# CONTROLLED-RELEASE TOPICAL POLYMERIC FORMULATIONS OF ANTHRALIN

A Thesis Presented to the
Faculty of the College of Pharmacy
University of Houston

In Partial Fulfillment of the
Requirement for the Degree of
Master of Science in Pharmacy

by

Tsu-I Wang

December, 1984

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## ABSTRACT

Five topical polymeric formulations of anthralin were fabricated with two types of silicone elastomers and various additives, containing 5% (W/W) of anthralin. The objectives were to provide a transdermal controlled-release mechanism with which the rate limiting step of percutaneous penetration was built into the polymeric device rather than stratum corneum as with conventional dosage forms, and to overcome the problems of chemical instability and irritation to normal skin, inherent to conventional dosage forms.

Product asscessments were conducted, including (1) weight, thickness and anthralin content uniformities, (2) *in vitro* anthralin release kinetics from the polymer and (3) *in vitro* anthralin penetration through and disposition in hairless mouse skin after 48 hours of application. The results provided guidelines for future product development and refinement.

An HPLC assay was developed with reversed-phase octyl column, mobile phase of acetonitrile/water mixture, capable of measuring amount as low as 5 ng of anthralin. The assay was employed in all studies of content uniformity, stability, drug release, and drug penetration.

The release process of anthralin from formulated polymer followed the first-order kinetics which is the characteristic of matrix-type of

controlled-release device. Anthralin had a larger diffusion coefficient through the 382 silicone elastomer than through MDX-4-4210 silicone polymer. The glycerol, azone, and propylene glycol incorporated in various polymeric formulations did not affect the diffusion coefficient of anthralin in test silicone elastomer.

The extent of anthralin penetration from 382 silicone elastomer into hairless mouse skin was significantly higher than that from MDX-4-4210 silicone elastomer. The incorporation of Azone and glycerol in 382 silicone polymer did not improve percutaneous penetration of anthralin from the polymer, while propylene glycol increased anthralin penetration from MDX-4-4210 silicone elastomer significantly.

Anthralin degradation might not be a simple first-order process where oxygen might play a role. Azone facilitated anthralin oxidation, and Vitamin C significantly stabilized anthralin in conventional vehicle. However, when anthralin was incorporated in polymeric formulations, the degradation process was further slowed down. All formulated polymers served the purpose of preventing anthralin from decomposition.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ANT | Anthralin |
| AZ | Azone |
| DAN | Danthron |
| IBU | Ibuprofen |
| IM | Isopropyl Myristate |
| PG | Propylene Glycol |
| Vit. C | Ascorbic Acid |

# I. INTRODUCTION

Psoriasis is a chronic proliferative epidermal disease which is estimated to affect 2-8 million people (1,2) in the United States, on about 1-3% of the American population. The true incidence may be even higher. In the United States, approximately 98% of psoriatic patients are caucasian. The patient population is rare in black, Oriental or American Indians. Men and women are equally affected.

The psoriatic lesion is characterized by well defined hyperproliferative erythematous plaques bearing large silvery scales. Acute lesions tend to be small and guttate. Lesions tend to occur most often on elbows, knees, scalp, genitalia and the upper gluteal fold. No portion of the skin surface enjoys freedom from the disease.

It may occur at any age but is rare before 3 years old, and commonest between 15-40. Regardless of the time of disease onset, the patient faces a life-long struggle to eradicate the erythematous scaling plaques that are a source of anxiety and embarrassment.

Psoriasis is considered to be multifactorial in origin (2). In many patients there is a genetic predisposition, although the disease only appears after the action of some additional precipitating factor. A child with one affected parent has a 25% chance of developing the disease, which rises to 60% if both parents are affected (3). If

nonpsoriatic parents have a psoriatic child, the risk for a subsequent child is 17%. The exact mode of inheritence is still debated (4-10). The precipitating factors include infections, trauma, drugs, light, and emotion.

Guttate psoriasis often follows an upper respiratory tract infection, especially streptococal tonsillitis or pharyngitis. Minor physical damage such as wounds or scratches to the skin of a psoriatic may lead to psoriasis appearing at the site of injury (Koebner's phenomenon). The lesions of a drug eruption may turn into psoriasis in a predisposed person. Antimalarials (especially chloroquine and mepacrine) and lithium may worsen psoriasis. Severe sunburn may be followed by lesion eruptions in the exposed area. Most physicians agree that the appearance and persistence of the disease is occasionally related to stress (11,12).

The basic abnormality in psoriasis is increased epidermal cell proliferation; epidermal cell turnover has been accepted to be 10 times faster than that in normal skin (13). The conventional explanation for this phenomenum is a dramatically reduced epidermal cell cycle time of 37 hours in psoriatic skin (14), compared with 457 hours in normal skin (15). However, the cell cycle times for both normal and psoriatic skin were recently determined to be 50-60 hours (16,17). The increased proliferation in psoriasis may be due to an increased number of germinative cells entering the cell cycle (18) rather than to a decrease in cell cycle time.

The decreased adhesiveness of keratinocytes might result from their poor surface coat of glycoproteins (19), and this could produce a lack of contact inhibition of growth leading to increased epidermal cell proliferation.

Since cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) have been shown to regulate glycogen metabolism and cellular proliferation in other tissues, several enzyme studies concerning cAMP and cGMP were conducted (20-25). The current evidence suggests that alterations of the cAMP:cGMP ratio and prostaglandin E: prostaglandin F balance are basic features of the hyperproliferative psoriatic epidermis.

Recent reports of immunological studies indicate that the function of T-lymphocyte is impaired (26-29); mean serum levels of IgG and IgA are increased (30). Antibodies directed to the stratum corneum of the epidermis are with complement in psoriatic lesions in vivo (31), and anti-basal-cell nuclear antibodies are bound to the membranes of circulating lymphocytes (26,32).

While the disease is treatable, there is no cure for the disease. The current treatments, including systemic and topical remedies are to control the lesions (33-38). The course is difficult to predict when psoriasis is being treated. Spontaneous remission and exacerbation may occur but are unpredictable.

The topical therapy involves the following agents:

a. Coal tar

Coal tar is a byproduct of the destructive carbonization and distillation of coal and the redistillation of its intermediate products. Tar has been used for centuries for the treatment of many skin diseases. It is known to be antipruritic, anti-bacterial, keratoclastic, and photosensitizing. Over the past century, coal tar preparations have been a very popular and effective treatment for psoriasis.

The Goeckerman regimen is a preferred method. It consists of daily tar baths to remove residual scales, exposure to increasing increments of ultraviolet light in suberythema doses, and the application of 1-5% crude coal tar U.S.P. in an ointment or zinc oxide base several times daily.

The actual mechanism of coal tar action in psoriasis has not been determined. Total remission of psoriasis lasting for months can be obtained after three to four weeks of a Goeckerman regimen. However, this is an extremely messy treatment, since the crude coal tar ointment is black and stains clothing permanently. As a result, the use of Goeckerman regimen is usually limited to hospital and is reserved for patients with extensive psoriasis.

Crude coal tar is a heterogeneous mixture of some 10,000 different compounds. Even after all the complicated processes of chemical extraction, only about 55% of the tars, approximately 400

compounds, have been indentified. Because the batch variation in constituent compounds, the therapeutic results in psoriasis vary.

Side effects of coal tar treatment include primary irritant reactions, folliculitis, allergic eczematous contact dermatitis, keratocystomatosis, induction of generalized pustular psoriasis, sunburn, photo contact dermatitis, herpes, and plantar warts. In addition, coal tar has been shown carcinogenic.

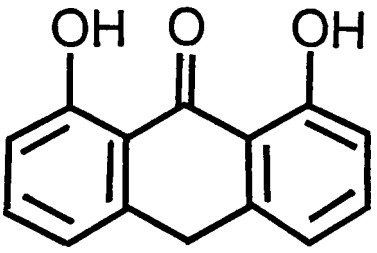
b. Anthralin

Anthralin (ANT), 1, 8, 9-anthracentriol (Fig. 1), was first synthesized in 1916 (39) to treat psoriasis. It causes a more rapid resolution of psoriatic plaques than crude coal tar and induces remissions lasting from weeks to two years.

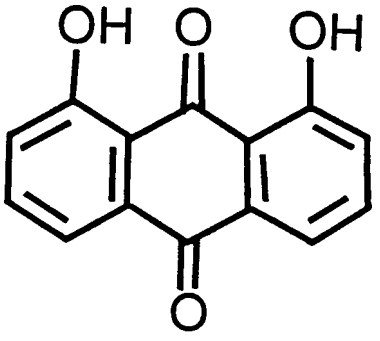
ANT has been proved to inhibit DNA synthesis and thereby inhibits protein synthesis. This results in depression of cell proliferation (40-47). It also inhibits the incorporation of deoxyuridine into DNA, oxygen consumption of cell, and the incorporation of amino acid and thymidine by growing cell (48-50).

Following daily libral application of 0.1 or 0.2% ANT with salicylic acid and 5% hard paraffin in zinc oxide paste everyday (Ingram regimen), the lesions will be cleared up in most patients within 2-3 weeks.

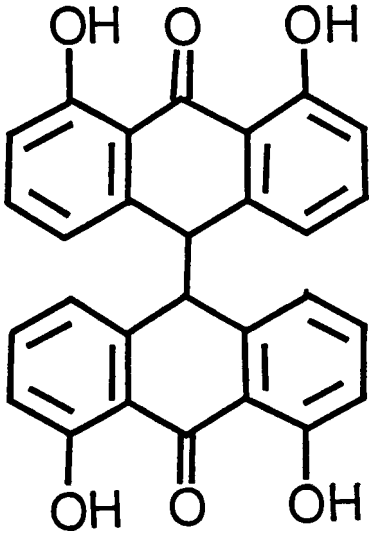
Figure 1. Chemical structures of anthralin and its decomposition products, danthron and dimer.




Anthralin




Danthron




Dimer

Several problems associated with ANT prevent its widespread use. First, severe burning may occur when the paste is applied to intertriginous areas and normal skin. Second, staining of skin, bedclothes and linen is common and caused by coloured oxidized compound of ANT, and complex of the ANT and salicylic acid. In addition, the instability of ANT molecule and its low water solubility make proper pharmaceutical formulation difficult.

c. Glucocorticoids

Topical glucocorticoids are epidermal mitotic inhibitors, anti-inflammatory agents, and vasoconstrictors. Glucocorticoids are cosmetically acceptable to a patient for they are available in cream, lotion, ointment, and gel bases. They are nonmessy and do not have an unpleasant odor. Topical glucocorticoid therapies are particularly effective if applied under occlusive plastic wraps which increase their penetration through the skin up to 100 fold. Potent fluorinated glucocorticoids often resolve psoriatic lesions within 1-2 weeks. Unfortunately there is usually prompt rebound if treatment is discontinued, and tachyphylaxis is common if treatment is continued beyond two to three weeks.

Topically applied steroids are relatively expensive and can produce local and systemic side effects. Local side effects include epidermal and dermal atrophy, vascular fragility with resulting eczematous. Systemic absorption of a topically applied glucocorticoid is possible, especially when occlusive dressings are

employed or it is applied to a large body surface area. Suppression of adrenocortical function could result.

Because of the side effects, glucocorticoids are often reserved for types and areas of psoriasis that will not tolerate ANT or tar, or when a rapid cosmetic response is desired such as psoriasis of the face, scalp, nails and intertriginous areas.

d. Keratolytics

Keratolytic drugs reduce thick scales, particularly on the palms of the hands and the soles of the feet. Salicylic acid ointment, cream, or gel in concentrations varying from 3 to 10 percent, urea cream 10-20%, lactic acid creams and lotions 5-10% are all effective keratolytics in the medical management of psoriasis.

The systemic therapy involves the following agents:

a. Methotrexate

Methotrexate is considered the most effective drug for life-ruining psoriasis that has resisted all other modes of therapy. It inhibits the dihydrofolate reductase, thus inhibits the metabolism of folic acid. Interference of this pathway blocks the formation of thymidine monophosphate which is necessary for the synthesis of DNA.

Needless to say, the associated risks of such therapy must be

carefully weighed against the benefits. Minor side effects such as gastro-intestinal disturbance are common, and the most serious side effect of prolonged therapy is hepatic fibrosis. Since a regular liver function test cannot reliably predict hepatic fibrosis, liver biopsy is needed during the therapy. Hemoglobin, renal function should be assessed, and white blood cell and platelet counts should also be determined to predict marrow suppression.

b. PUVA

PUVA refers to ingestion of 8-methoxypsoralen followed by exposure to long-wave length UV light (UVA, 320-400 nm). Psoralen molecules form photoadducts with DNA pyrimidine bases and cross-link between complementary DNA strands in the presence of UVA, resulting in inhibition of DNA synthesis and cell division. PUVA has considerable advantages of being effective, clean and quick, but the requirement for an elaborate light source is likely to restrict the treatment to the hospital.

Side effects include pruritus, nausea, erythema, and cataracts. Meanwhile, the binding of a drug to DNA after ultraviolet irradiation poses a theoretical risk of cytogenetic changes with resultant carcinogenesis. For this reason, approval of PUVA therapy for psoriasis by the Food and Drug Administration has been delayed, pending long term follow-up studies on PUVA treated patients. Because of the potential for long term adverse side effects, PUVA treatment is limited to patients with severe and extensive psoriasis

that has not responded to conventional topical therapies.

As summarized in Table I, most of the therapies have above 80% of patients reporting clearing of lesions. The average therapy time required is 2-3 weeks except topically applied steroids that require 1-2 weeks only. Remission periods of coal tar and ANT treatments are quite long but the treatments are messy and require hospitalization and skilled nurses to apply. Topically applied steroids have a rapid response but the remission period is very short. Methotrexate has several severe side effects, and PUVA does not have a promising long term safety.

By weighing the merits of individual treatments against their disadvantages, many dermatologists have been encouraged to revert to the use of ANT. In addition, reports of liver damage associated with methotrexate therapy and some disillusionment with the long term results of corticosteroids treatment have led to a resurgence of ANT popularity. Topically ANT not only provides a proven efficaceous treatment for psoriasis but it is also free from systemic toxicity.

Nowadays, the available conventional pharmaceutical preparations (35) of ANT are (a) Anthra ointment containing 0.2% each of ANT and salicylic acid in petrolatum base, (b) Anthra-Derm ointment, with 0.1, 0.25, 0.5, and 1% ANT in white petrolatum and fatty acid ester, (c) Lasan Unguent consisting 0.4% each of ANT and salicylic acid in a water-washable base, and (d) Lassar's paste containing ANT 0.1-0.8%, salicylic acid 2% in white paraffin base stiffened with zinc oxide.

Table I. Summary of treatment regimens for psoriasis.

| | Goeckerman | Ingram | Topical Steroids | Methotrexate | PUVA |
|---|---|---|---|---|---|
| % patients reporting clearing of lesions | 80-90% | 80-90% | 95-100% | 75-80% | 85-90% |
| Average time required for clearing of lesions | 21 days | 11-20 days | 7-14 days | ? | 34 days |
| Length of remission achieved | Up to 12 months | 10 months | 3 weeks | ? | 5-10 weeks |
| Advantages | Long term safety<br>Consistently effective, long remissions | Long term safety<br>CCosistently effective | Rapid response<br>Effective intertrigenous area | Outpatient therapy<br>Not messy | Outpatient therapy<br>Not messy |
| Disadvantages | Time consuming, requires hospitalization | Messy, requires skilled nurses to apply; requires skill in compounding paste | Expensive, tachyphylaxis may occurs; relapses are prompt after therapy is discontinued | Hepatotoxic, requires close monitoring | Potential for oncogenicity, long term risks unknown |

Problems inherent to the conventional dosage forms are (a) chemical instability. ANT is subject to autoxidation in the presence of light, moisture and atmospheric oxygen, especially in alkaline conditions. (b) low water solubility of ANT. ANT must be incorporated in a highly lipophilic vehicle such as petrolatum. (c) staining and irritation problems. The oxidized compound of ANT and ANT-salicylic acid complex are known to stain skin, clothes, linen, etc. and irritates normal skin. (d) hospitalization and skilled nursing are required to apply the ointment or paste for patients. (e) poor patient acceptance and compliance as a result of messiness of the preparation, inconvenience of the treatment and staining problems.

Several approaches have been conducted to solve the problems inherent in ANT preparations. Because of the staining and irritation characteristics of ANT, starch and zinc oxide have been incorporated in the ointment for many years as stiffeners. They give a precise location of the paste when the paste is applied, and prevent the irritation to normal skin. However, it is difficult to apply the stiffened paste. Meanwhile, zinc oxide facilitates the oxidation of ANT (51-56). The incorporation of salicylic acid stabilizes ANT (54-56). Benzoic acid, m- and p-hydroxy benzoic acids, and tartaric acid have also been tried as a stabilizer (56). Only benzoic acid has a stabilizing effect on ANT equal to that of salicylic acid. Salicylic acid is still prefered since it acts as a keratolytic agent as well.

A new formulation was developed recently (57), 0.25% ANT was incorporated in white petrolatum and ascorbic acid was dissolved in

water to make an oil in water emulsion-type ointment. ANT in the oil phase is protected by ascorbic acid in the water phase against oxidation reactions, and it is more acceptable by patients for it is an emulsion type dosage form which is not so messy as an ointment or paste. However, it has been demonstrated that the penetration and distribution of ANT within the skin layer was considerably influenced by the carrier (58). A lipophlic vehicle allowed much better penetration of ANT into epidermis and dermis than a hydrophilic one. The quantity of ANT wich penetrats into epidermis decreases with increasing water content of hydrophilic ointment base. Therefore, it is not favorable to use an oil in water ointment in view of therapeutic effect. Up to now, the Lassar's paste is still the most popular preparation. However, the chemical stability of ANT in Lassar's paste is not satisfactory as it is prone to oxidation even in the presence of salicylic acid.

Low strength ANT (0.01-0.25%) treatment with ointment (59-61) and short-contact ANT therapy (62-65) with higher concentrations (1-3%) of ANT in ointment were developed to reduce the irritation of ANT to normal skin, but it takes a longer time to clear the psoriatic lesions. A wax stick preparation of ANT was developed (66). It could be applied more rapidly and simply, but again, that required longer time to clear the psoriatic lesions.

Some $C_{10}$-substituted analogues of ANT have been tried as a prodrug to reduce irritation, staining, and increase chemical stability of ANT (67-69), but the therapeutic efficacy is still under investigation, and needs to be confirmed.

Until now, none of the ANT preparations is perfect, with respect to therapeutic effect, chemical stability and patient compliance. This study was intended to develop a drug delivery system for ANT with satisfactory characteristics which provides controlled and reproducible rate of drug release, improvement on chemical stability of ANT, free of ANT irritation to normal skin, and better patient compliance.

