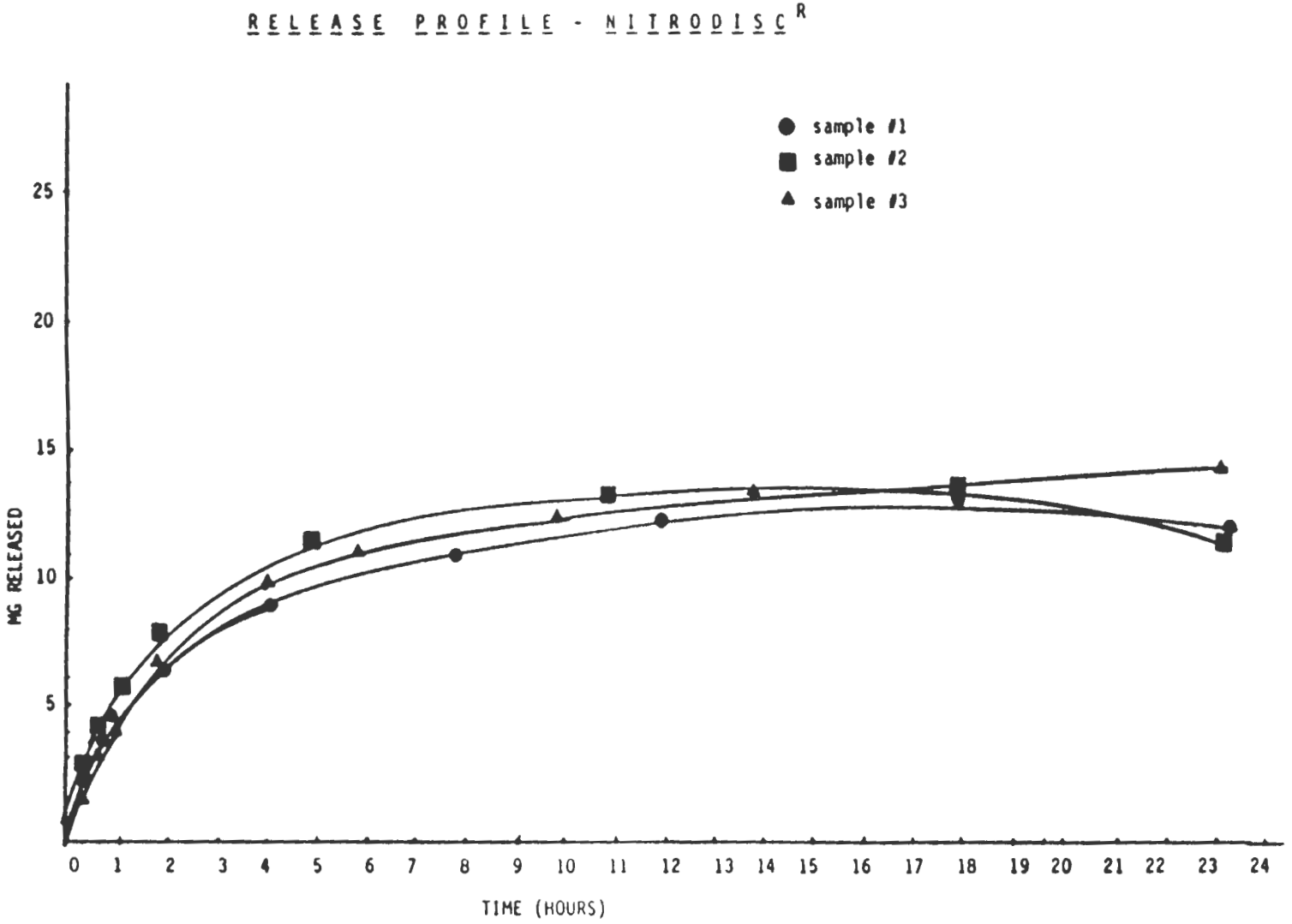


Figure 10. Release profile of Nitrodisc (Searle) run on 3 separate dates.

RELEASE PROFILES (BATCHES 1, 2, 3)

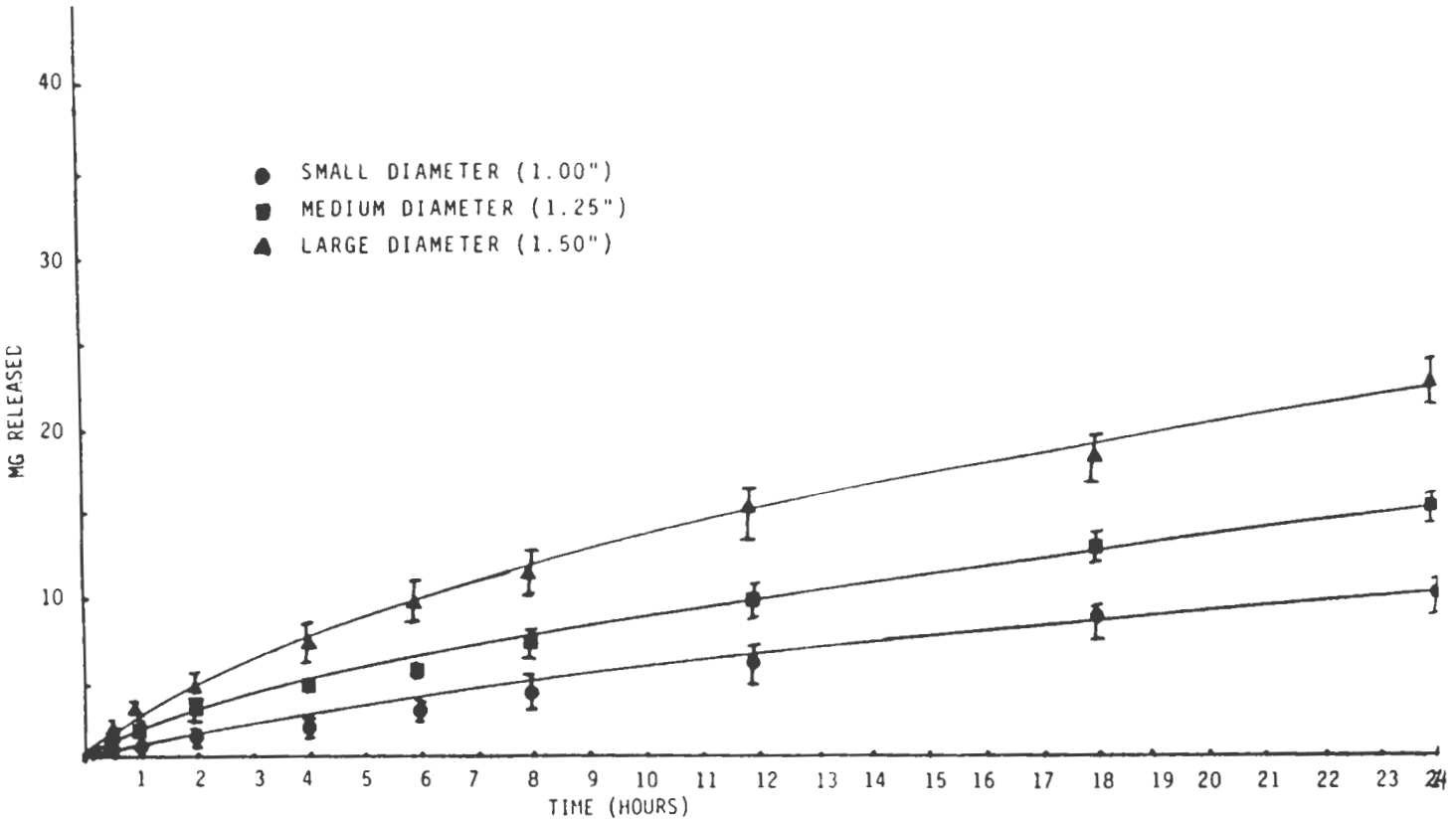


Figure 11. Release profiles of batches 1, 2, and 3.

RELEASE PROFILES (BATCHES 4, 5, 6)

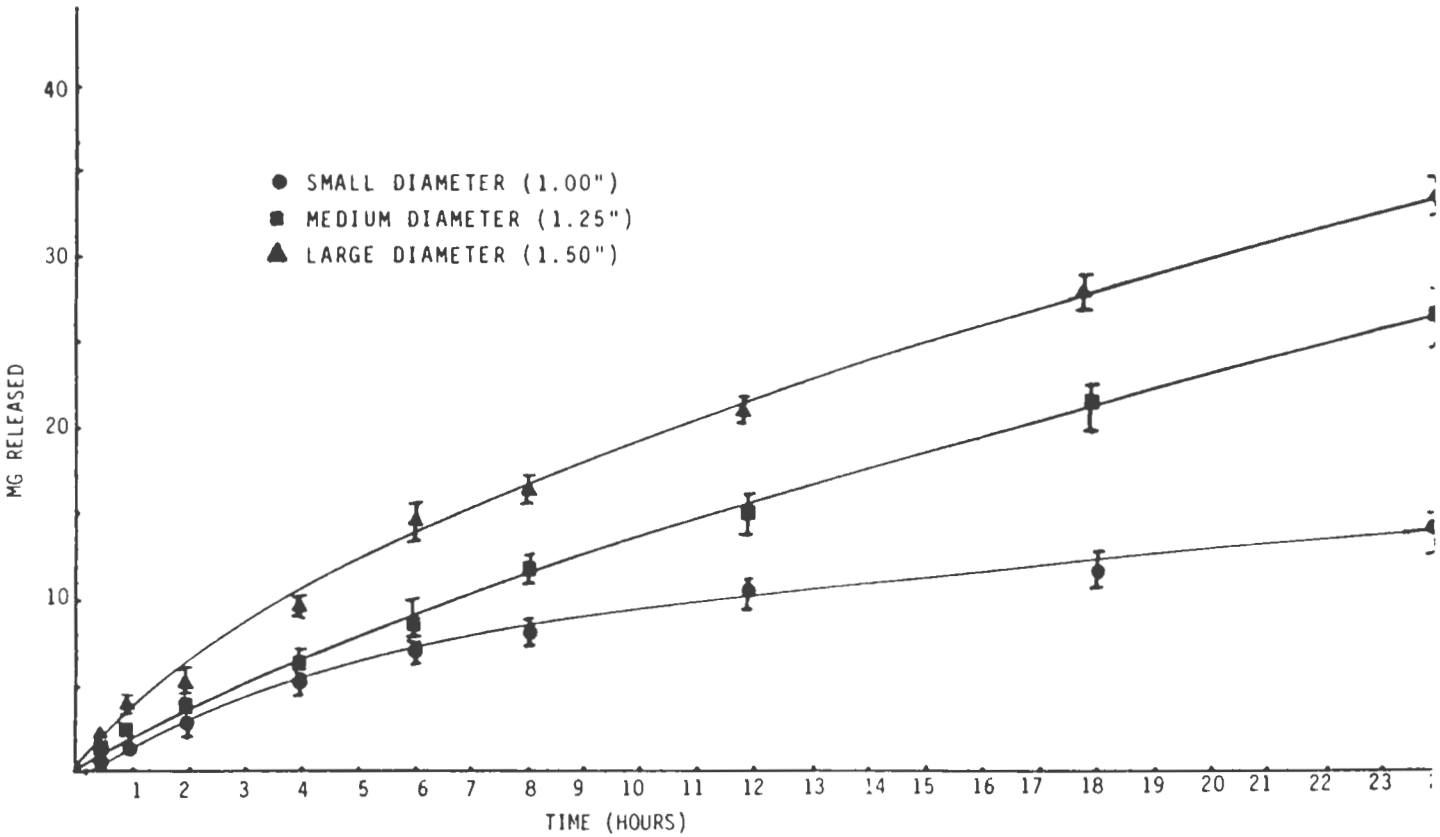


Figure 12. Release profiles of batches 4, 5, and 6.