Silicone elastomers are selected to fabricate the controlled-release dosage form of ANT. They are essentially inert, stable, and diffusable for lipophilic drug. Silicon elastomers are one type of the biocompatible polymers and have been used in reconstructive surgery and maxillofacial surgery as tissue or organ substitute for many years (70,71). They have also been demonstrated to provide a controlled and reproducible release rate in a capsule-type design for drugs, such as several classes of steroids (72), and contraceptive progesterones (73,74) by either subcutaneous implantation (75) or intrauterine (76,77) application. The controlled release properties of silicone elastomer were also applied to the development of matrix type drug delivery systems for many contraceptive progesterones (78-83).

In the controlled-release polymeric delivery system, the therapeutically active agents are either encapsulated in a closed compartment surrounded by a polymeric membrane, or dispersed monolithically in a matrix system formed by the cross-linked polymer chains. The agent molecules diffuse through the polymer phase at controlled-release rate (71,84,85).

Basically, the controlled-release rate of drug from both capsule and matrix-type polymeric delivery systems are governed by Fick's law of diffusion:

$$\frac{dQ}{dt} = - D \frac{dC}{dh} \qquad (1)$$

Where dQ/dt is the the flux of diffusion across a plane surface of a unit area, dC/dh is the concentration gradient of a therapeutic agent across the diffusion path of h, D is the diffusivity of the therapeutic agent in a given medium, and the negative sign accounts for the fact that the therapeutic agent diffuses from a high concentration region to a low concentration region until an equilibrium state is reached. However, the release kinetics of drug from these two system is controlled by two different mechanisms, which result from the time dependency of the diffusional flux defined in equation 1.

In a capsule-type drug delivery system, the concentration gradient dC/dh of drug across a thickness of polymeric membrane is essentially constant with time as long as the amount of drug remained is more than its solubility in the undiffusable medium or the drug powder still exists. The drug molecules have to dissociate themselves first, then dissolve into either the liquid medium or the polymer structure, followed by diffusion through it, and final partition into the tissue fluid surrounding the drug delivery system.

The cumulative amount of drug released from a unit surface area of

a capsule-type drug delivery system is defined by

$$Q = \frac{Cp\ K\ Ds\ Dp}{K\ Ds\ Hp + Dp\ Hd}\ t \qquad (2)$$

Where Cp is the solubility of drug in the polymeric membrane, K is the partition coefficient of drug between polymeric device and the surrounding tissue fluid, Ds is diffusion coefficient of drug in the surrounding tissue fluid, Dp is diffusion coefficient of drug in the polymeric membrane, Hp is thickness of polymeric membrane, Hd is thickness of the diffusion layer.

The rate of drug release is usually described by zero order kinetics since

$$\frac{Q}{t} = \frac{Cp\ K\ Ds\ Dp}{K\ Ds\ Hp + Dp\ Hd} \qquad (3)$$

When K Ds Hp >> Dp Hd, equation 3 is simplified to:

$$\frac{Q}{t} = \frac{Cp\ Dp}{Hp} \qquad (4)$$

and release of drug is a polymer membrane-limiting process.

When K Ds Hp << Dp Hd, equation 3 is simplified to:

$$\frac{Q}{t} = \frac{Cs\ Ds}{Hd} \qquad (K\ Cp = Cs) \qquad (5)$$

and release of drug is a diffusion layer-limiting process.

In a matrix-type drug delivery system, the dispersed drug particles must be eluted from the matrix structure first by dissolution and then by diffusion through the polymer network. The solid drug particles on the surface layer of the polymeric device will be eluted first; when this layer becomes exhausted, the next layer begins to deplete. A depletion zone thus forms and becomes thicker as elution continues. The release of drug is usually a first-order process. Cumulative amount of drug released from a matrix device is defined by

$$Q = [(2A-Cp)\ Cp\ Dp\ t\ ]^{1/2} \qquad (6)$$

Where A is initial amount of drug incorporated in a unit volume of matrix-type polymeric device, Cp is the solubility of drug in the polymer phase, and Dp is the diffusion coefficient of drug in the polymer matrix. Rate of drug release is therfore dependent upon both time and drug content as follows:

$$dQ/dt = [(2A-Cp)\ Cp\ Dp/4t]^{1/2} \qquad (7)$$

Because of these promising characteristics, silicone elastomers

were employed in this study. The rate of drug release from polymer matrix is dependent on drug content; a larger initial amount will result in a faster rate of release. Therfore, ANT is incorporated in polymer at a concentration of 5% (W/W), which is higher than the concentration in conventional dosage form, 0.1-3%.

In the study, ANT is incorporated in silicone polymer to make a controlled-release delivery system, in view of several potential advantages over the conventional dosage form. First, a reproducible and predictable controlled-rate of release will be achieved. Second, an improved chemical stability will result since ANT is incorporated in inert polymeric matrix. The molecules are free from contact with moisture and atmospheric oxygen and light is prevented by an opaque cover. Third, the shape and the size of polymer can be tailored for individual lesions; therefore, normal skin surrounding the psoriatic lesions will not be irritated. Fourth, better patient acceptance and compliance will result from the non-mess, convenience, and lack of irritation to normal skin.

Product assessment and comparison of several polymeric formulations of ANT with various additives are conducted. The parameters include thickness, weight and content uniformities, stability, drug release kinetics and the extent of percutaneous penetration.

## II. OBJECTIVES

1. To develop an HPLC assay for ANT and its decomposition products, danthron (DAN) and dimer, in order to monitor the ANT stability in test formula, drug release from polymer, and drug levels in the skin.

2. To formulate controlled-release polymeric dosage forms of ANT.

3. To verify the improved chemical stability of ANT in the polymeric delivery system over that in a conventional vehicle.

4. To evaluate the polymeric delivery system in terms of:
   a. Thickness.
   b. Weight and content uniformities.
   c. Stability.
   d. Drug release profile.
   e. In vitro percutaneous penetration.

5. To qualitatively identify the chemical structure of the unknown metabolite, one of the major component extracted from ANT-treated skin, with 300 MHz NMR.

# III. EXPERIMENTAL

## A. Materials

### 1. Chemicals and reagents

a. Acetone (Aldrich Chemical Company Inc., Milwaukee, WI.) was tried as an extracting solvent for skin sample.

b. Acetonitrile (HPLC grade, Fisher Scientific Company, Fair Lawn, NJ.) was used to prepare the mobile phase of HPLC.

c. Anthralin powder (Lot. No. 736636, American Dermal, Sumerset, NJ.) was used to prepare ANT standard solutions.

d. L-Ascorbic acid (Lot. No. 533912, J.T. Baker Chemical Co., Phillipsburg, NJ.) was used as an antioxidant for stabilizing ANT solutions.

e. Azone (Lot. No. 0486K0610, Nelson Research, Irvine, CA.) was used as a penetration enhancer for the in vitro percutaneous penetration study.

f. Benzene (HPLC grade, J.T. Baker Chemical Co., Philipsburg, NJ.) was tried as an internal standard for HPLC assay.

g. Benzoic acid (Lot. No. 319166, Baker Analyzed Reagent, J. T. Baker Chemical Co., Philipsburg, NJ.) was tried as an internal standard for HPLC assay.

h. Chloroform (HPLC grade, Fisher Scientific Company, Fair Lawn, NJ.)

was used as a solvent of ANT and DAN stock solutions,as well as to extract the skin sample of in vitro percutaneous penetration study and to reconstitute the sample prior to injection into the HPLC.

i. Cyclohexane (Mallinckrodt Inc., St. Louis, MO.) was tried as an extracting solvent for skin sample.

j. Danthron powder (Lot. No. 61F-0641, Sigma Chemical Company, St. Louis, MO.) was used to make DAN standard solutions.

k. Ethyl acetate (HPLC grade, J.T. Baker Chemical Co., Philipsburg, NJ.) was tried as an extracting solvent for skin sample.

l. Ethyl ether (Anhydrous, Fisher Scientific Company, Fair Lawn, NJ.) was used to sacrifice animal by the method of ether inhalation.

m. Ibuprofen powder (U-18, 573, Lot. No. 0749L, The Upjohn Company, Kalamazoo, MI.) was used as the internal standard for HPLC assay.

n. Isopropyl myristate (Lot. No. 102F-0255, Sigma Chemical Company, St. Louis, MO.) was used as the medium of the drug release study, and was used to improve the attachment between polymer and the skin in the in vitro percutaneous penetration study.

o. Methanol (HPLC grade, Fisher Scientific Company, Fair Lawn, NJ.) was used as a solvent of ANT working standard solution and for reconstituting the sample prior to injection into the HPLC.

p. Methylene chloride (Fisher Scientific Company, Fair Lawn, NJ.) was tried as an extracting solvent for skin sample.

q. Naphthalene (Lot. No. 772096, Fisher Scientific Company, Fair Lawn, NJ.) was tried as an internal standard for HPLC assay.

r. Phosphoric acid (Lot. No. 740532, Fisher Scientific Company, Fair Lawn, NJ.) was used to adjust the pH value of HPLC mobile phase.

s. Potassium phosphate dibasic (Lot. No. B5L 18, MCB Manufacturing

Chemists, Inc., Cincinnati, OH.) was used to prepare the buffered saline stock solution.

t. Potassium phosphate monobasic (Lot. No. 1N16, MCB Manufacturing Chemists, Inc., Cincinnati, OH.) was used to prepare the buffered saline stock solution.

u. Probenecid (Imperial Chemical Industries, Cheshine, England) was tried as an internal standard.

v. Sodium Chloride (Lot. No. 532659, J.T. Baker Chemical Co., Philipsburg, NJ.) was used to prepare the buffered saline solution.

w. Sodium Clofibrate (Imperial Chemical Industries, Cheshine, England) was tried as an internal standard.

x. Water, deionized and distilled, was used for the preparation of buffered saline stock solution and for diluting the saline stock solution.

## 2. Instrumentation and apparatus

a. Filter holder (Millipore, Cat. No. XX1004703, Millipore Corp., Bedford, MA.) was used to filter and de-gas the HPLC mobile phase.

b. Filter paper (Qualitative #1, 11 um, Whatman Limited, England.)

c. Accumet pH meter (Model 292, Fisher Science Company, Fair Lawn, NJ.) was used to measure the pH value of the mobile phase.

d. Freeze dryer (Labconco Corporation, Kansas City, MO.) was used to concentrate saline diffusate of the penetration study, and the unknown metabolite collected from HPLC eluent.

e. Vacuum Pump (Model JQB 56C17D886A P, Marathon Electric, Wausau,

WI.) was connected with freeze dryer for lyophylization.

f. Polytron Homogenizer (Kinematica GmbH, Luzern, Steinhofhalde, Switzerland.) was used to homogenize the skin sample of the in vitro percutaneous penetration study.

g. 2095 Bath & Circulator (Forma Scientific, Marietta, OH.) was used for temperature control during the experiments of in vitro percutaneous penetration study and the drug release study.

h. Vortex Genie Mixer S8223 (Scientific Products, McGaw Park, IL.) was used during the polymer extraction procedure, and was used to mix mobile phase, working standard solutions and all sample injected into HPLC.

i. Magnetic Stirrer (Fisher Scientific Company, Fair Lawn, NJ.) was used to mix mobile phase for HPLC assay.

j. TI-55 Desk Calculator (Texas Instruments, Dalas, TX.) was used for linear regression analysis.

k. Computer (NAS AS/9000N) was used for data treatment. Package of SAS was available on line, and employed.

l. Hi-Precision Micrometer (The L.S. Starrett Company, Athol, MA.) was used to measure the thickness of polymeric formulations.

m. Spectrophotometer (Model 26, Backman, Irvine, CA.) equipped with a recorder (Model 24-25ACC, Beckman, Irvine, CA.) was used for UV scanning to find the maximum absorption wavelength of ANT and DAN.

n. The HPLC system: A liquid chromatograph (Constant-Metric I, Laboratories Data Control, Riviera Beach, FL.) equipped with a 50 ul sample loop (Rheodyne, Barkeley, CA.), a variable wavelength UV detector (Spectral Monitor III, Laboratories Data Control, Riviera Beach, FL.), a reversed-phase octyl column (5 um, 25 cm x 4.6 mm

I.D., Custom LC, Inc., Houston, TX.), or a reversed-phase octyl column (3 um, 10 cm x 4.6 mm I.D., Rainin Instrument Co. Inc., Woburn, MA.), and a chart recorder (Linear Instruments Corp., Irvine, CA.) was used.

o. Electrical Balance (Mettler H51AR, Mettler Instrument Corp., Hightstown, NJ.) was used to weigh all the solid chemicals needed in the study.

p. Franz diffusion cell (O-ring 9mm I.D.; receptor chamber 5 ml) and driving unit (Grown Glass Co. Inc., Somerville, NJ.) was used in the in vitro percutaneous penetration study to simulate the physiological condition.

q. Foxy fraction collector (Instrumentation Specialties Co., Lincoln, NB.) was used to collect the HPLC eluent for isolating the unknown metabolite.

r. Micropipets 2-10ul, 10-100ul, 100-1000ul (Fisher Scientific, Fair Lawn, NJ.) were used to pipette the liquids used in this study.

## 3. Polymeric formulations

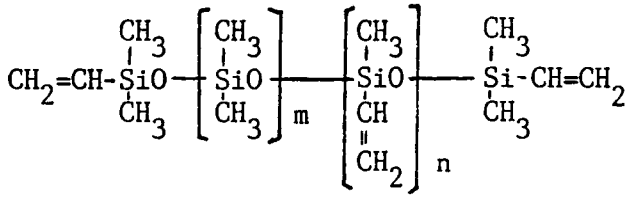
### a. Formula selection

Two types of silicone elastomer, the dimethyl silicone elstomer (382 silicone elastomer) and the vinyl methyl silicone elastomer (MDX-4-4210 silicone elastomer), were used in this study. Their chemical structures are shown in Figures 2a and 2b.

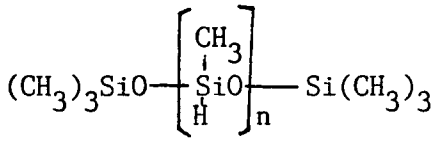
Five polymeric formulations of ANT were prepared (Table II).

Figure 2. Chemical structures of silicone elastomers

a. The 382 silicone elastomer



(polymer)

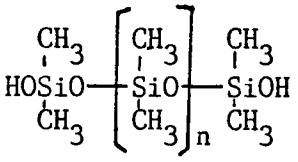


Platinum

(crosslinker)

(catalyst)

b. The MDX-4-4210 silicone elastomer



(polymer)

$Si(OCH_2CH_2CH_3)_4$

Stannous octoate

(crosslinker)

(Catalyst)

Table II. Formula characteristics and rationale of formulation.

| Batch | Formula | Rationale |
|---|---|---|
| A | 0.5 gm ANT (5%)<br>7.6 gm 382 silicone elastomer<br>1.9 gm 360 silicone elastomer (catalyst) | |
| B&F | 0.5 gm ANT (5%)<br>8.55 gm 382 silicone elastomer<br>0.95 glycerol<br>(with appropriate amount of catalyst) | less lipophlilic than batch A. Glycerol decreases acativation energy of drug release from polymer. |
| C | 0.5 gm ANT (5%)<br>8.5 gm 382 silicone elastomer<br>0.9 gm glycerol<br>0.1 gm Azone (1%)<br>(with appropriate amount of catalyst) | Azone, penetration enhancer polymer. |
| D | 0.5 gm ANT (4.76%)<br>9.0 gm MDX-4-4210 silicone elastomer<br>1.0 gm MDX-4-4210 catalyst | |
| E | 0.5 gm ANT (4.76%)<br>8.0 gm MDX-4-4210 silicone elastomer<br>1.0 gm MDX-4-4210 catalyst<br>1.0 gm MDX-4-4210 propylene glycol | Propylene glycol, good solvent for topical drug, decreases activation energy of drug release from polymer. |

Products A, B, and C were fabricated with the polymer of 382 silicone elastomer. They all contained 5% ANT. In addition, 9.5% glycerol was incorporated in product B, and product C contained 1% Azone (AZ) and 9% glycerol. Glycerol is known to decrease the activation energy of drug release from the polymer, and AZ is a penetration enhancer.

Products D and E were fabricated with MDX-4-4210 silicone elastomer which was a more lipophilic polymer. Propylene glycol (PG) was incorporated in product E, because it is a good solvent for lipophilic drugs, and it decreases the activation energy of drug release from the polymer.

b. Fabrication

Polymeric formulations of ANT were fabricated with the procedures summarized in Table III, by Dr. D. Hsieh at Rutgers University.

## B. Procedures

### 1. Preparation of stock and working standard solutions

a. Anthralin solutions

Table III. Procedure of preparing polymeric anthralin dosage forms.

---

⇓ Required amount of silicone elastomer(s)

⇓

⇓ add 0.5 gram of anthralin

⇓ blended with stirrer

⇓

⇓ add one drop of catalyst

⇓

⇓ thorough mixing

⇓ deaerated under vacuum

⇓

⇓ cast within two glass plates

⇓

⇓ kept at room temperature overnight for crosslinking

⇓ uncast

⇓

wraped the polymer sheet with aluminum foil

---

* Fabrication of polymeric dosage form were conducted by Dr. D. Hsieh at Rutgers University.

Because ANT solubility is much lower in methanol than in chloroform, the solutions of ANT were prepared as follows:

(i) The high concentration solution was prepared by dissolving 2 mg of ANT in 2 ml of chloroform resulting in a concentration of 1 mg/ml.

(ii) The low concentration solution was prepared by diluting 100 ul of the previous solution to 10 ml with methanol. The final concentration was 10 ug/ml.

All ANT solutions were freshly prepared before use for its known chemical instability.

b. Danthron stock solutions

Because DAN solubility is much lower in methanol than in chloroform, the stock solutions of DAN were prepared as follows:

(i) The high concentration solution was prepared by dissolving 100 mg of DAN in 100 ml of chloroform resulting in a concentration of 1 mg/ml.

(ii) The low concentration solution was prepared by diluting 2 ml of the previous solution to 200 ml with methanol. The final concentration was 10 ug/ml.

c. Sodium clofibrate stock solution

The stock solution was prepared by dissolving 80 mg of sodium clofibrate in 100 ml of methanol resulting in a concentration of 800 ug/ml.

d. Ibuprofen stock solution

The stock solution was prepared by dissolving 500 mg of ibuprofen (IBU) in 100 ml of methanol resulting in a concentration of 5 mg/ml.

e. Ascorbic acid stock solution

The stock solution of ascorbic acid (Vit. C) was freshly prepared within one week to ensure its antioxident effect. Five hundred mg of ascorbic acid was dissolved in 50 ml of methanol. The concentration was 10 mg/ml.

f. Saline phosphate buffer stock solution

The stock solution was prepared by dissolving 40.915 gm of sodium chloride, 1.7418 gm of potassium phosphate monobasic, and 0.2722 gm of potassium phosphate dibasic in 500 ml of distilled water. The pH value was adjusted with phosphoric acid to 7.38.

g. Buffered saline solution

The buffered saline solution was prepared by diluting one part of saline stock solution with nine parts of distilled water.

h. Working standard solutions of anthralin and danthron

(i) For content uniformity study:

One ml each of a series of working standards was obtained by transferring 50, 100, 400 ul of low concentration solutions of ANT and DAN, 10, 50 ul of high concentration solutions of ANT and DAN into 5 separate 10 ml test tubes. Each test tube was brought to volume of 1 ml by adding 100 ul of internal standard stock solution, 100 ul ascorbic acid stock solution, and appropriate volume of methanol (700, 600, 0, 780, 700 ul, respectively), resulting in final concentrations of 0.5, 1, 4, 10, 50 ug/ml each of ANT and DAN in methanol.

(ii) For drug release study:

One ml each of a series of working standards were obtained by transferring 50, 100, 400 ul of low concentration solutions of ANT and DAN, 10, 50 ul of high concentration solutions of ANT and DAN into 5 separate 10 ml test tubes. Each test tube was brought to volume of 1 ml by adding 100 ul of internal standard stock solution, 50 ul of isopropyl myristate (IM), and appropriate volume of methanol (650, 550, 0, 730, 650 ul, respectively), resulting in concentrations of 0.5-50 ug/ml.

(iii) For <u>in vitro</u> penetration study:

A series of working standards were obtained by transferring 50, 100, 400 ul of low concentration solutions of ANT and DAN, 10, 50 ul of high concentration solutions of ANT and DAN into 5 separate 20 ml test tubes representing concentrations range of 0.5-50 ug/ml. Each test tube was brought to volume of 3 ml by adding 100 ul of internal standard stock solution and appropriate volume of chloroform (2.7, 2.6, 2, 2.78, 2.7 ml, respectively), and 70 mg of minced hairless mouse skin was added to each test tube.

i. Preparation of mobile phase

The composition of the mobile phase was 47:53 (acetonitrile : water, V/V) for sample assay and 40:60 for isolation of unknown peak. Phosphoric acid was added until pH value was equal to 2.5. The solution was thoroughly mixed, and de-gased by filtration three times.

2. Measurement of polymer thickness

Thickness of polymer was measured with a Hi-precision micrometer at random locations. Each batch of polymer was measured at 20 different sites.

3. HPLC conditions

In order to carry out the product assessment in terms of content uniformity, ANT stability, ANT release from polymer, percutaneous

penetration of ANT from polymer into skin, and to isolate the unknown metabolite from the skin extract, three HPLC conditions was developed.

For all samples analyzed, except the sample for isolating unknown metabolite, a reversed-phase spherisorb octyl column (5 um, 25cm x 4.6 mm I.D.) was used. The mobil phase was composed of acetonitrile and water at a ratio of 47:53 (V/V), the pH value was 2.5, and the flow rate was 2 ml/min. For isolating the unknown metabolite from skin extract, a shorter reversed-phase microsorb octyl column (3 um, 10cm x 4.6 mm I.D.) was used. The mobil phase was acetonitrile/water 40:60 (V/V), flow rate was 1 ml/min, other conditions were the same as aforementioned ones.

4. Selection of internal standard

Benzene and naphthalene were tried at the beginning as the internal standards. Benzene was eluted with solvent front, and the retention time of naphthalene was 7.2 minutes, superimposed with DAN. Therefore, the structure of a suitable internal standard was inferred that of substituted benzene ring.

Benzoic acid, probenecid, sodium clofibrate, and IBU were then tried. Benzoic acid, probenacid, and sodium clofibrate were eluted with endogenous peaks of skin extract. IBU was finally chosen as the internal standard, because it did not fuse with other peaks, and had a reasonable retention time, 6.6 minutes, with respect to those of ANT and DAN, 7.5 and 9.0 minutes, respectively.

5. Construction of calibration curves

In order to validate the HPLC assay, a study of within-day and between-day variations of calibration curves was carried out.

The calibration curves of ANT (or DAN) were constructed at concentration range of 0.5 to 5 ug/ml. The peak height ratios of ANT (or DAN) to internal standard were plotted versus concentrations of ANT (or DAN) to give a standard curve.

Five sets of standard curves were constructed within one day for the within-day comparison. Seven sets of standard curves constructed on separate days just prior to sample analysis were also cumulated for between-day comparison.

For all calibration curves, the linear regression was done, and the slopes were calculated. The mean slope and its standard deviation of calibration curves constructed in one day were calculated for comparison of within-day variation, and those of calibration curves constructed on separate days were calculated for comparison of between-day variation.

6. Weight and content uniformities

For each batch of polymer, five pieces of polymeric dosage forms of 1.43 $cm^2$ from randomly selected sites were cut by a metal cap and weighed. Theoretical content of ANT was calculated based on the weight

of polymer cut and the formula of that particular batch.

Each piece of polymer was minced and extracted three times, with 5 ml of chloroform each containing 5 mg of ascorbic acid as antioxidant in a test tube. The chloroform extract was transferred to another test tube, and evaporated to dryness with the aid of an air stream. The residue was reconstituted with 5 ml of chloroform. Ten ul of the extract was mixed with 100 ul internal standard stock solution and 100 ul ascorbic acid in the third tube, and subjected to HPLC assay.

The amount of ANT quantitated by HPLC was corrected for dilution factor to give the total recovery of ANT.

7. Stability study

The ANT stability study was carried out in both conventional vehicle and test formula. For each batch of formula, three pieces of polymer matrix of 1.43 $cm^2$ were cut by a metal cap and extracted following the aformentioned extraction procedure. The polymeric dosage forms were tested every three months.

In the case of conventional vehicle, ANT and DAN were spiked separately in methanol at 0.5, 1, 4, 10, 30, and 50 ug/ml. Four sets of ANT and DAN solutions were tested to compare the effect of ascorbic acid and AZ on ANT or DAN stability. The first set contained ANT (or DAN) only; the second set contained ANT (or DAN) with ascorbic acid 1 mg/ml; the third set contained ANT (or DAN) with AZ 20 ug/ml, and the

fourth set contained ANT (or DAN), ascorbic acid 1 mg/ml, and AZ 20 ul/ml. ANT and DAN solutions were analyzed by HPLC periodically for 40 days.

8. In vitro drug release study

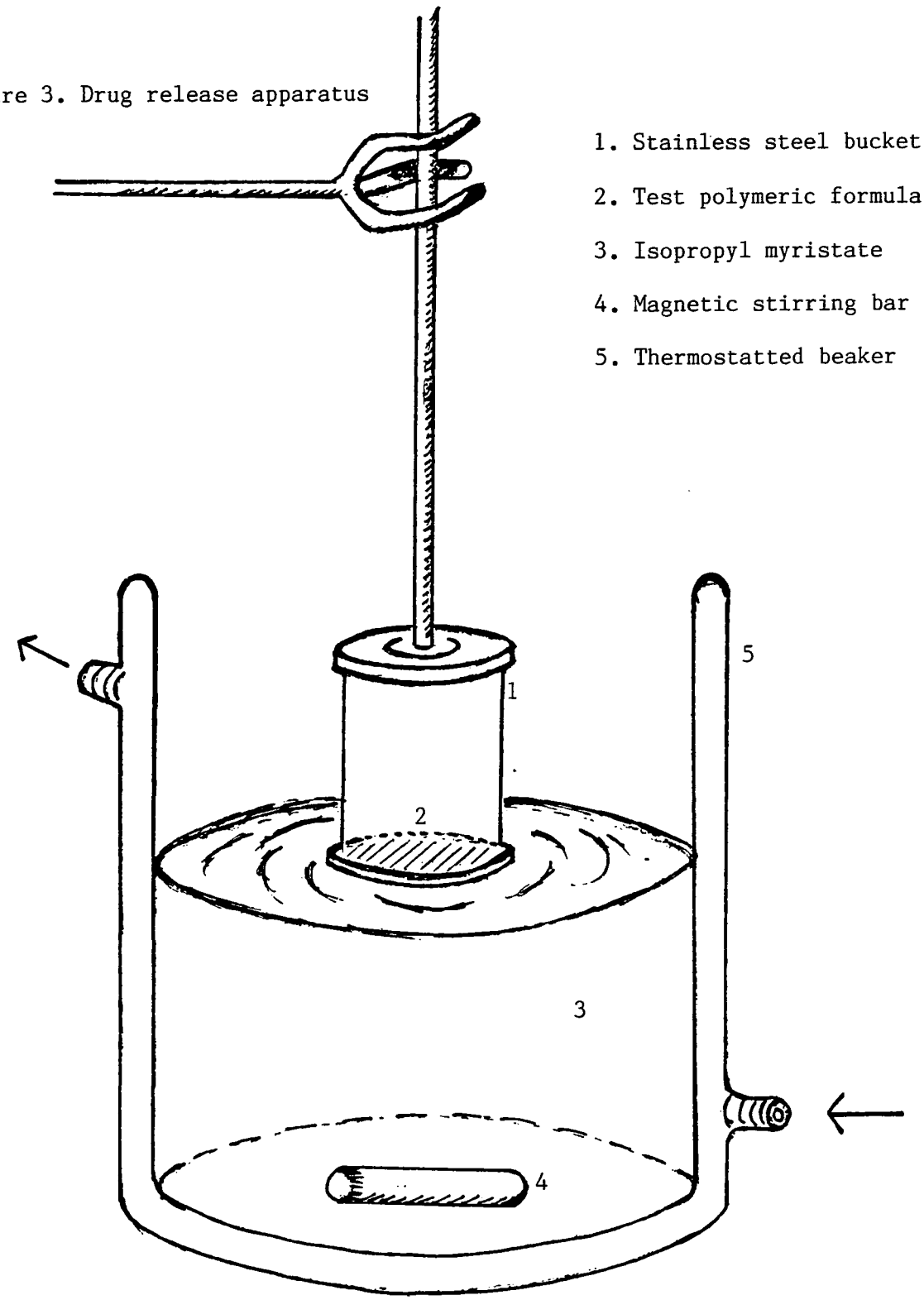
The evaluation of in vitro ANT release from the polymeric system was made with a membraneless model. A stainless steel bucket was fixed on the top of an organic sink of IM which was in a thermostatted beaker (Fig. 3). The polymer in the bucket was in contact with 30 ml of IM equilibrated at 34°C. A magnetic stirring bar was positioned at the bottom of the beaker, and driven by a magnetic stirrer to mix the medium.

9. Analysis of sample from drug release study

One-tenth ml of IM was removed with a syringe from the sink to a vial every 15 minutes for the first two hours and hourly for the following 6 hours. Samples at 23rd, 24th, 25th, and 30.25th hour were also taken. An equal volume of IM equilibrated at the same temperature was replaced. Fifty ul out of 0.1 ml of sample was transferred from the vial to a test tube with a micropipet, mixed with 1 mg of ascorbic acid and 500 ug of the internal standard, diluted with methanol to 1 ml, and subject to HPLC assay.

10. Skin preparation

Figure 3. Drug release apparatus




1. Stainless steel bucket
2. Test polymeric formula
3. Isopropyl myristate
4. Magnetic stirring bar
5. Thermostatted beaker

Homozygous, Hr/Hr, hairless mice of the HRS/J strain was used. Animal between 6 to 12 weeks of age was selected and whose skin was grossly normal and free of bites, scratches or bruises. The mice were sacrificed with the technique of ether inhalation, and the skin was dissected. The full thickness skin was used for penetration study.

11. In vitro percutaneous penetration study

The Franz diffusion cell was used for the study (Fig. 4). The skin sample was mounted between the donor and receptor chambers of the Franz cell. The stratum corneum was thus exposed to ambient conditions and the dermal side was oriented toward the saline phosphate buffer (86), pH 7.4, simulating the physiological pH of the dermal side, 7.3-7.4 (87), in the receptor chamber. The temperature of solution in the receptor chamber was equilibrated by circulating water through a jacket surrounding the chamber at $34^{o}$C, which reflected the temperature of the stratum corneum (88), prior to the application of the test formula. Stirring of the solution of the receptor chamber was accomplished by a teflon-coated magnetic stirring bar driven by a magnetic stirrer. Thirty ul of IM was spread on the entire exposed stratum corneum surface of the mounted skin to improve the attachment of the polymeric formulation to the skin, prior to the application of weighed polymer matrix.

12. Analysis of saline sample from the in vitro penetration study

Two-tenth ml of normal saline diffusate was removed with a syringe

Figure 4. Franz diffusion cell

a. Cell

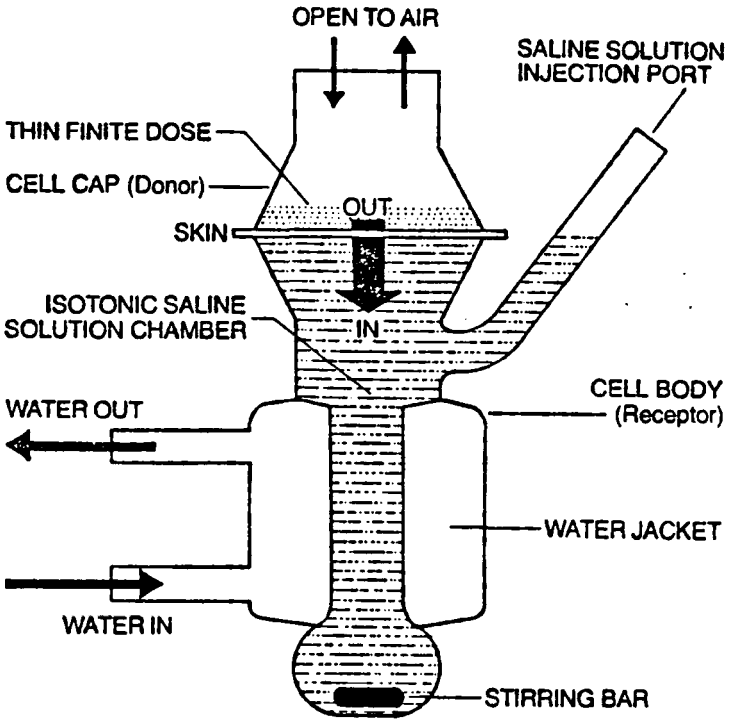
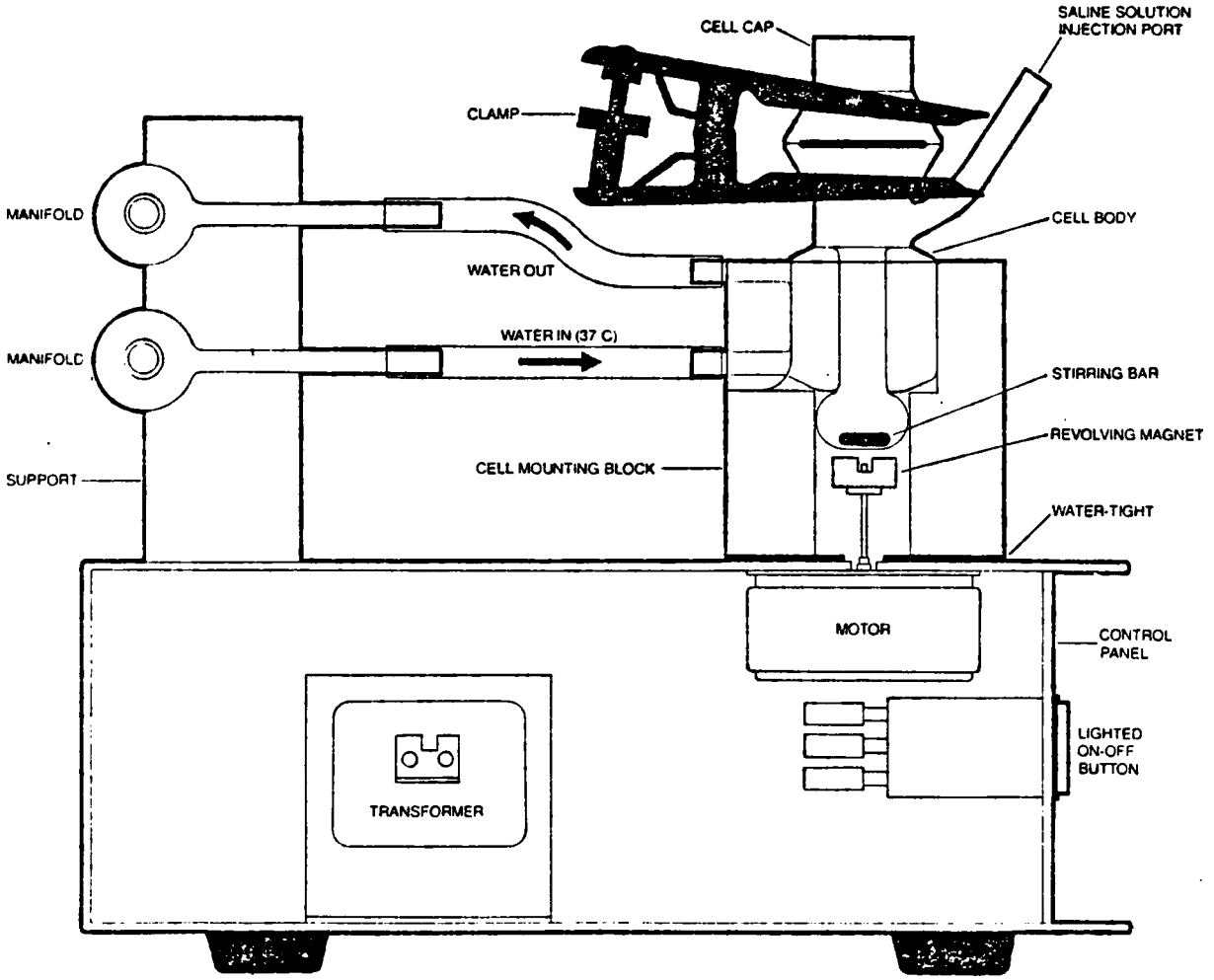


Figure 4. Franz diffusion cell

b. Eight-cell drive unit/mounting assembly



from the side arm of Franz diffusion cell every hour for 8 hours. Samples at 24th, 28th, 32nd, and 48th hours were taken. An equal volume of normal saline equilibrated at the same temperature was replaced. The sample was mixed with 100 ul of internal standard stock solution, 100 ul of ascorbic acid stock solution, ready for HPLC assay. The whole diffusate after 48 hour penetration (5 ml) was lyophilized, and reconstituted with 0.5 ml of methanol containing 100 ul of internal standard and 100 ul of ascorbic acid for HPLC assay.