RELEASE PROFILES (BATCHES 7, 8, 9)

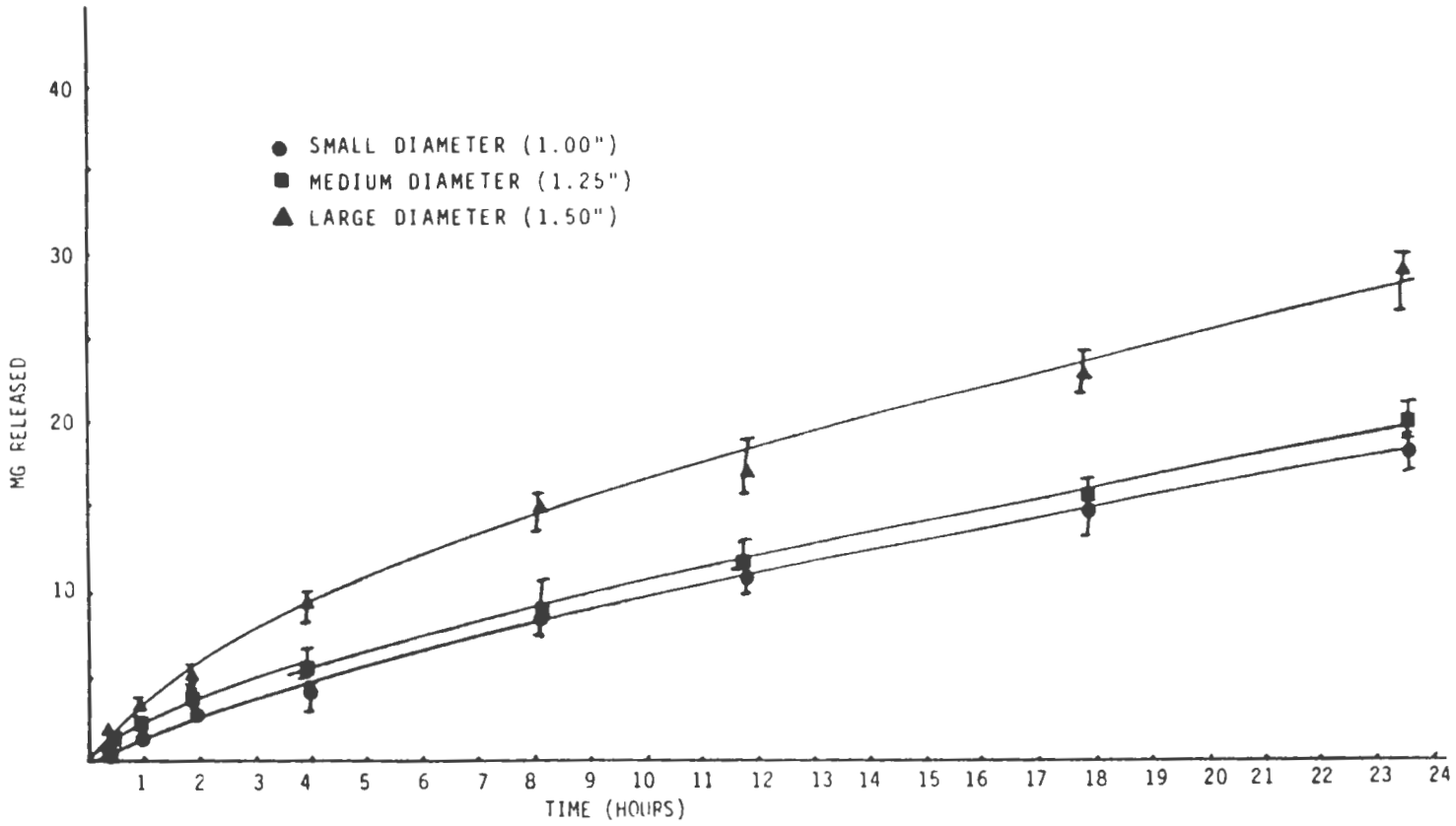


Figure 13. Release profiles of batches 7, 8, and 9.