13. Extraction procedure of skin sample

a. Solvent selection

Methylene chloride, ethyl acetate, cyclohexane, acetone, ether and chloroform had been examined for extracting solvents. ANT was unstable in methylene chloride. Ethyl acetate and ether extracts contained an endogenous skin component with a retention time of 8.4 minutes superimposed with peaks of ANT and DAN. Acetone also extracted a skin component whose peak fused with internal standard. The solubility of ANT and DAN were low in cyclohexane. Therefore, chloroform was determined to be the extracting solvent of choice.

b. The extraction procedure

When blank skin sample was homogenized in buffered normal saline spiked with Vit. C, different concentrations of ANT and DAN, and extracted with chloroform, the recoveries of both ANT and DAN were

within 50 to 60%. When the blank skin sample was homogenized in chloroform spiked with Vit. C, ANT, and DAN, the recoveries of ANT and DAN were 85 to 95%. Therefore, all skin samples from the in vitro percutaneous penetration study was extracted with chloroform directly.

The skin sample after 48hr penetration was minced and homogenized by a Polytron homogenizer in the presence of 500 ug of internal standard and 5 mg of ascorbic acid in 3 ml of chloroform. The homogenates were pooled and mixed with a Vortex-Genie mixer for 2 minutes followed by filtration through a disposable pipet plugged with a pack of cotton at the end. The filtrate was evaporated to dryness and reconstituted with 0.5 ml of methanol and chloroform mixture (1:1, V/V), and subjected to HPLC assay.

14. Pretreatment

The full thickness hairless mouse skin was mounted on the diffusion cell as in the regular penetration study. Two-tenth ml of 1% or 2% AZ in PG solution was applied to the stratum corneum surface of the mounted skin. After 8 hr exposure of AZ to skin, the AZ solution was removed completely and the stratum corneum surface wiped dry. weighed polymer matrix was applied to the same stratum corneum surface. The skin sample after 48 hr penetration was extracted and subject to HPLC assay. In the case of controls, two-tenth ml of propylene glycol was applied to the stratum corneum surface for 8 hours, weighed matrix was applied to the same surface. The skin sample after 48 hours

penetration was extracted and subject to HPLC assay.

15. Isolation of the unknown metabolite

When the polymeric formulations, saturated ANT in PG, and saturated DAN in PG were used as a penetrant in the in vitro percutaneous penetration study, an unknown peak was observed in the chromatogram as one of the major recovered components in the skin extract. Its retention time, 7.8 minutes, was between those of ANT and DAN, 9.0 and 7.5 minutes, respectively. It was suspected to be a metabolite of DAN. The unknown metabolite was isolated in order to elucidate the chemical structure with 300 MHz NMR.

Eight pieces of hairless-mouse skin after 48 hours in vitro percutaneous penetration of ANT from polymer batch B were collected. The skin sample was extracted following the aforementioned extraction procedure except that the internal standard was omitted. The eight tubes of filtered homogenate were pooled to one tube where they were evaporated to dryness by the aid of an air stream. The dried residue was then reconstituted with 1 ml of chloroform and methanol mixture (1:1, V/V).

The skin extract was first examined by the HPLC condition-A (Table IV). Only DAN and the unknown metabolite, but no ANT, were recovered. ANT might have had decomposed. The HPLC condition-B (Table IV) was tried to attain better resolution between DAN and the unknown metabolite. The retention times of DAN and unknown metabolite were 8.7

Table IV. HPLC conditions.

| HPLC condition | | A | B | C |
|---|---|---|---|---|
| Column dimension | | Octyl Column<br>5 um<br>25 cm<br>4.6 mm I.D. | Octyl Column<br>3 um<br>10 cm<br>4.6 mm I.D. | Octyl Column<br>3 um<br>10 cm<br>4.6 mm I.D. |
| Mobile phase | | $CH_3CN$ : $H_2O$<br>47 : 53<br>pH 2.5 | $CH_3CN$ : $H_2O$<br>44 : 56<br>pH 2.5 | $CH_3CN$ : $H_2O$<br>40 : 60<br>pH 2.5 |
| Flow rate | | 2 ml/min | 1 ml/min | 1 ml/min |
| Max. absorption wave length | | 260 nm | 260 nm | 260 nm |
| Chart speed | | 10 cm/hr | 10 cm/hr | 10 cm/hr |
| Retention time | ANT | 9.0 min | 11.4 min | 16.8 min |
| | DAN | 7.5 min | 8.7 min | 12.0 min |
| | unknown metabolite | 8.1 min | 11.4 min | 16.5 min |

and 11.4 minutes, respectively. The HPLC eluents were collected every 30 seconds in numbered vials with a fraction collector. Fractions of eluent No. 19 to 22 were reinjected into HPLC to check the separation. It was found that DAN and the unknown metabolite were still eluted together, with larger amount of DAN in fractions No. 19 and 20, and larger amount of unknown metabolite in fractions No. 21 and 22. Therefore, the HPLC condition-C (Table IV) was used, and the tube connecting the UV detector and the fraction collector was shortened to a minimum length in order to minimize the post-column fusion of DAN and the unknown metabolite.

The retention times were 12 and 16.5 minutes for DAN and unknown metabolite, respectively, using condition-C. The HPLC eluents were collected by a fraction collector every 30 seconds in separate vials. DAN was eluted in fractions No. 24 to 27, and the unknown metabolite was eluted in fractions No. 33 to 37.

After all the skin extract were injected into HPLC, eluent fractions No. 24 to 27 were lyophlized in 6 vials, fractions No. 33 to 37 were also lyophylized in 6 vials. The unknown metabolite in the vials were pooled together using 1 ml of chloroform to transfer the content of each vial, and evaporated to dryness with the aid of an air stream. The dried residual was reconstituted with 0.2 ml of chloroform and methanol misture (1:1, V/V), and ready for structure elucidation by 300 MHz NMR.

16. Data treatment

a. Thickness of polymer

In order to compare the within-batch and between-batch variations in polymer thickness, the mean and standard deviation of the twenty measurements for each batch were calculated.

b. Weight and content uniformities

In order to compare the within-batch and between-batch variations in polymer weight, the mean and standard deviation of five measurements for each batch of polymer were calculated. Polymer weights were also divided by the mean polymer thickness to give the weight of polymer per 0.143 $cm^3$ of volume. Mean and standard deviations of corrected weight were then calculated for each batch for between-batch comparison.

For each piece of polymer cut by a metal cap, theoretical ANT content was calculated by multiplying the weight of polymer and the percentage (W/W) of ANT incorporated in the particular batch. The amount of ANT quantitated by HPLC was multiplied by 500 to give the total recovery, and then devided by the the theoretical content and multiplied by 100 to yield the percentage recovery for comparison.

c. Stability study

(i) In conventional vehicle:

The time profiles of remaining ANT were constructed for four sets of ANT solutions whose compositions were described in section III-B-7 (page 35). The amount of remaining ANT (ug) quantitated by HPLC were plotted versus time (days) on both quadratic coordinate and semi-log papers. The degradation kinetics of ANT was determined to be an apparent first-order process based on the exponential curves and linear curves observed on the quadratic coordinate paper and the semi-log paper, respectively. Four sets of solutions were plotted separately. The slopes of the straight lines on semi-log paper were obtained by linear regression, and the mean negative slope for different concentration solutions in one set was determined as the apparent first-order decomposition rate constant of ANT.

In the case of DAN solutions, amounts of remaining DAN (ug) quantitated by HPLC were also plotted versus time (days). Four sets of DAN solutions were plotted separately, and first-order decomposition rate constant was similarly determined.

(ii) In polymeric formulations:

The mean value of percentage recoveries was determined by extracting three pieces of polymer at each sampling time, and plotted versus time (days) on semi-log paper. Each batch of polymer was plotted separately. The linear regression was done, and the negative value of the slope was taken as the first-order degradation rate constant of ANT in polymeric formulations.

d. In vitro drug release study

The amount of ANT (ug) quantitated by HPLC was multiplied by 600 to yield the ANT amount (ug) in 30 ml of IM, and divided by 1000 to have the unit in mg.

A cumulative correction was made for the previously removed samples (89) in determining the total amount of released ANT by using the formula:

$$An = Anm + \frac{1}{300} \left[ \sum_{i=1}^{n-1} Aim \right] \qquad ( 8 )$$

Where Anm denotes the amount of ANT measured by HPLC, and An is the amount of ANT expected in the medium at the nth sampling time if the previous samples had not been removed. A FORTRAN computer program was written to do the correction (Appendix A).

Since the products were matrix type of preparation, the release kinetics followed the time-square root equation of Higuchi (90)

$$q = 2C_o \left[ Dt/\Pi \right]^{1/2} \qquad ( 9 )$$

Where q is the amount of drug released per unit area, $C_o$ is the initial drug concentration in the formulation, D is the apparent diffusion coefficient, t is time. When q is replaced by Q/A, and $C_o$

is replaced by $Q_o/hA$, the equation is

$$Q/A = 2Q_o/hA \left[ Dt/\Pi \right]^{1/2} \qquad (10)$$

where Q, $Q_o$, h, and A are amount of drug released from polymer at time t, initial amount of drug contained in polymer, thickness of polymer, and area of tested polymer, respectively. When the area term, A, in both sides of equation 10 was canceled, the equation is

$$Q = 2Q_o/h \left[ Dt/\Pi \right]^{1/2} \qquad (11)$$

When amount of drug released is plotted versus square root of time,

$$S = 2Q_o/h \left[ D/\Pi \right]^{1/2} \qquad (12)$$

where S is slope of the straight line. After rearranging equation 12,

$$D^{1/2} = \left( Sh/2Q_o \right) \Pi^{1/2} \qquad (13)$$

therefore,

$$D = \left( Sh/2Q_o \right)^2 \Pi \qquad (14)$$

The mean polymer thickness and the determined ANT content of the tested polymer were used when calculating the diffusion coefficient of ANT. A FORTRAN computer program (Appendix B) was written to do the linear regression for data points in the first 8 hours, and to calculate the apparent diffusion coefficient with the determined slope.

In order to illustrate the within-batch variation, mean and standard deviation of apparent diffusion cofficients for each batch were calculated. For comparison of ANT diffusion in two types of polymer, the program of one way analysis of variance of SAS package was used to analyze the mean diffusion coefficients of ANT in batch A and D, which both contained no additive. It was also used to compare batch A with B, batch B with C, and batch D with E to varify the effects of glycerol, azone, and PG on ANT diffusion respectively.

For each batch of polymer, the ANT released at each sampling time was normalized by an equal initial amount of ANT, 15 mg. The mean and standard deviation of the normalized values for each sampling time were calculated, and the characteristic drug release profile for that particular batch was constructed by plotting mean normalized amount of released ANT with standard deviation versus square root of time. A straight line was obtained in the first eight hours.

e. In vitro percutaneous penetration study

ANT in normal saline diffusate was not detectable by HPLC. Amounts of ANT, DAN and unknown metabolite penetrated into hairless mouse skin were quantitated at nanomole levels by HPLC, and normalized by an equal initial amount of ANT, 5 mg. The amount of the unknown metabolite was estimated using the DAN calibration curve.

In oredr to compare the percutaneous penetration of ANT from two types of polymer, the program of one way analysis of variance of SAS package was used to analyze the mean amounts of ANT penetrated from batch A and D. It was also used to compare batch A with B, batch B with C, and batch D with E to varify the effects of glycerol, azone, and PG on the percutaneous penetration of ANT.

# IV. RESULTS AND DISCUSSION

## A. Thickness variation

Mean thickness of polymeric formulations varied from 0.9 to 1.4 mm (Table V). The within-batch coefficients of variation of bach A and F were less than 5%, and those of the rest were within 14%. Analysis of variance (Table VI) showed that the mean thickness of polymeric formulations were not all the same (p=0.0001); therefore, Student-Newman-Keuls' test (Table VII) was used for further comparison. Six batches of polymer were divided into four categories because of different thickness. However, the thickness of polymer was taken into account in calculating the diffusion coefficient (Equation 14), and the variations in thickness among batches would not affect the determination of diffusion coefficient of ANT in polymeric formulations.

## B. Variation in polymer weight

The mean weights of 1.43 $cm^2$ of polymeric formulations ranged from 155 to 224 mg. The within-batch coefficient of variation of batch A was 0.8%, and those of others varied from 6% to 20%. The variation of

Table V. Thickness of polymeric formulations.

| | Thickness, mm | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | F |
| 1 | 0.947 | 1.034 | 1.128 | 1.061 | 1.172 | 1.254 |
| 2 | 0.881 | 0.894 | 1.298 | 1.135 | 1.243 | 1.267 |
| 3 | 0.892 | 0.886 | 1.326 | 1.188 | 1.176 | 1.315 |
| 4 | 0.912 | 0.836 | 1.209 | 1.189 | 1.516 | 1.374 |
| 5 | 0.945 | 0.907 | 1.171 | 1.295 | 1.544 | 1.441 |
| 6 | 0.858 | 0.907 | 1.120 | 1.163 | 1.680 | 1.426 |
| 7 | 0.935 | 0.846 | 1.110 | 1.210 | 1.395 | 1.460 |
| 8 | 0.927 | 0.968 | 0.935 | 1.285 | 1.343 | 1.495 |
| 9 | 0.912 | 1.057 | 1.067 | 1.234 | 1.376 | 1.441 |
| 10 | 0.920 | 1.077 | 1.219 | 1.251 | 1.289 | 1.476 |
| 11 | 1.011 | 1.020 | 0.965 | 1.301 | 1.080 | 1.492 |
| 12 | 0.996 | 1.021 | 0.909 | 1.424 | 1.060 | 1.478 |
| 13 | 0.990 | 0.998 | 1.036 | 1.377 | 1.224 | 1.426 |
| 14 | 0.998 | 1.046 | 1.012 | 1.399 | 1.318 | 1.422 |
| 15 | 0.976 | 0.889 | 1.076 | 1.268 | 1.456 | 1.435 |
| 16 | 1.010 | 1.021 | 1.118 | 1.258 | 1.615 | 1.389 |
| 17 | 0.983 | 0.942 | 1.443 | 1.294 | 1.550 | 1.401 |
| 18 | 0.908 | 1.017 | 1.401 | 1.229 | 1.636 | 1.419 |
| 19 | 0.983 | 1.033 | 1.320 | 1.308 | 1.564 | 1.512 |
| 20 | 0.984 | 1.066 | 1.177 | 1.298 | 1.391 | 1.400 |
| Mean | 0.948 | 0.973 | 1.152 | 1.258 | 1.382 | 1.416 |
| S.D. | 0.046 | 0.078 | 0.150 | 0.088 | 0.186 | 0.070 |
| C.V.% | 4.8 | 7.9 | 13.0 | 7.0 | 13.5 | 5.0 |

Table VI. Analysis of variance for polymer thickness.

| Analysis of variance | | | |
|---|---|---|---|
| Source | DF | Sum of squares | Mean square |
| Model | 5 | 3.98532800 | 0.79706560 |
| Error | 114 | 1.48056435 | 0.01298741 |
| Corrected total | 119 | 5.46589234 | |

| ANOVA SS | F value | PR > F |
|---|---|---|
| 3.98532800 | 61.37 | 0.0001 |

Table VII. Student-Newman-Keuls' test for polymer thickness.

$\alpha$=0.05 DF=114 MSE=0.0129874

| SNK Grouping * | Mean | N | Batch |
|---|---|---|---|
| A | 1.4161 | 20 | F |
| A | 1.3815 | 20 | E |
| B | 1.2582 | 20 | D |
| C | 1.1520 | 20 | C |
| D | 0.9732 | 20 | B |
| D | 0.9484 | 20 | A |

* Means with the same letter are not significantly different.

polymer weight resulted in part from the thickness difference among six batches. When the mean weight of each batch was normalized with mean thickness of the polymer, the between-batch variation dropped from 153 to 192 mg (Table VIII).

Analysis of variance for polymer weight (Table IX) showed that the mean weights of tested formula for each batch were all the same ($p=0.097$), and the difference among six batches was statistically insignificant.

C. HPLC analysis of sample

Ultraviolet spectra of ANT and DAN (Fig. 5a & 5b) showed that the maximum absorption wavelength of these two compounds were 260 and 255 nm, respectively. The wavelength of 260 nm was selected for sample detection in HPLC assay.

Authentic chromatograms are shown in Fig. 6a-6c. When eluted with $CH_3CN/H_2O$ (47:53, V/V) through a spherisorb octyl column (5 um, 25cm x 4.6 mm I.D.), the retention times of ANT, DAN, and IBU were 9.0, 7.5, and 6.3 minutes, respectively.

Table VIII. Weight uniformity of polymeric formulations.

| | Weight[a], mg | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | F |
| 1 | 163.8 | 170.6 | 140.6 | 154.1 | 122.5 | 144.1 |
| 2 | 165.6 | 177.6 | 168.5 | 157.7 | 154.5 | 165.3 |
| 3 | 162.2 | 255.4 | 186.4 | 131.2 | 193.5 | 163.1 |
| 4 | 163.1 | 197.8 | 231.9 | 160.4 | 147.4 | 151.7 |
| 5 | 163.2 | 156.2 | 173.8 | 161.0 | 147.8 | 168.3 |
| Mean | 163.6 | 191.5 | 180.2 | 152.9 | 153.1 | 158.5 |
| S.D. | 1.3 | 38.7 | 33.4 | 12.4 | 25.6 | 10.2 |
| C.V.% | 0.8 | 20.2 | 18.5 | 8.1 | 16.8 | 6.4 |

a. Volume of tested formula was 0.143 $cm^3$.

Table IX. Analysis of variance for weight uniformity.