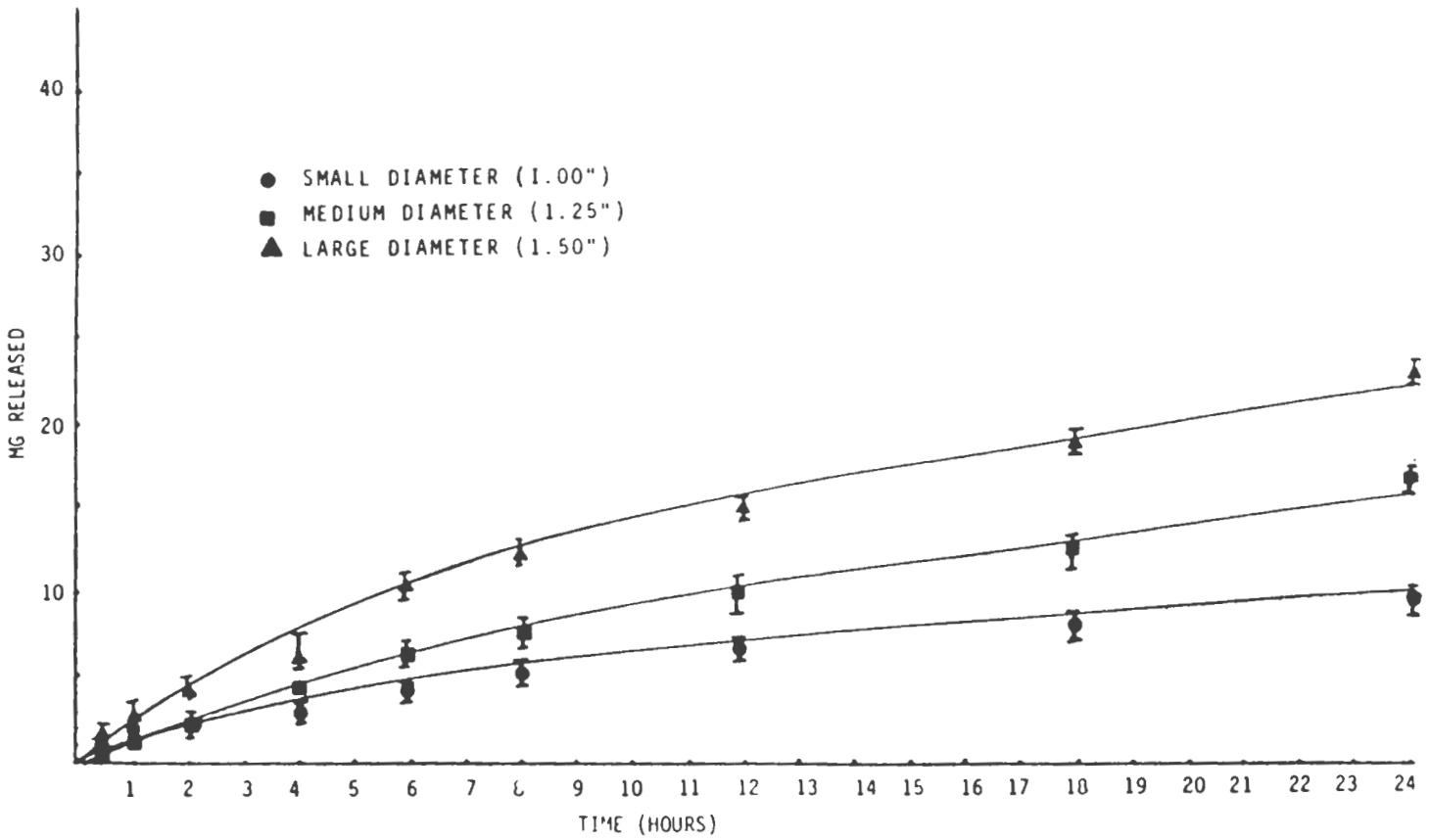


Figure 14. Release profiles of batches 10, 11, and 12.

RELEASE PROFILES (BATCHES 13, 14, 15)

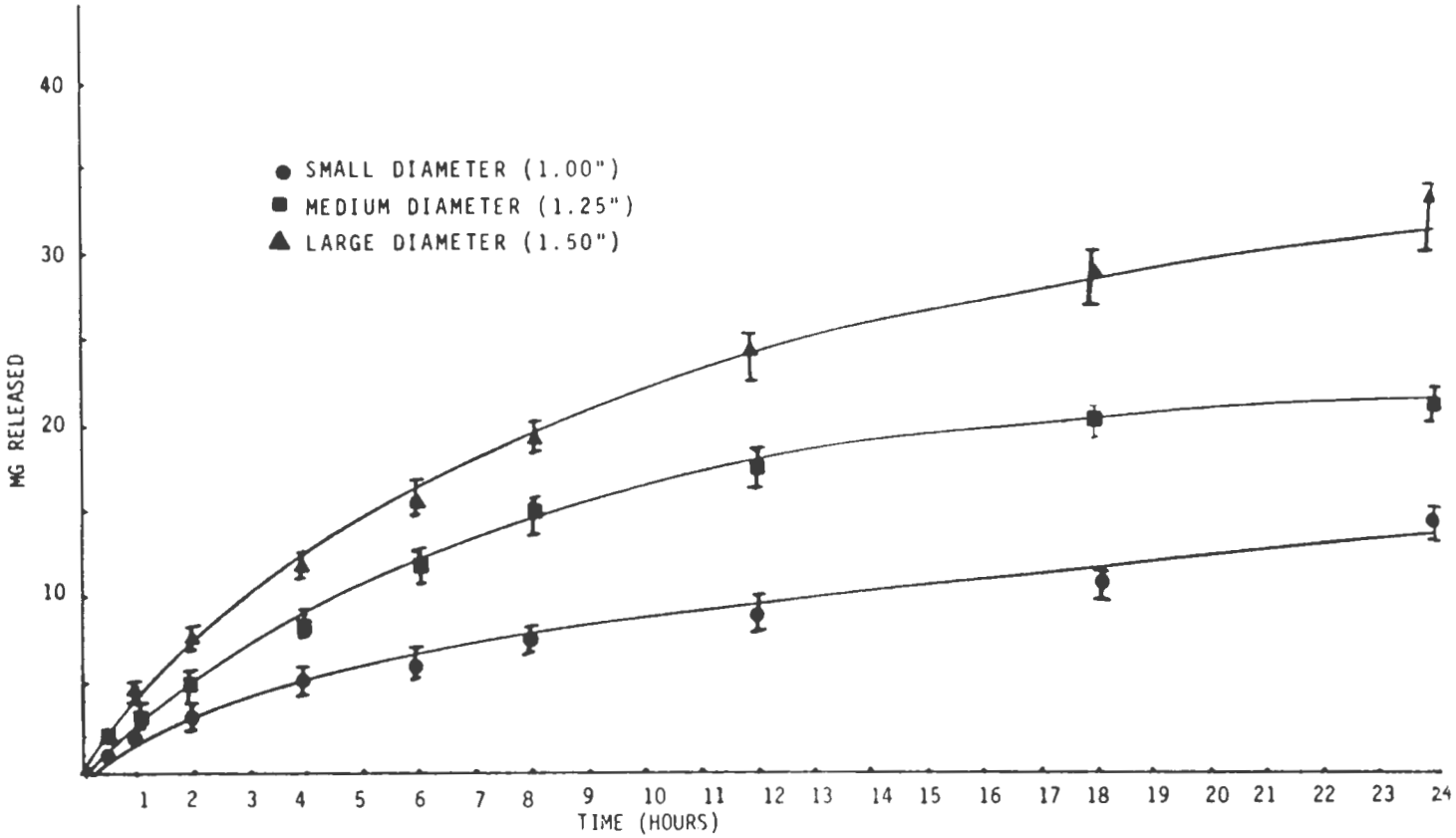


Figure 15. Release profiles of batches 13, 14, and 15.

RELEASE PROFILES (BATCHES 16, 17, 18)