Analysis of variance

| Source | DF | Sum of squares | Mean square |
|---|---|---|---|
| Model | 5 | 6255.92566667 | 1251.185133333 |
| Error | 24 | 14128.06800000 | 588.669500000 |
| Corrected total | 29 | 20383.99366667 | |

| ANOVA SS | F value | PR > F |
|---|---|---|
| 6255.92566667 | 2.13 | 0.0970 |

Figure 5. UV spectra

a. Anthralin

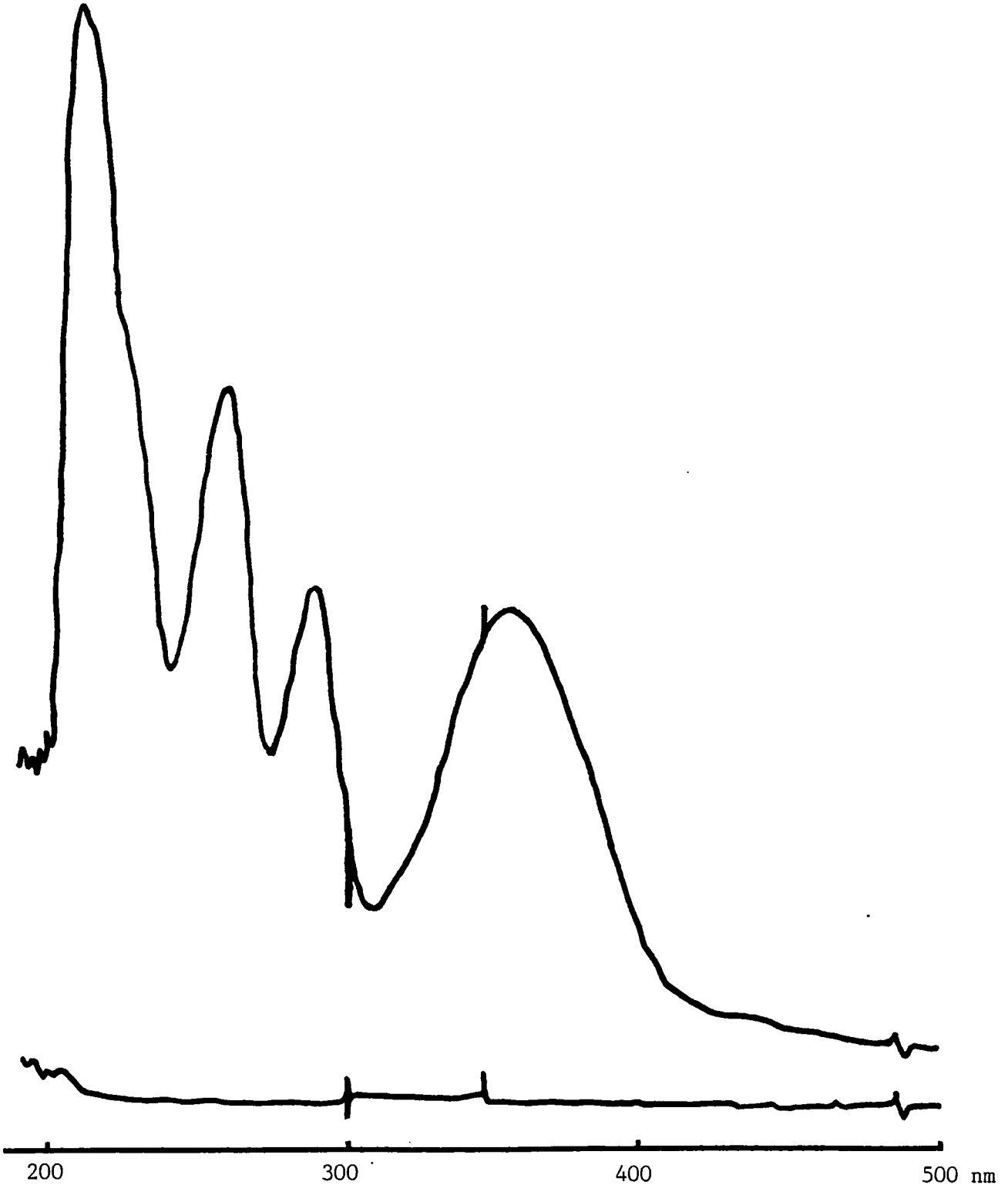


Figure 5. UV spectra

b. Danthron

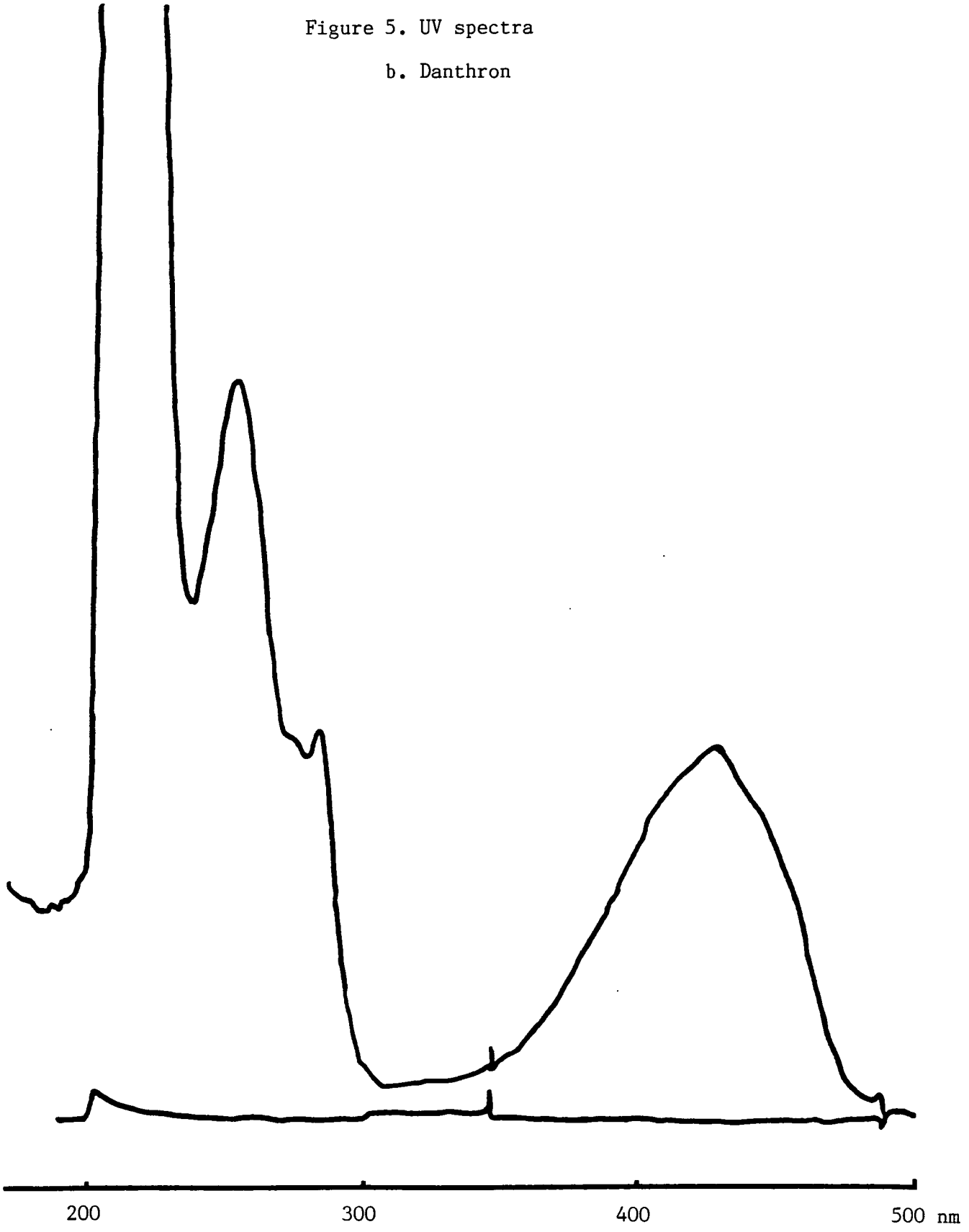
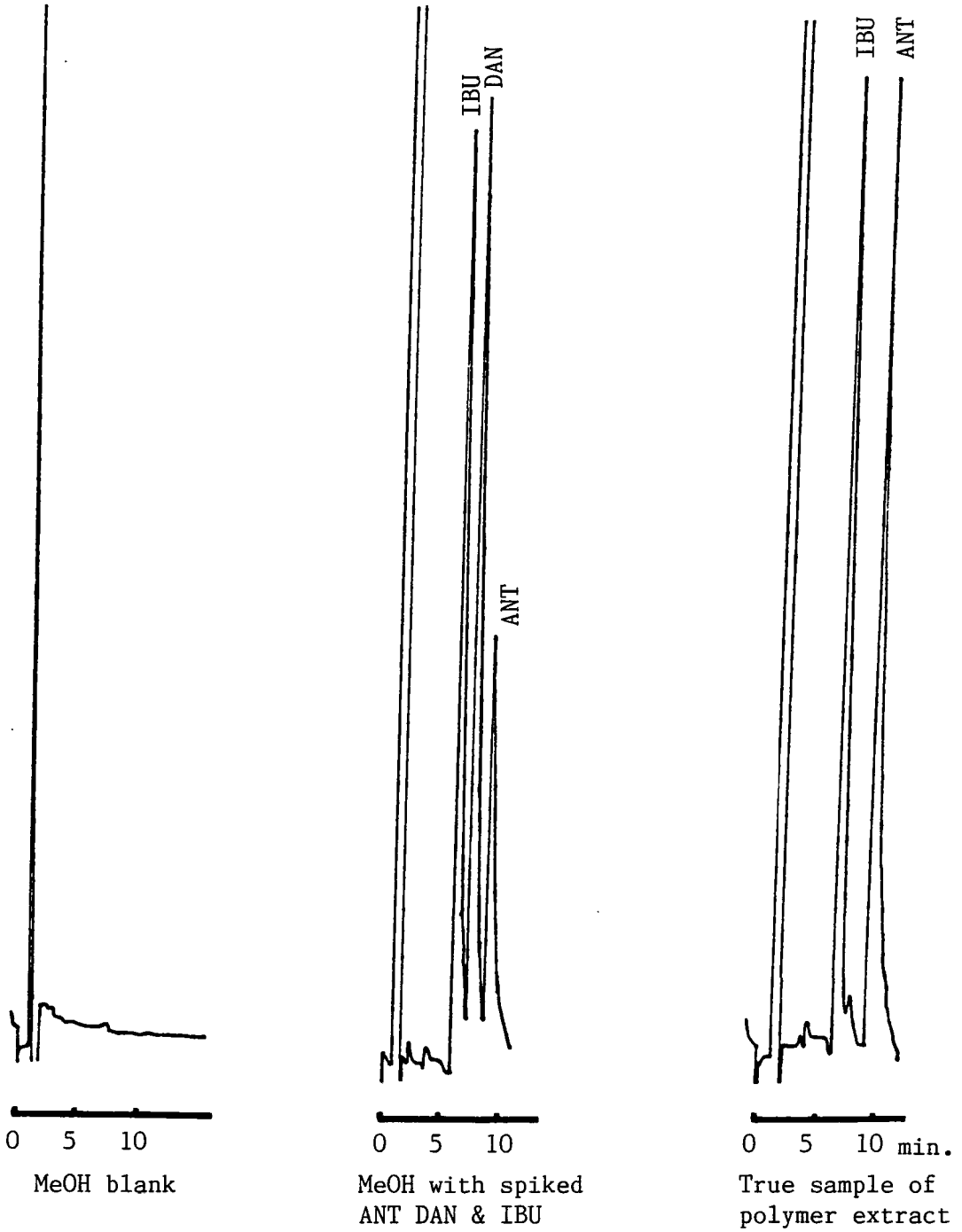


Figure 6. Authentic chromatograms

a. For content uniformity study




MeOH blank

MeOH with spiked ANT DAN & IBU

True sample of polymer extract

Figure 6. Authentic chromatograms

b. For drug release study

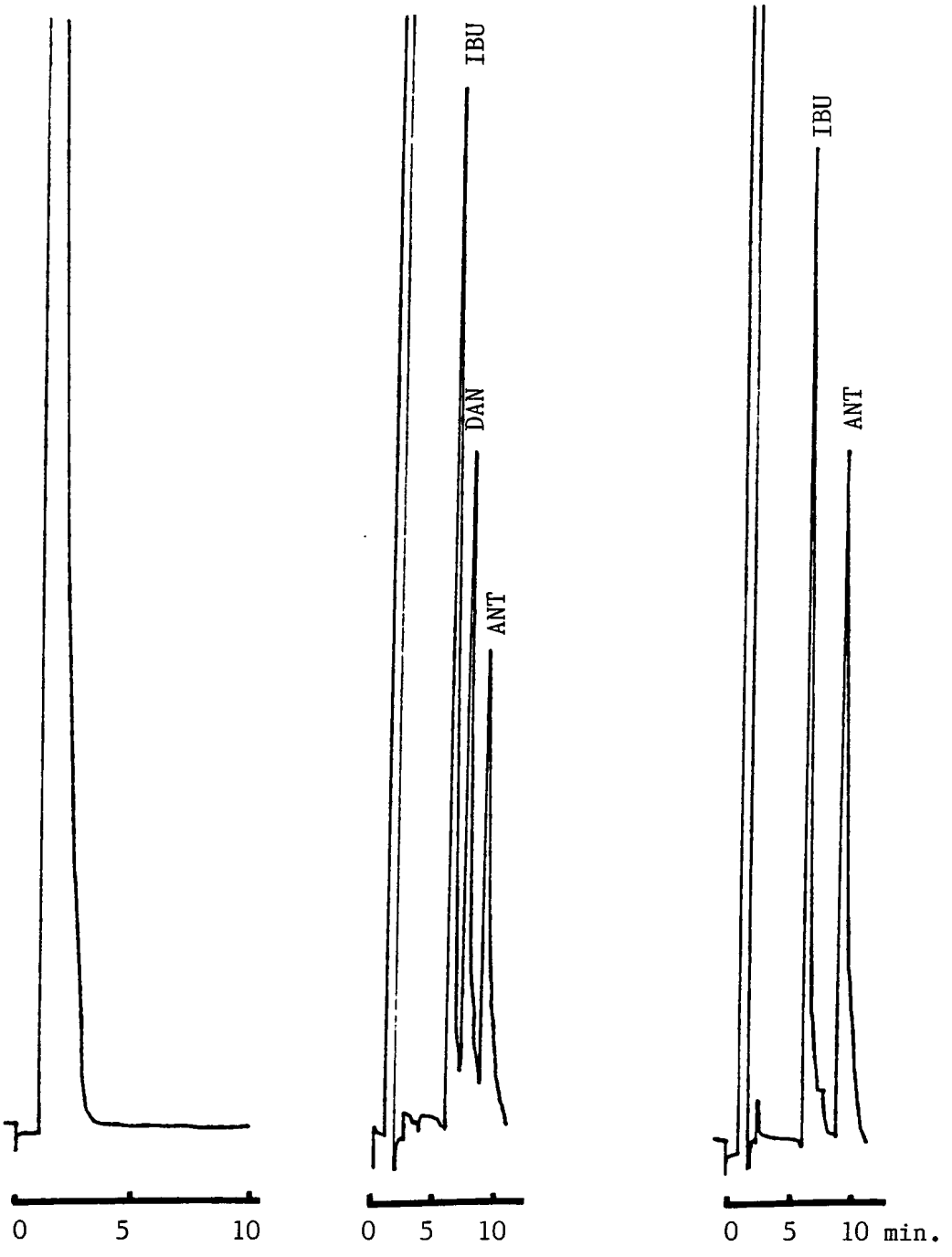
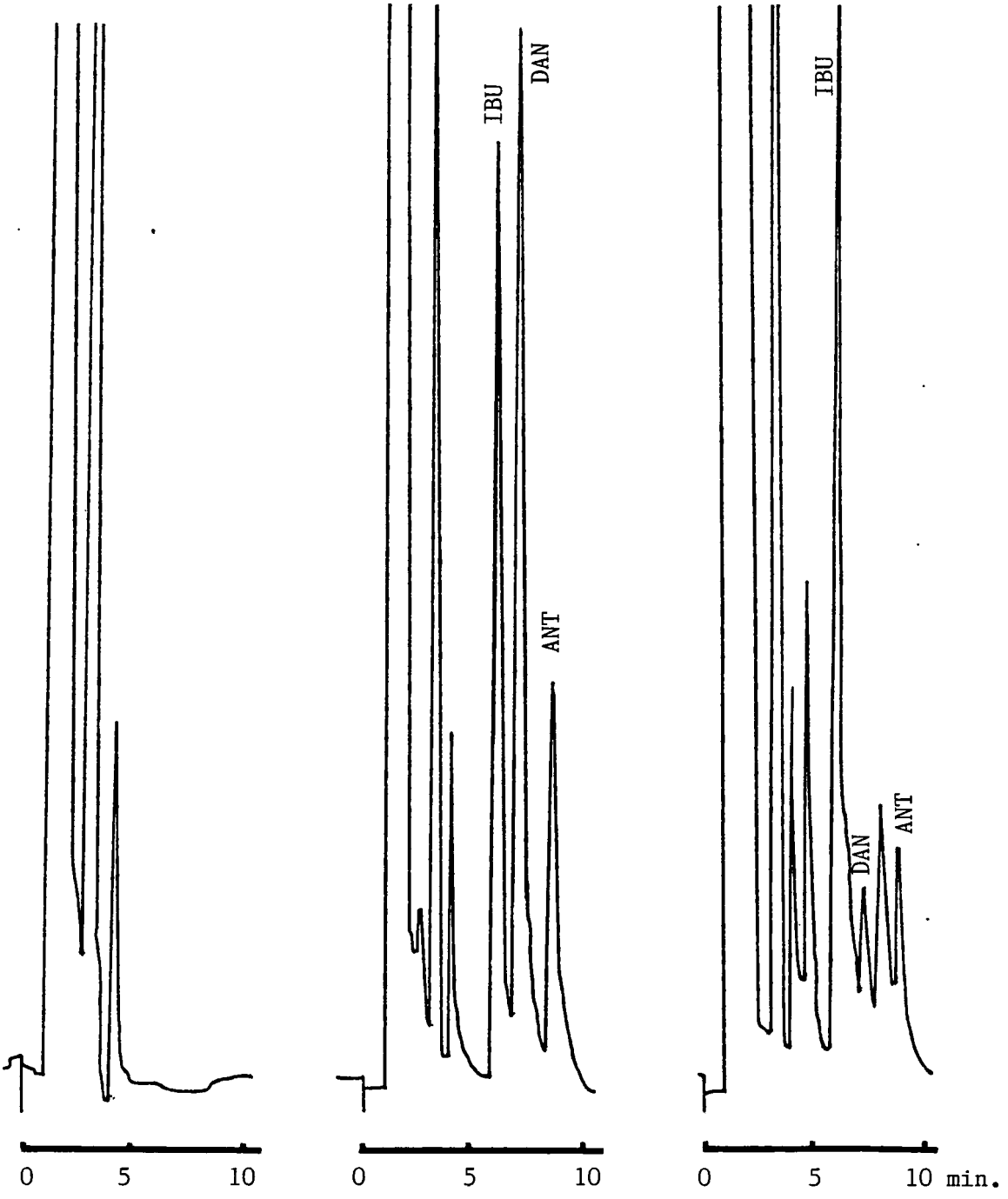


Figure 6. Authentic chromatograms

c. For percutaneous penetration study



D. Validation of the assay

The concentration range of the calibration curves of both ANT and DAN was 0.5-50 ug/ml. Typical calibration curves for sample of content uniformity study, drug release study, and skin extract sample of percutaneous penetration study were shown in Fig. 7a to 7c. The square of correlation coefficients of the calibration curves were all larger than 0.999.

The within-day variation of calibration curves of ANT and DAN were all less than 5% (Table X). As concerning between-day variation, the calibration curves of both compounds for content uniformity and drug release study had coefficients of variation of 4-7%. However, the calibration curves of both compounds for skin extract had larger variations, 11.7-22.7%; this might be due to the greater variability in biological samples.

E. Content uniformity of polymeric formulations

Batch B, C, and D contained more than 90% of claimed ANT, other batches contained 75-82% of the claimed amount (Table XI). Analysis of variance (Table XII) showed that the mean percentages of claimed ANT contained in the six batches were not all the same, and they were ranked

Figure 7. Typical calibration curves of anthralin and danthron

a. For content uniformity study

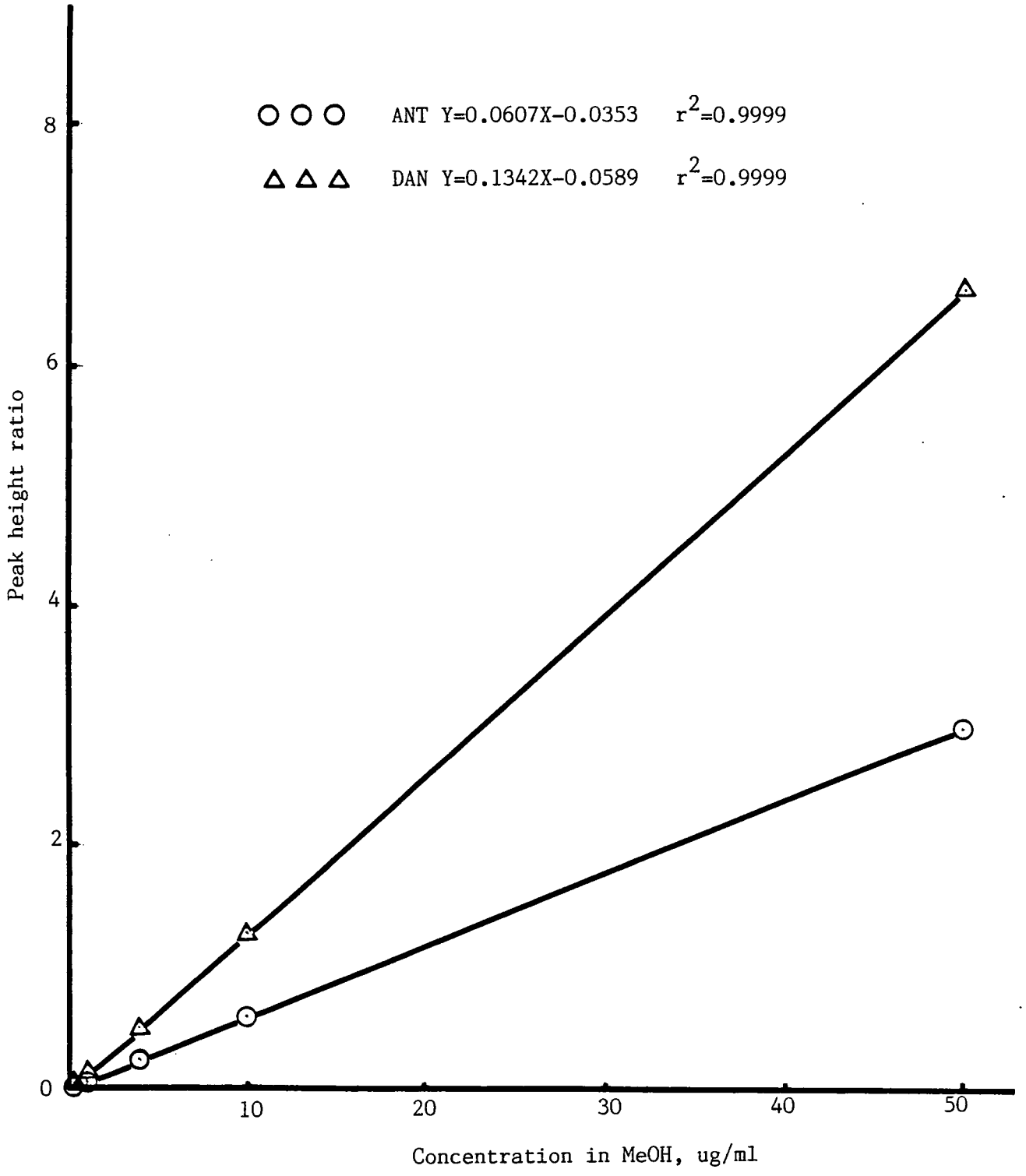


Figure 7. Typical calibration curves of anthralin and danthron

b. For drug release study (ANT, DAN with IM)

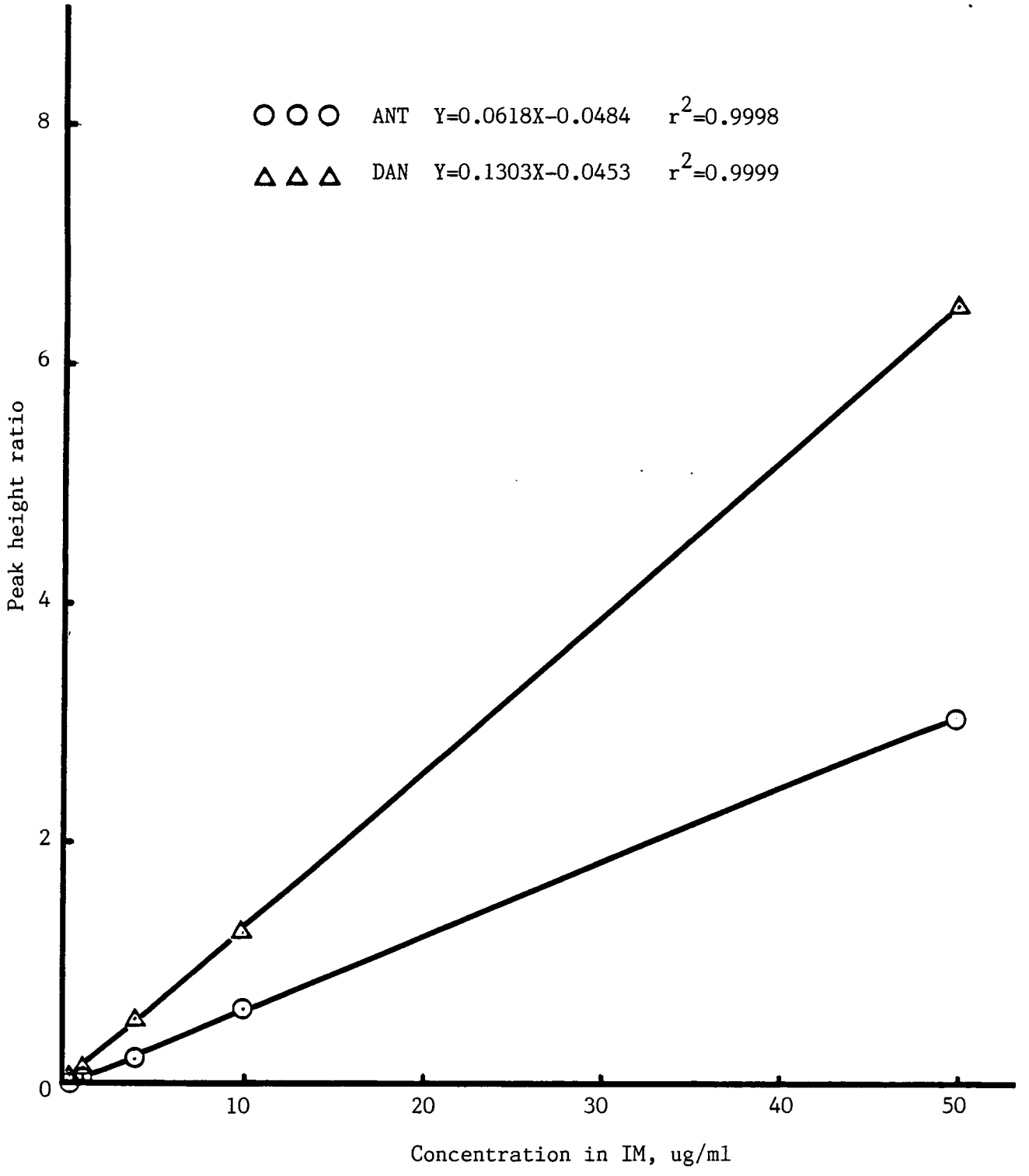


Figure 7. Typical calibration curves of anthralin and danthron

c. For percutaneous penetration study

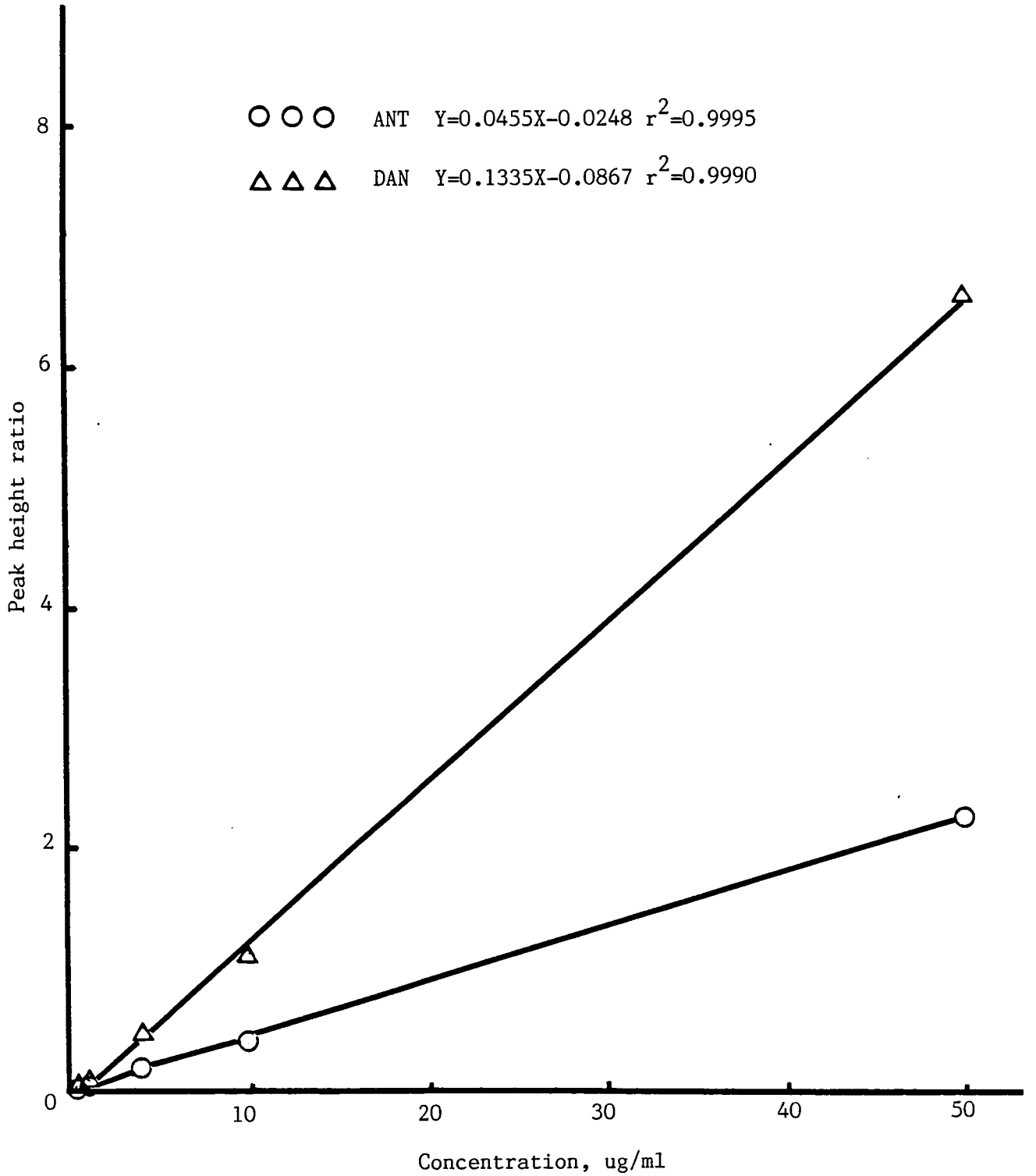


Table X. Within-day and between-day variations of calibration curves.

| | Within-day variation | | Between-day variation | |
|---|---|---|---|---|
| | ANT | DAN | ANT | DAN |
| [a] For content uniformity | | | | |
| N | 4 | 4 | 4 | 4 |
| Mean slope | 0.0621 | 0.1398 | 0.0665 | 0.1474 |
| S.D. | 0.0030 | 0.0040 | 0.0050 | 0.0087 |
| C.V.% | 4.8 | 2.9 | 7.5 | 5.9 |
| [b] For drug release study | | | | |
| N | 5 | 5 | 7 | 7 |
| Mean slope | 0.0635 | 0.1339 | 0.0639 | 0.1370 |
| S.D. | 0.0014 | 0.0062 | 0.0027 | 0.0077 |
| C.V.% | 2.2 | 4.7 | 4.2 | 5.6 |
| [c] For skin extract | | | | |
| N | 4 | 4 | 6 | 6 |
| Mean slope | 0.0490 | 0.1298 | 0.0514 | 0.1262 |
| S.D. | 0.0023 | 0.0026 | 0.0117 | 0.0148 |
| C.V.% | 4.7 | 2.0 | 22.7 | 11.7 |

a. Methanol was used as solvent.

b. ANT and DAN were spiked in methanol with 50 ul IM.

c. Methanol and chloroform mixture (1:1) was used as solvent.

Table XI. Content uniformity of polymeric formulations.

| | content, % claimed anthralin | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | F |
| 1 | 70.09 | 96.13 | 102.70 | 88.35 | 75.55 | 77.98 |
| 2 | 87.95 | 89.92 | 100.54 | 88.49 | 83.61 | 80.56 |
| 3 | 69.34 | 86.75 | 97.05 | 90.40 | 80.21 | 83.89 |
| 4 | 79.01 | 90.32 | 91.77 | 92.60 | 84.59 | 89.25 |
| 5 | 69.48 | 87.34 | 97.12 | 90.35 | 58.67 | 80.42 |
| Mean | 75.17 | 90.09 | 97.84 | 90.04 | 76.53 | 82.42 |
| S.D. | 8.2 | 3.7 | 4.2 | 1.7 | 10.6 | 4.36 |
| C.V.% | 10.9 | 4.1 | 4.3 | 1.9 | 13.84 | 5.3 |

Table XII. Analysis of variance for content uniformity.

| Analysis of variance | | | |
|---|---|---|---|
| Source | DF | Sum of squares | Mean square |
| Model | 5 | 1951.81461667 | 390.36292333 |
| Error | 24 | 931.01692000 | 38.79237167 |
| Corrected total | 29 | 2882.83153667 | |

| ANOVA SS | F value | PR > F |
|---|---|---|
| 1951.81461667 | 10.06 | 0.0001 |

Table XIII. Student-Newman-Keuls' test for content uniformity.

$\alpha$=0.05 DF=24 MSE=38.7924

| SNK Grouping * | Mean | N | Batch |
|---|---|---|---|
| A | 97.836 | 5 | C |
| B A | 90.092 | 5 | B |
| B A | 90.038 | 5 | D |
| B C | 82.420 | 5 | F |
| C | 76.526 | 5 | E |
| C | 75.174 | 5 | A |

* Means with the same letter are not significantly different.

into three classes when Student-Newman-Keuls' test was used for further comparison (Table XIII). Because each batch of formula carried a different percentage of claimed content, the actual amount of ANT was used in all calculations for the drug release and percutaneous penetration studies.

## F. Stability study

### 1. Anthralin stability

Four sets of solutions, described in section III-B-7 (page 35), were tested in the study. The degradation profile of ANT was constructed by plotting amount of remaining ANT versus time (Fig. 8a to 8d). The straight line observed suggested that the degradation of ANT was an apparent first-order process.

Table XIV summerized apparent first-order degradation rate constants of ANT in conventional vehicle with four different conditions and in polymeric formulations. When ANT was spiked in the absence of Vit. C without AZ, the degradation rate constants were 0.52 $day^{-1}$ for low concentration solutions (4 and 10 ug/ml), and 0.17 $day^{-1}$ for high concentration solutions (30 and 50 ug/ml). With AZ, the rate constants became 0.74 and 0.33 $day^{-1}$ for low and high concentrations, respectively.

Figure 8. Degradation profiles of anthralin in MeOH at 4°C.