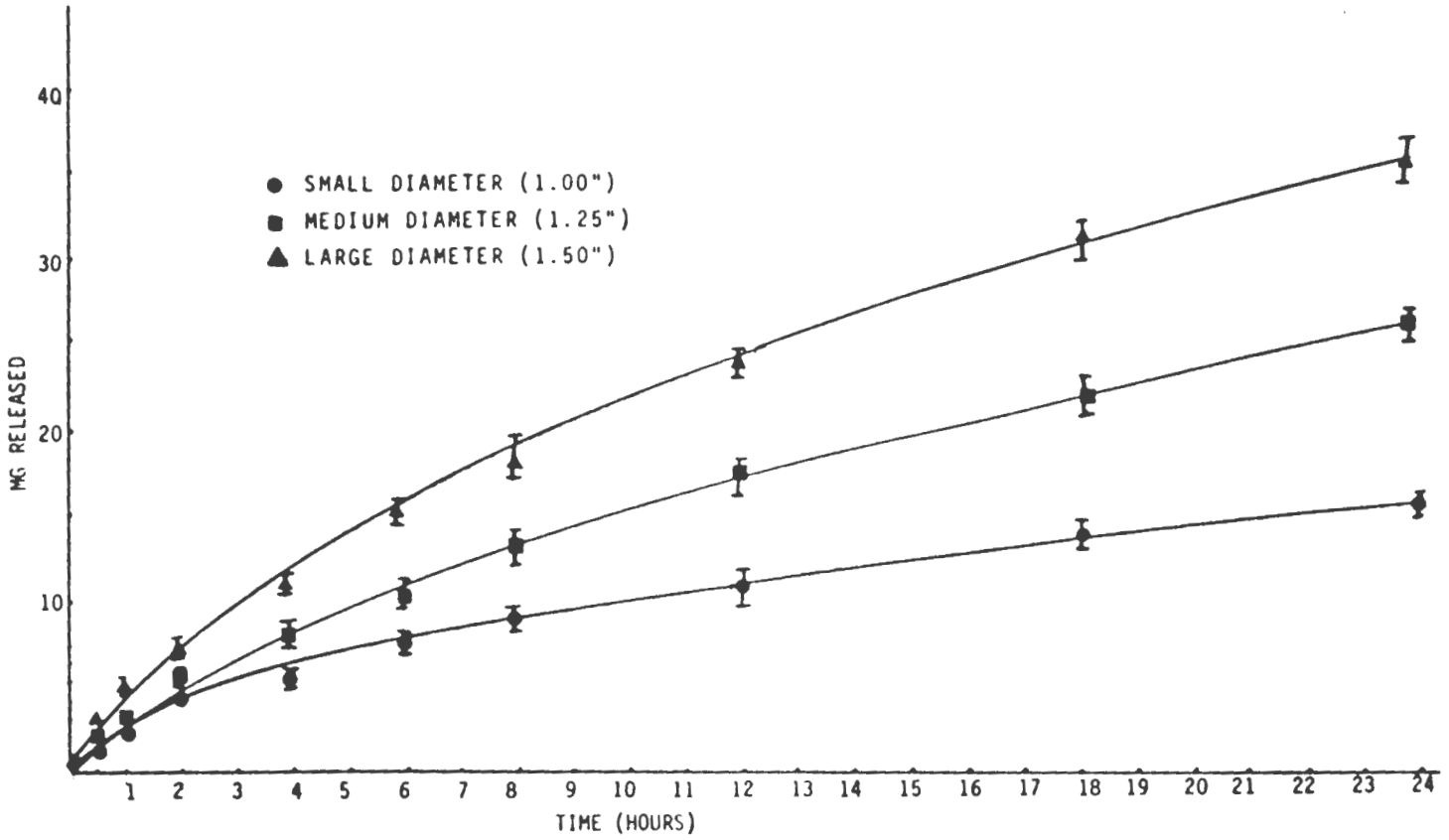


Figure 16. Release profiles of batches 16, 17, and 18.

RELEASE PROFILES (BATCHES 19, 20, 21)

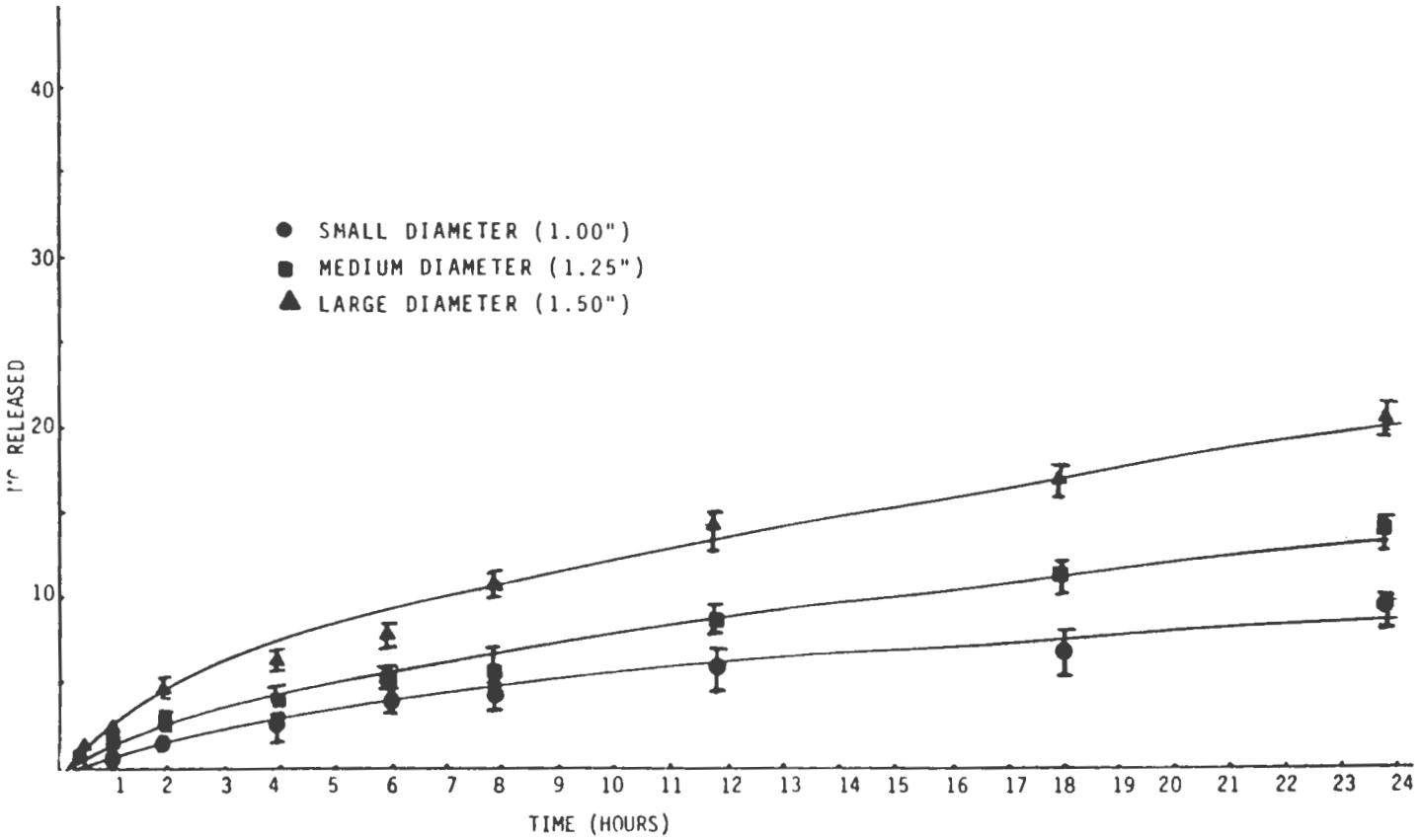


Figure 17. Release profiles of batches 19, 20, and 21.