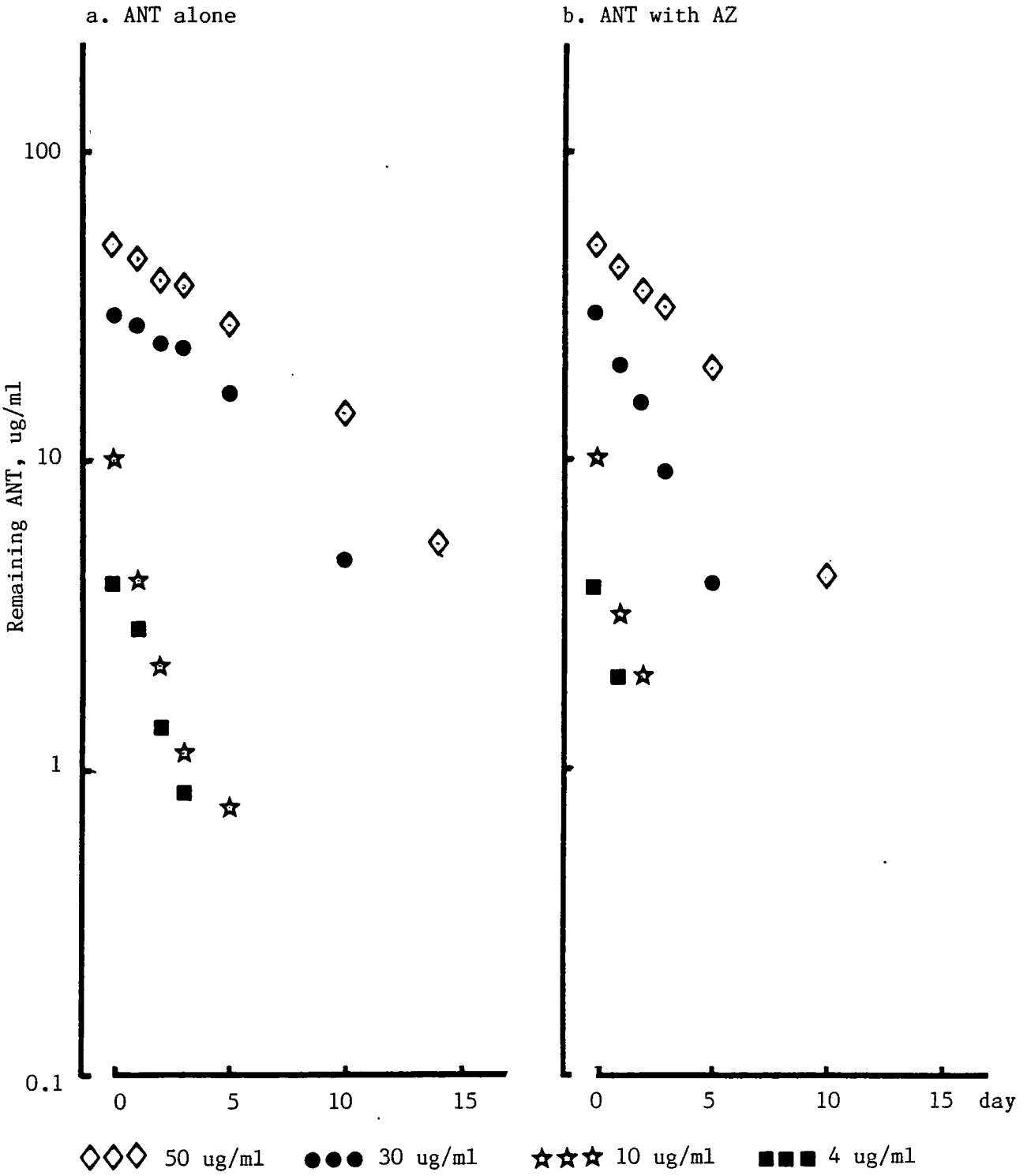


Figure 8. Degradation profiles of anthralin in MeOH at 4°C.

c. ANT with Vit. C

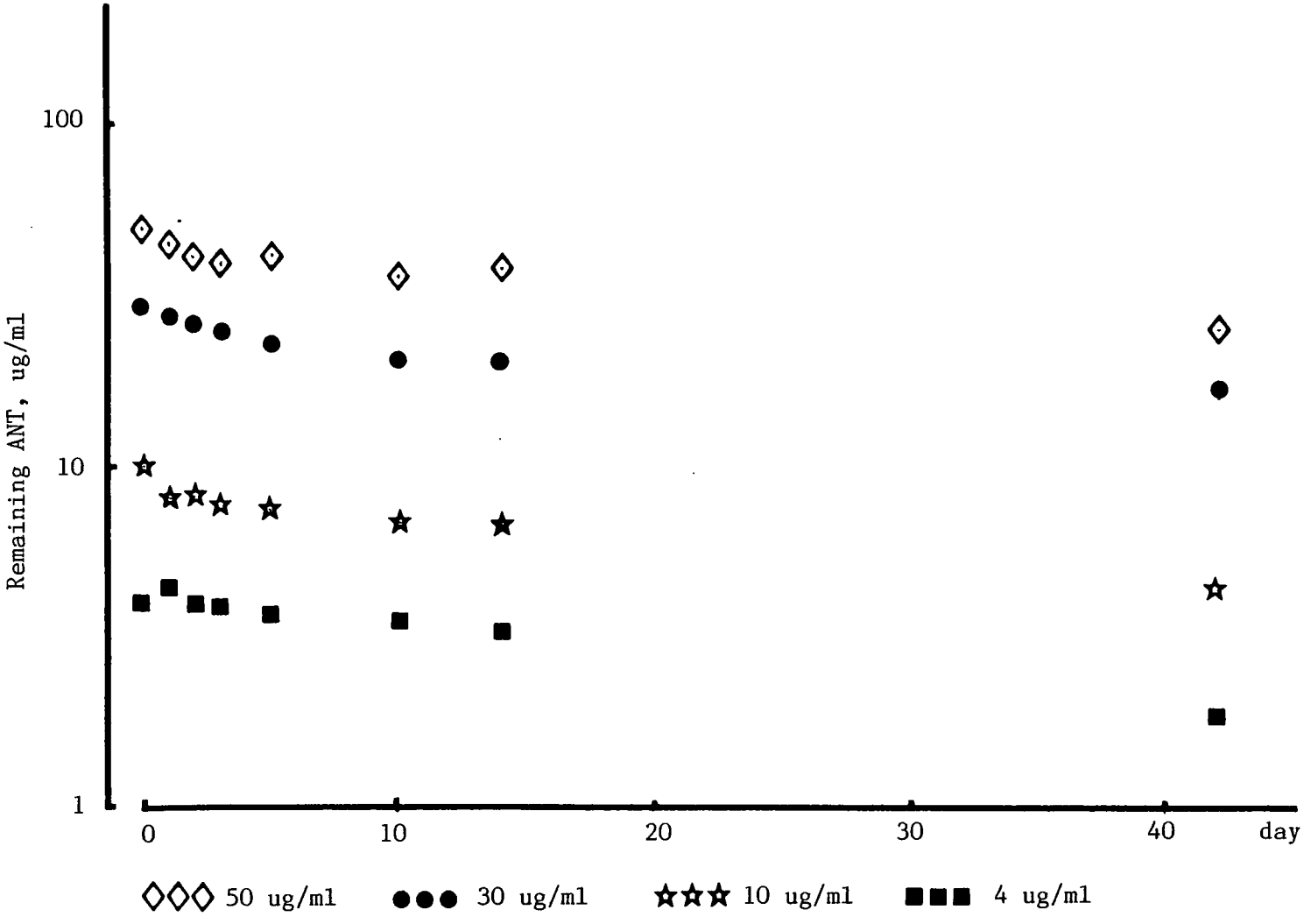


Figure 8. Degradation profiles of anthralin in MeOH at 4°C.

d. ANT with 2% AZ and Vit. C.

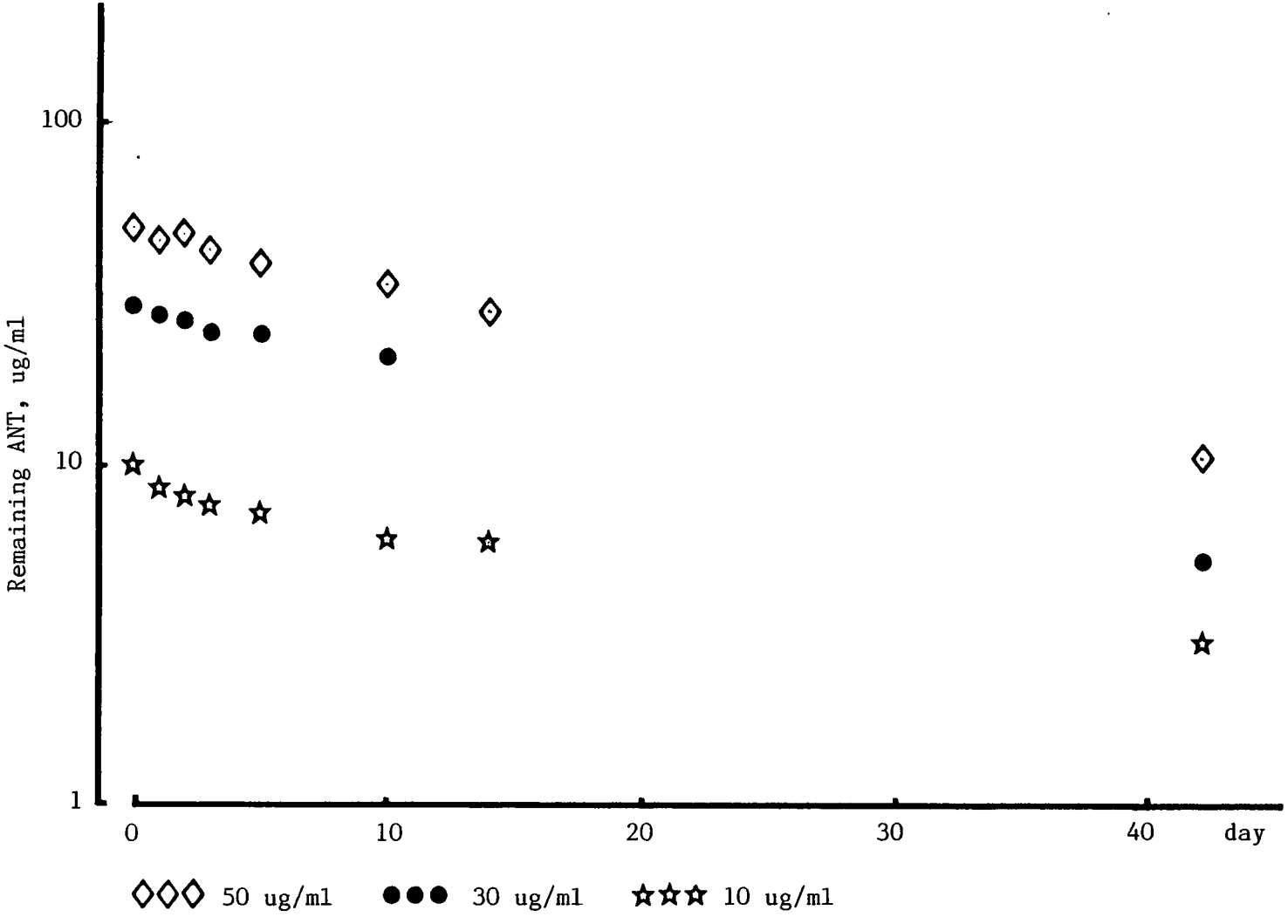


Table XIV. Apparent first order degradation rate constant of anthralin in methanol with various additives, stored at 4°C.

| | K, day$^{-1}$ | | | | | | |
|---|---|---|---|---|---|---|---|
| | ANT alone | | | | ANT with Vit.C | | |
| conc. | low conc. | | high conc. | | | | a |
| ug/ml | no AZ | 2% AZ | no AZ | 2% AZ | no AZ | 2% AZ | polymer |
| 4 | 0.527 | 0.685 | | | 0.0189 | | A b |
| 10 | 0.510 | 0.798 | | | 0.0165 | 0.0255 | B 0.001324 |
| 30 | | | 0.184 | 0.405 | 0.0117 | 0.0403 | C 0.002005 |
| 50 | | | 0.156 | 0.248 | 0.0142 | 0.0361 | D 0.001248 |
| | | | | | | | E 0.000567 |
| Mean | 0.5185 | 0.7415 | 0.1700 | 0.3265 | 0.0153 | 0.0340 | 0.001286 |
| S.D. | 0.0120 | 0.0799 | 0.0198 | 0.1110 | 0.0031 | 0.0076 | 0.000588 |
| C.V.% | 2.3 | 10.8 | 11.6 | 34.0 | 20.2 | 22.4 | 45.7 |
| Students' t-test | | NS[c] | | NS[c] | | S[c] | |
| | | | S[d] | | S[e] | | |
| | S[f] | S[f] | S[f] | S[f] | | | |

a. Concentration of anthralin in polymer was 5% W/W.

b. Negligible.

c. Compared with corresponding case without Azone.

d. Compared with corresponding case with low concentration.

e. Compared with the case of polymer.

f. Compared with corresponding case with Vitamin C.

When ANT was spiked with Vit. C, the degradation rate constants were 0.034 and 0.015 $day^{-1}$ for samples with and without AZ, respectively. When ANT was incorporated in polymeric formulations, the degradation rate constant was extremely small, ranging 0.6-2.0 x $10^{-3}$ $day^{-1}$.

ANT degradation, in the absence of Vit.C., either with or without AZ, was slower in higher concentration (30 and 50 ug/ml) than in lower concentration solutions (10 and 4 ug/ml) as demonstrated in Fig. 8a and 8b. The Students'-t-test showed that the apparent first order degradation rate constants of ANT in high and low concentration solutions were significantly different when ANT was spiked alone without AZ. The concentration-dependent degradation rate constants strongly suggested the ANT degradation was not a simple first order process. Another determinent might exist.

When Vit. C was incorporated in ANT solutions (Fig. 8c & 8d), the degradation rate constants of ANT, with or without the presence of AZ, were significantly smaller than the corresponding case when Vit. C was not incorporated. It clearly demonstrated that ANT was stabilized by Vit. C. The presence of AZ in ANT solution with Vit. C significantly increased the degradation rate constant of ANT. That suggested the possible acceleration of AZ to ANT decomposition.

When ANT was incorporated in polymeric formulations, the degradation rate constant was even much smaller than those when ANT was spiked with Vit. C in conventional vehicle. The fabrication of

polymeric ANT dosage form proved to significantly improve the stability of ANT (Table XIV).

When ANT was spiked alone in methanol, the degradation profile showed exponential curves when constructed by plotting amount of remaining ANT versus time on quadratic paper. When degradation profile was plotted on semi-log paper, straight lines were observed as shown in Fig. 8a. Since slopes of straight lines representing different concentrations are significantly different, the decomposition of ANT seemed to be a concentration-dependent, pseudo first-order process. This implied that another substance was involved in the decomposition of ANT. Because oxidation was the major route of ANT decomposition, very possibly oxygen played an important role in ANT oxidation. Therefore, the maximum amount of oxygen available in 1 ml of MeOH at 1 atm, $4^{o}C$ was calculated.

According to Henrys' law, $p = Hx$, where x is mole fraction of the solute in the liquid phase; p is partial pressure of the solute in the gas phase, expressed in atmospheres; H is a proportionality constant and in unit of atmospheres of solute pressure in the gas phase per unit concentration of the solute in the liquid phase. According to "International Critical Tables" (Vol. 3, P. 257. Pray, Scheveickert, and Minnich), H is $2.55 \times 10^{4}$ at $0^{o}C$, $2.91 \times 10^{4}$ at $5^{o}C$. By interpolation, H will be $2.838 \times 10^{4}$ at $4^{o}C$. The partial pressure of oxygen in air is close to 0.2 atm., thus the maximum mole fraction of oxygen in MeOH is $7.047 \times 10^{-6}$. Density of methanol at $4^{o}C$ is 0.8102 g/ml, and molar volume of methanol is 39.50 ml. Therefore, the maximum

amount of oxygen available in one ml of MeOH is $1.784 \times 10^{-7}$ mole.

Meanwhile, the ANT solutions at a concentration of 50 ug/ml contains $2.210 \times 10^{-7}$ mole, 30 ug/ml contains $1.326 \times 10^{-7}$ mole, 10 ug/ml contains $4.420 \times 10^{-8}$ mole, 4 ug/ml contains $1.768 \times 10^{-8}$ mole of ANT.

Since the molarity number of oxygen was between that of high and low concentrations of ANT solutions, it will make ANT degrade faster at a relatively lower concentration and slower at a higher concentration if oxygen reacted with ANT in an equal molar base. That is, degradation rate = K [ANT] [$O_2$]. When ANT concentration was 10 or 4 ug/ml, oxygen concentration was much higher than that of ANT, ANT degradation became a pseudo first-order process.

When Vit. C was incorporated, most oxygen was consumed by reacting with Vit. C. Therefore, the rate of ANT oxidation became much slower, and four different concentrations had very similar degradation rate constants (Table XIV).

The incorporation of AZ accelerated ANT decomposition since the degradation rate constant of ANT in the presence of AZ and Vit. C was significantly larger than that without AZ when Vit. C was incorporated. When Vit. C was not incorporated, the accelerating effect of AZ on ANT oxidation was not obvious, but the possibility could not be excluded. The accelerating effect of AZ on ANT oxidation might be due to the surface activity of AZ which would decrease the surface tension between

methanol and oxygen, thus increased solubility of oxygen in methanol and resulted in faster rate of ANT oxidation.

ANT is subject to autoxidation reactions in the presence of light, moisture, and atmospheric oxygen, especially in alkaline conditions. However, when ANT was incorporated in polymeric formulations, moisture and atmospheric oxygen diffused into polymer was drastically minimized, and light was also prevented if stored under an opaque cover. Therefore, the degradation rate constant of ANT was negligibly small. Both types of silicone elastomer equally prevented ANT from oxidation.

2. Danthron stability

The decomposition of DAN was negligible in all cases when DAN was spiked alone, with Vit. C, with 2% AZ, or with both Vit. C and AZ (Fig. 9a-9d). When first-order degradation process was assumed, the rate constants ranged from 0 to 8 x $10^{-3}$ $day^{-1}$ for all cases.

G. In vitro drug release study

The drug release data of each batch of polymer was compiled in Tables XVa to XVe. The mean values of drug release data of all batches were summarized in Table XVI. Characteristic drug release profile of each batch was constructed by plotting mean amount of ANT released

Figure 9. Degradation profiles of danthron in MeOH at 4°C.

a. DAN alone.