RELEASE PROFILES (BATCHES 22, 23, 24)

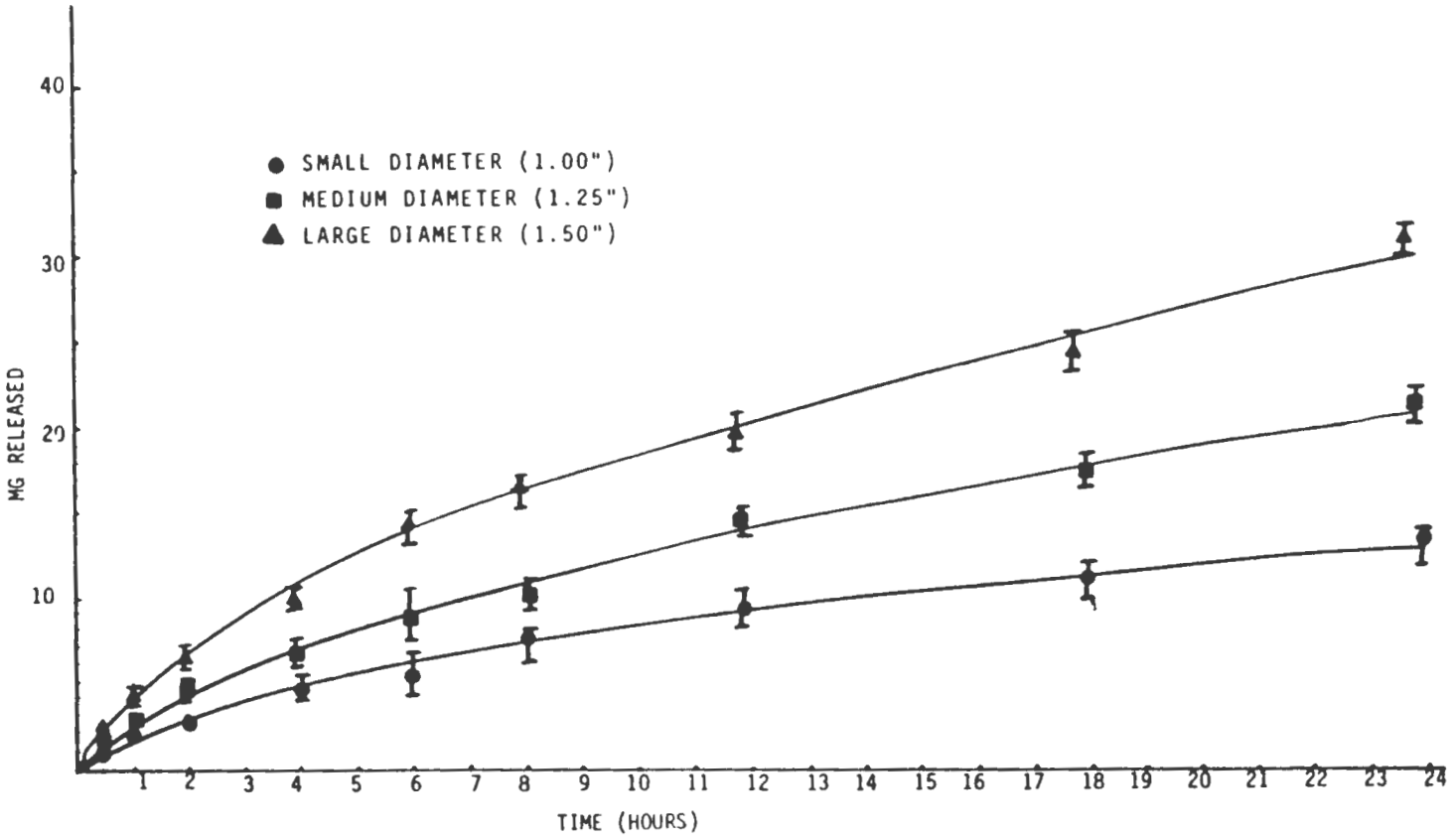


Figure 18. Release profiles of batches 22, 23, and 24.

RELEASE PROFILES (BATCHES 25, 26, 27)

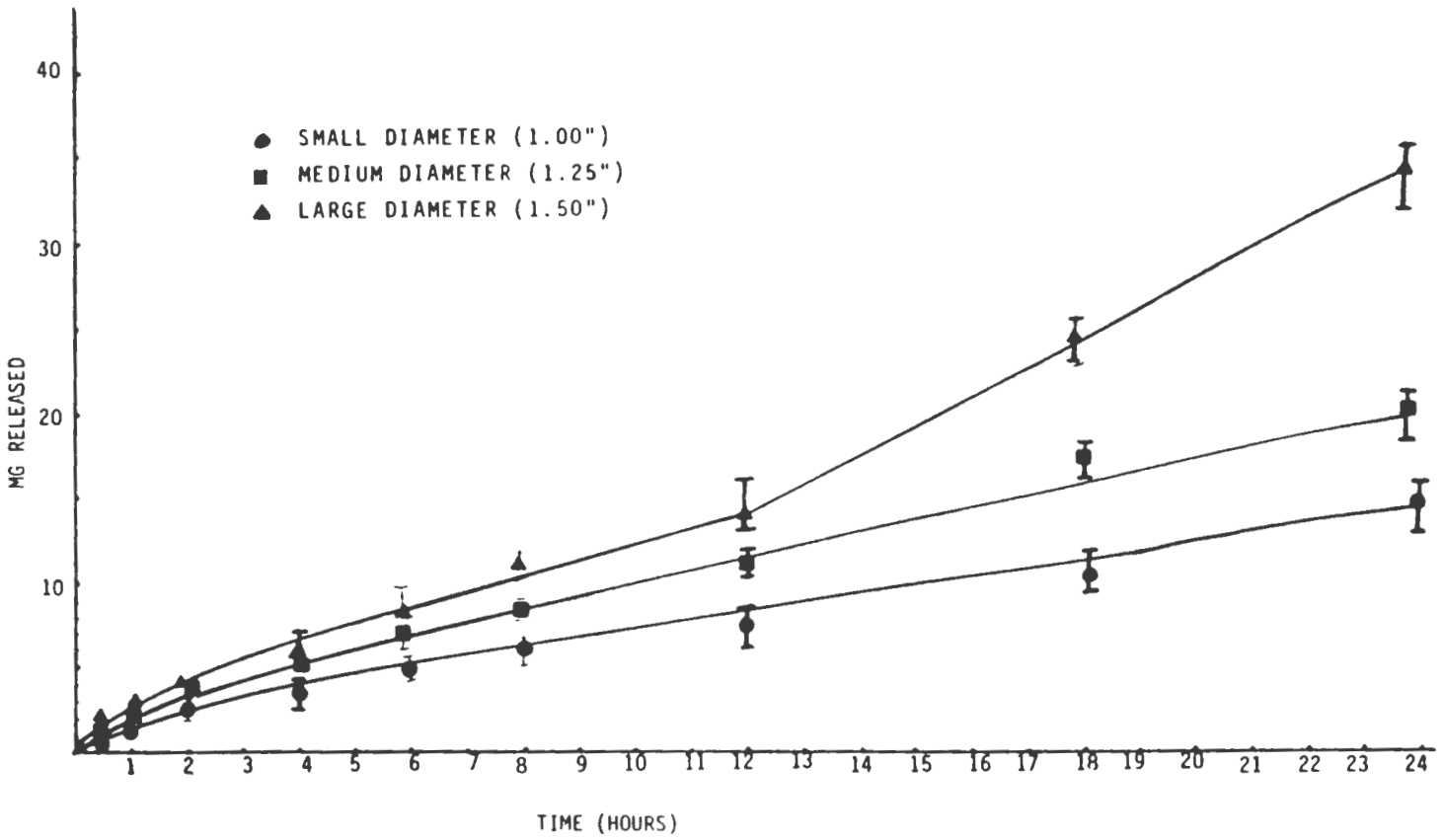


Figure 19. Release profiles of batches 25, 26, and 27.

Jaminet, 1984), and indeed this was the case. An overall linear correlation of 0.989 ± 0.013 was observed. Table 8 summarizes drug release as a function of the square-root of time. It is obvious from this table that drug concentration seemed to affect the slope of each plot. Concentration and slope were found to be inversely proportional, that is, an increase in drug concentration was associated with a decrease in the slope of the line. Percent release over 24 hours was directly proportional to the slope of the line.

Close observation of table 8 reveals that, in most cases, the total percent release of nitroglycerin over 24 hours decreased with increases in concentration. This appears to be due to a function of the driving force of diffusion and the total drug load. As the total drug load was increased, a smaller percentage of drug was actually released since some drug must remain inside the matrix as the driving force. Table 8 also demonstrates the insignificance of the effect of firmness on drug release from these systems. For a given size and drug concentration, a change in firmness did not result in a significant ($p < 0.01$) change in drug release.

Flux calculations were made from two portions of each dissolution profile. These two portions were those represented by the release of drug during the first 2 hours (f_1) and the final 12 hours (f_2). The values of these two fluxes appear in Table 9. It is difficult to determine the extent of the interacting effects of concentration and surface area on the flux of drug from these systems. Subsequent statistical analysis makes these points clearer. Overall, it appears that concentration had the greatest influence on flux. However, a

Table 8. Drug release as a function of the square-root of time. (See table 7 for explanation of batch numbers)

Batch #	Total % rel./24 hr.	Correlation (r)	Slope
1	68.56	0.982	16.32
2	62.91	0.996	14.43
3	64.45	0.995	13.47
4	49.66	0.997	11.39
5	74.17	0.987	17.09
6	51.06	0.996	11.62
7	40.19	0.996	9.38
8	28.25	0.989	6.27
9	28.4	0.998	6.32
10	64.23	0.994	13.02
11	71.67	0.988	15.64
12	66.16	0.997	15.12
13	30.24	0.999	6.77
14	45.93	0.944	10.71
15	51.13	0.998	11.62
16	33.90	0.997	7.59
17	35.99	0.995	8.07
18	35.89	0.997	8.29
19	63.45	0.993	13.72
20	61.61	0.985	13.53
21	70.39	0.983	13.79
22	44.90	0.995	10.47
23	45.87	0.995	10.24
24	45.80	0.998	9.97
25	35.47	0.977	7.57
26	30.18	0.981	6.49
27	34.39	0.955	7.36

Table 9. $Flux_1$ and $flux_2$ for release of NTG from silicone discs.

Batch* #	f_1 (0-2 hr) ($mg/cm^2/hr$)	f_2 (12-24 hr) ($mg/cm^2/hr$)	Ratio $f_1:f_2$	mg released per 24 hr.
1	0.146	0.071	2.07 :1	10.28
2	0.223	0.053	4.24	14.99
3	0.213	0.063	3.39	23.59
4	0.248	0.061	4.08	14.82
5	0.256	0.130	1.96	27.27
6	0.246	0.097	2.53	34.31
7	0.242	0.115	2.56	18.08
8	0.162	0.084	1.83	19.95
9	0.172	0.146	1.55	30.03
10	0.242	0.062	3.88	9.99
11	0.162	0.080	2.03	17.56
12	0.172	0.067	2.59	24.23
13	0.307	0.079	3.89	14.30
14	0.302	0.063	4.80	23.28
15	0.333	0.069	4.80	30.33
16	0.392	0.074	5.30	15.44
17	0.302	0.101	3.00	27.36
18	0.302	0.084	3.60	35.96
19	0.178	0.059	3.01	9.54
20	0.192	0.061	3.16	14.30
21	0.188	0.056	3.42	21.93
22	0.248	0.066	3.77	13.00
23	0.250	0.083	2.98	27.75
24	0.270	0.034	7.84	31.04
25	0.243	0.135	1.81	16.15
26	0.266	0.106	2.52	21.27
27	0.172	0.156	1.10	35.32

closer look at the two flux rates in comparison with the other batches suggests another possibility.

In comparing batches having the same value of f_1, we see a common factor of surface area. For example, batches 9, 12 and 27 have a flux rate of 0.172 mg/cm^2/hr, and have the same surface area of 11.94 cm^2. Similarly, batches 7, 10 and 25 have f_1's of 0.242 mg/cm^2/hr and have a common surface area of 5.07 cm^2. Since no other common formulation factors were noted between these batches, it appears that the initial flux of nitroglycerin from these systems is most influenced by the surface area of the discs.

On the other hand, f_2 appeared to be influenced the most by the concentration of drug in the disc. For example, if we examine batches 3, 10, 19 and 20, which had f_2's of 0.063, 0.062, 0.059 and 0.061 mg/cm^2/hr, respectively, we find a common factor of low drug concentration (10mg/cm^3).

The ratios of f_1 to f_2 are also listed in Table 9. As can be seen from the data, no generalizations can be made with regards to increases in any formulation parameters, suggesting a possible interactive effect of these parameters.

Analysis of variance (Figure 20) of the release data showed surface area ($p < 0.01$) and concentration ($p < 0.01$) to be significant factors in the total release of nitroglycerin from the silicone discs. The firmness of the discs had no significant effect on the release of drug from these systems. Table 10 further illustrates this lack of significance of the firmness of the disc on total drug release over a 24 hour period. The only apparent exception to this conclusion was with two groups of discs- batches 1, 10 and 19, and batches 4, 13 and 22,

Figure 20 . ANOVA (Analysis of Variance)* for 24-hour drug release

Source	df	SS (sums of squares)	F (SS ÷ df)	PR< F
Concentration	2	1058.29	21.00	*** 0.0001
Firmness	2	58.58	1.16	0.3214
Surface Area	2	2616.52	51.92	*** 0.0001
Conc. x S.Area	4	204.70	2.03	0.1049
Conc. x Firm.	4	104.85	1.04	0.3963
Firm x S.Area	4	86.70	0.86	0.4946
Conc x Firm. x SA	8	92.83	0.46	0.8776

* Using Statistical Analysis computer System (SAS)

*** Significant (< 0.05 is significant)

Table 10. Effect of firmness on mg NTG released per 24 hours.

Firmness	Conc. (mg/cm^3)	Surface area (cm^2)	Batch #	mg rel./24 hr.
1	10	5.07	1	10.28
2	"	"	10	9.99
3	"	"	19	9.54
1	"	7.94	2	14.99
2	"	"	11	15.56
3	"	"	20	14.30
1	"	11.40	3	23.59
2	"	"	12	24.23
3	"	"	21	21.93
1	20	5.07	4	14.82
2	"	"	13	14.30
3	"	"	22	13.00
1	"	7.94	5	27.27
2	"	"	14	23.28
3	"	"	23	27.75
1	"	11.40	6	34.31
2	"	"	15	30.31
3	"	"	24	31.04
1	30	5.07	7	18.08
2	"	"	16	15.44
3	"	"	25	16.15
1	"	7.94	8	19.95
2	"	"	17	27.36
3	"	"	26	21.27
1	"	11.40	9	30.03
2	"	"	18	35.96
3	"	"	27	35.32

where a subtle decrease in total milligrams released was noted as the firmness increased. These two groups were both of the small surface area (5.07 cm^2) and the low and medium (10 and 20 mg/cm^3) concentrations.

5. Comparative

Scopolamine base could not be uniformly dispersed into this silicone system. The base appeared as droplets of various sizes which did not dissolve when broken with a glass stirring rod. For this reason, reproducible release characteristics could not be expected. This is evident in the results of the release studies which show virtually no release of scopolamine from any sample during the 24 hour time period.

The scopolamine base is a sticky, viscous liquid and is difficult to handle. Accurate weighing and mixing are essential in an evaluation of this type, but can not be guaranteed.

Vigorous mixing with a glass stirring rod reduced the droplet size, however this method of mixing introduced air bubbles, which are undesirable and could not be removed. The scopolamine base soon settled back to the bottom of the beaker.

6. Penetration enhancer study

The addition of dimethylsulfoxide (DMSO) to silicone/ nitroglycerin formulations was expected to improve the release of nitroglycerin from systems which showed relatively slow release characteristics. However, at the levels tested, this did not occur. In fact, the addition of DMSO to these formulations prevented the cross-linking of the polymer, and

the formulation remained in a semi-liquid state. The Dow-Corning literature for the product used in these studies lists several compounds which are incompatible with polydimethylsiloxane, but DMSO was not one of them.

Due to the lack of resources in the area of penetration enhancers, this portion of the study was not pursued. Other researchers in the field of transdermal drug release have indicated that the application of a penetration enhancer directly to the surface of the device (attempting to directly affect the stratum corneum) is a more successful approach than the encorporation of the penetration enhancer into the matrix (Sanders, 1985).

CONCLUSIONS

1. The firmness (as measured by force to produce 50 percent deformation) of silicone disc matrices containing nitroglycerin on lactose increased as the concentration increased.

2. The firmness of the discs did not significantly affect the release of nitroglycerin in the range studied.

3. Release from these systems was not zero-order.

4. An in-vitro dissolution method was developed to study the release characteristics of drug from silicone discs. The method appeared reliable and reproducible in that there was good correlation ($r=0.920$) of dissolution profiles in both commercial transdermal products and test systems.

5. Surface area had the most significant effect on flux and total release of nitroglycerin. This effect was observed in systems at all three firmness and concentration levels. Flux was most affected by surface area during the first 2 hours of release, and by concentration during the final 12 hours of release.

6. Flux was directly related to concentration (drug load). As the drug concentration was increased, the flux also increased.

7. In all cases, not all of the drug was released from the discs. At most, 60 percent of the nitroglycerin was extracted from the silicone, indicating the an interaction between the drug and the silicone matrix.

FUTURE WORK

This study provided insight into how the release of nitroglycerin is affected by the design of the disc. Several possibilities exist for further investigation in the following areas:

1. In retrospect, a wider range of firmness levels could probably more clearly define the significance of this factor on drug release.

2. To evaluate a system using a more potent drug, i.e., one which would require a smaller drug load in the device, such as timolol or clonidine.

3. To investigate the possibility of encorporating a more compatible penetration enhancer into these systems. Compatible penetration enhancers are currently being studied by other investigators and will find an increased use in commercial transdermal products.

4. To compare this in-vitro method of drug release study to one utilizing diffusion cells with either excised skin or silastic membranes.

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