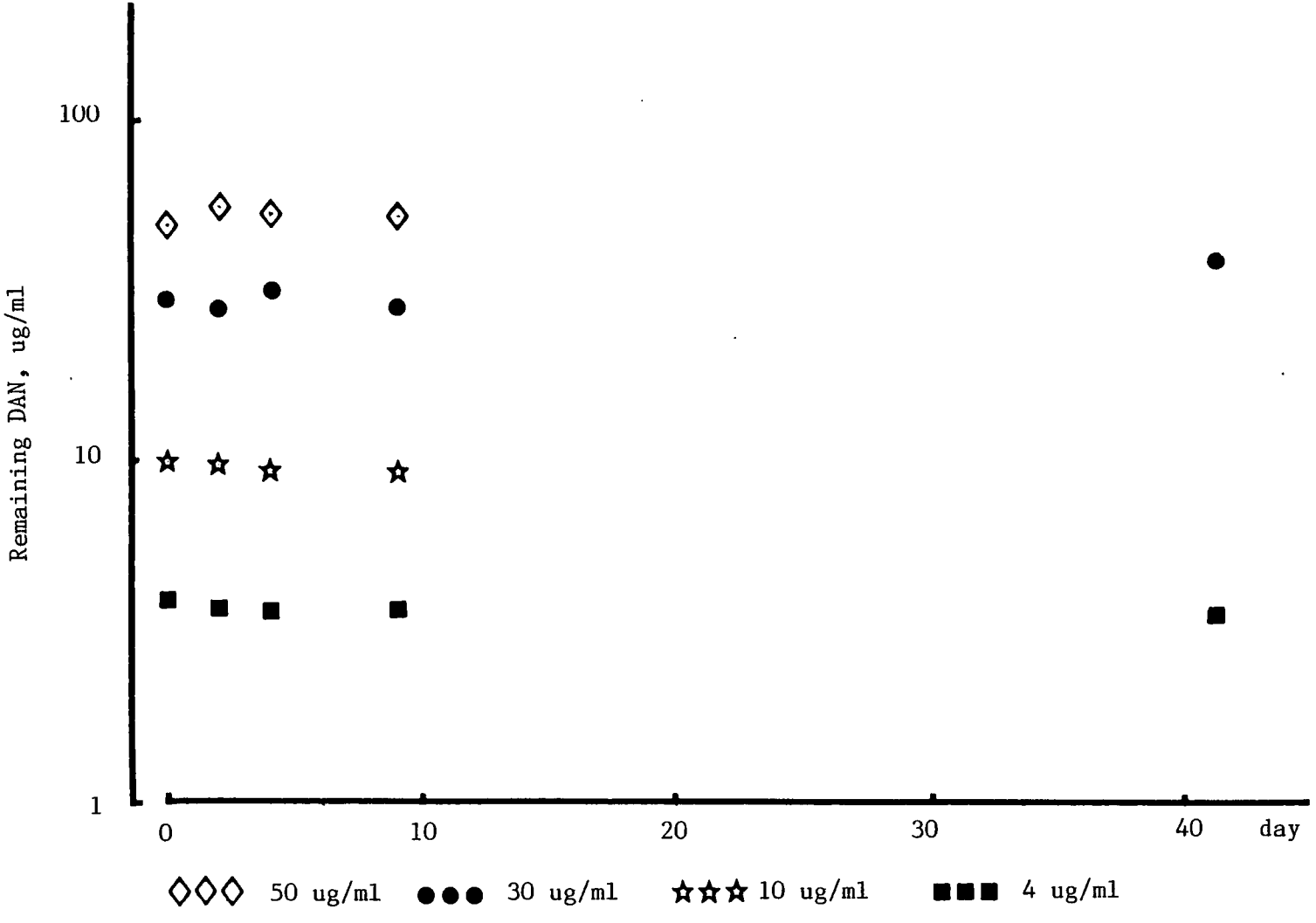


Figure 9. Degradation profiles of danthron in MeOH at 4°C.

b. DAN with 2% AZ.

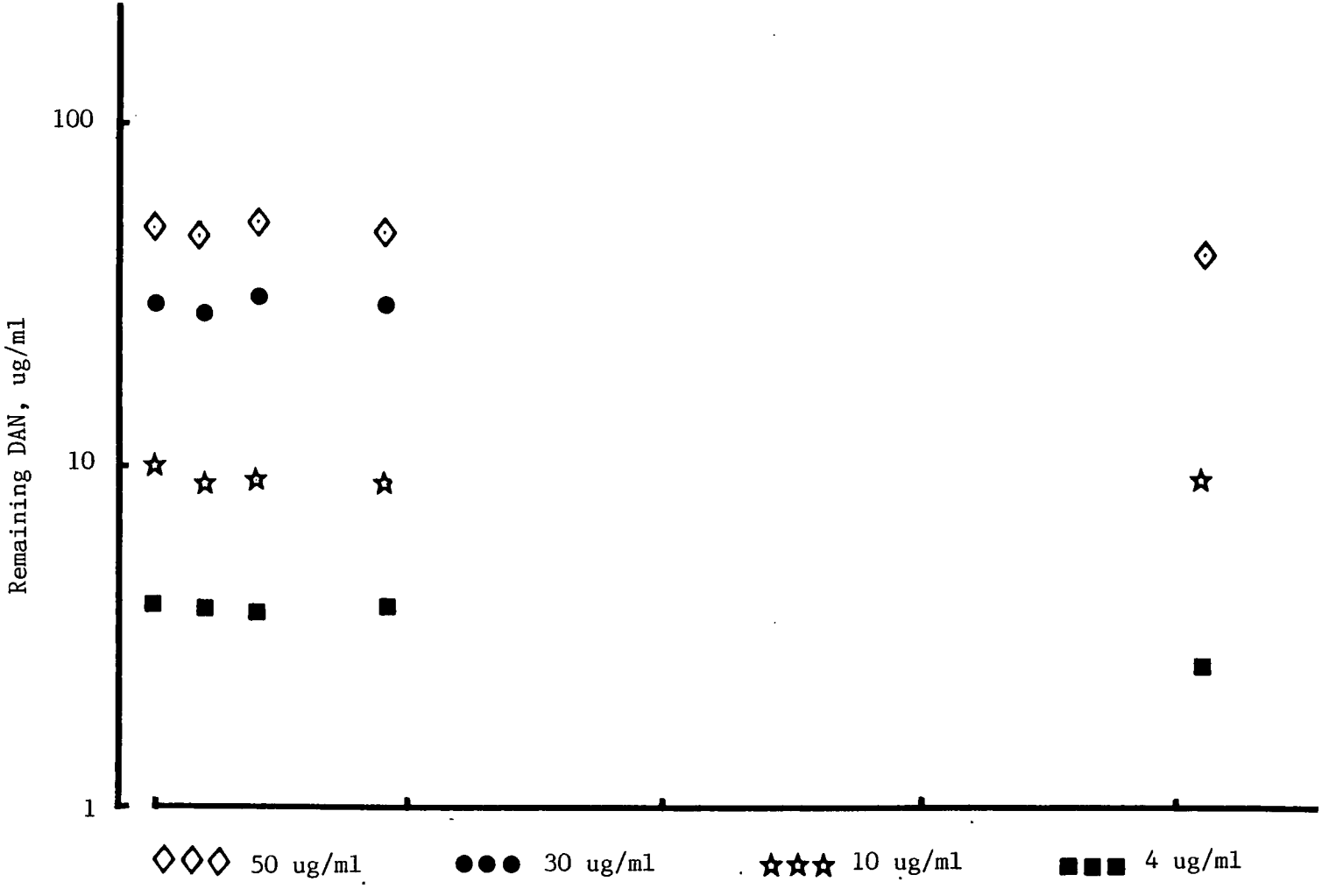


Figure 9. Degradation profiles of danthron in MeOH at 4°C.

c. DAN with Vit. C.

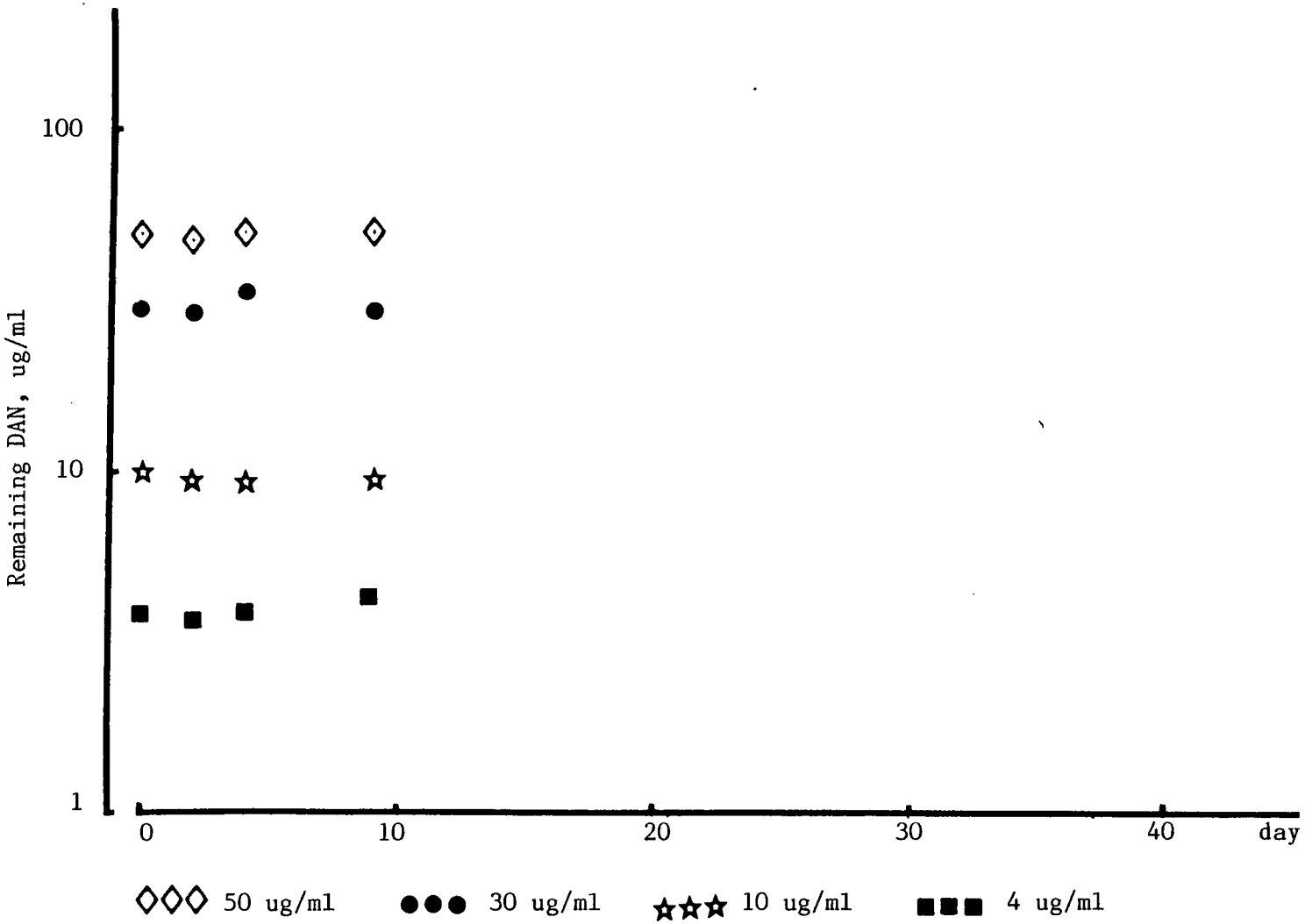


Figure 9. Degradation profiles of danthron in MeOH at 4°C.

d. DAN with 2% AZ and Vit. C.

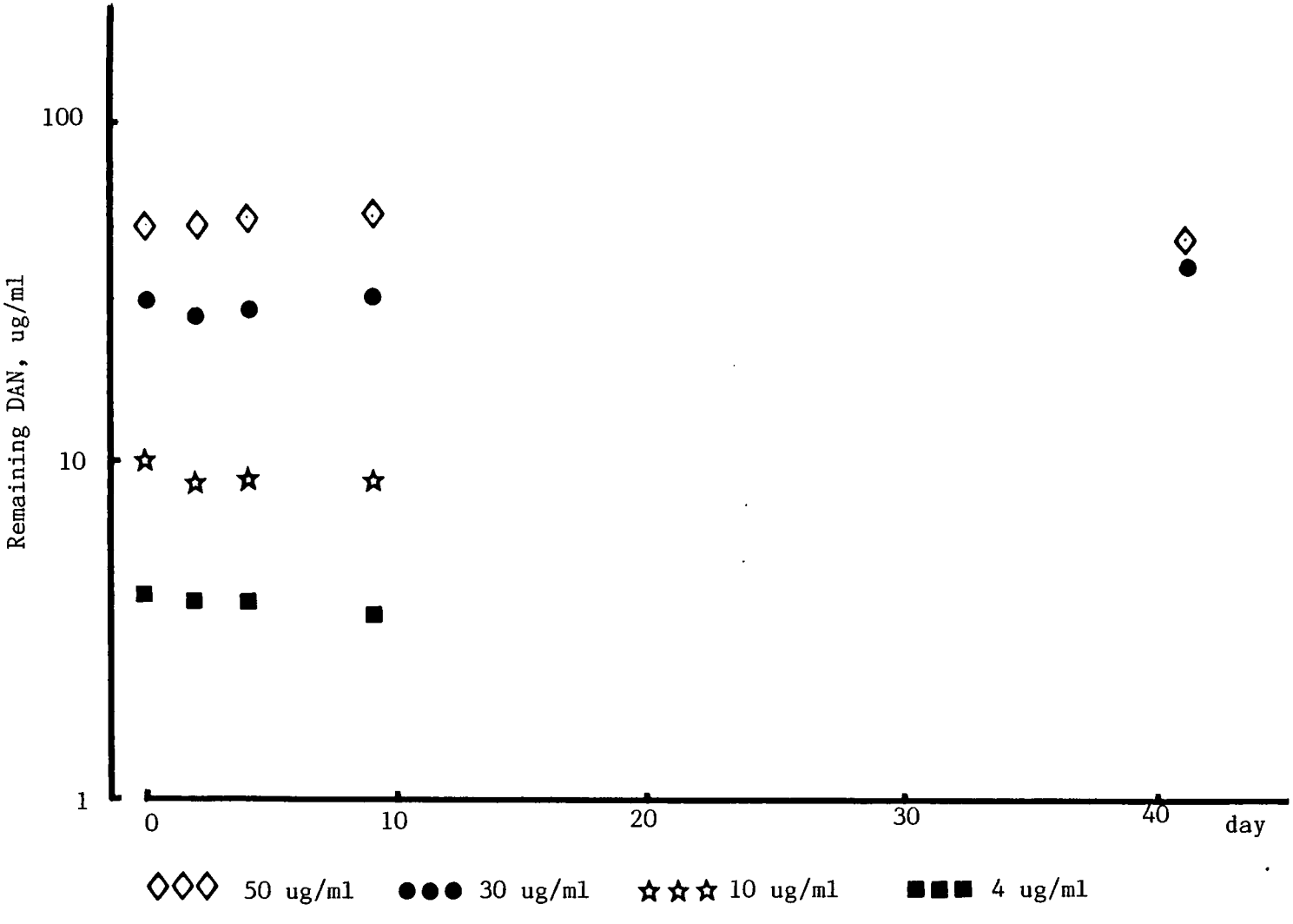


Table XV. Drug release data of polymeric formulations.

a. Batch A

| | Amount released[a], mg | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| hr | 1 | 2 | 3 | 4 | 5 | Mean | S.D. | C.V.% |
| 0.25 | | 3.347 | 2.202 | 1.648 | 2.017 | 2.304 | 0.733 | 31.8 |
| 0.50 | 1.843 | 3.298 | 2.440 | 2.744 | 2.882 | 2.647 | 0.543 | 20.5 |
| 0.75 | | 3.664 | 3.554 | 3.386 | 3.732 | 3.584 | 0.151 | 4.2 |
| 1.00 | 2.374 | 3.867 | 3.990 | 4.492 | 4.332 | 3.811 | 0.842 | 22.1 |
| 1.25 | | 4.654 | 4.644 | 4.731 | 5.031 | 4.765 | 0.182 | 3.8 |
| 1.50 | | 5.130 | 4.293 | 5.340 | 5.221 | 4.996 | 0.476 | 9.5 |
| 1.75 | | 5.664 | 5.216 | 5.617 | 6.023 | 5.682 | 0.417 | 7.3 |
| 2.00 | 3.387 | 5.797 | 5.034 | 6.001 | 6.798 | 5.403 | 1.290 | 23.9 |
| 3.00 | 4.860 | 7.263 | 6.622 | 8.268 | 8.475 | 7.098 | 1.460 | 20.6 |
| 4.00 | 6.102 | 8.484 | 7.587 | 9.249 | 9.443 | 8.173 | 1.369 | 16.7 |
| 5.00 | 7.385 | 9.260 | 8.599 | 10.172 | 9.882 | 9.060 | 1.115 | 12.3 |
| 6.00 | 11.502 | 10.478 | 9.627 | 12.440 | 10.898 | 10.989 | 1.060 | 9.6 |
| 7.00 | 15.370 | 9.951 | 9.832 | 12.166 | 12.917 | 12.045 | 2.300 | 19.1 |
| 8.00 | 14.018 | 11.013 | 10.712 | 12.541 | 13.067 | 12.270 | 1.394 | 11.4 |
| 23.00 | | 12.824 | 11.636 | 13.593 | 15.135 | 13.297 | 1.466 | 11.0 |
| 24.00 | | 13.760 | 11.153 | 13.996 | 15.020 | 13.482 | 1.646 | 12.2 |
| 25.00 | 16.844 | 13.685 | 12.190 | 14.110 | 15.024 | 14.371 | 1.720 | 12.0 |
| 30.25 | | 12.648 | 11.524 | 14.744 | 15.063 | 13.495 | 1.695 | 12.6 |

a. Values normalized by 15mg ANT as initial amount.

Table XV. Drug release data of polymeric formulations.

b. Batch B&F

| | Amount released[a], mg | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| hr | 1 | 2 | 3 | 4 | 5 | 6 | Mean | S.D. | C.V.% |
| 0.25 | | 1.339 | 1.303 | 1.439 | 1.205 | 1.085 | 1.274 | 0.135 | 10.6 |
| 0.50 | 1.367 | 2.135 | 1.895 | 1.901 | 1.540 | 1.540 | 1.730 | 0.291 | 16.8 |
| 0.75 | | 2.506 | 2.116 | 2.436 | 1.656 | 1.823 | 2.107 | 0.371 | 17.6 |
| 1.00 | 2.630 | 2.603 | 2.398 | 2.699 | 1.878 | 2.003 | 2.369 | 0.349 | 14.7 |
| 1.25 | | 3.293 | 2.668 | 2.763 | 2.211 | 2.082 | 2.603 | 0.482 | 18.5 |
| 1.50 | | 3.158 | 2.432 | 2.932 | 2.463 | 2.723 | 2.742 | 0.310 | 11.3 |
| 1.75 | | 3.745 | 3.021 | 2.985 | 1.967 | 2.995 | 2.943 | 0.634 | 21.5 |
| 2.00 | | 3.488 | 3.446 | 3.629 | 2.116 | 3.212 | 3.178 | 0.612 | 19.3 |
| 3.00 | 4.516 | 3.552 | 4.033 | 4.375 | 2.383 | 4.237 | 3.849 | 0.793 | 20.6 |
| 4.00 | 4.974 | 6.572 | 4.180 | 4.993 | 1.843 | 4.888 | 4.575 | 1.552 | 33.9 |
| 5.00 | 5.434 | 6.868 | 4.925 | 4.603 | 2.051 | 5.503 | 4.897 | 1.595 | 32.6 |
| 6.00 | 5.663 | 7.280 | 4.739 | 5.991 | 2.819 | 6.080 | 5.429 | 1.517 | 27.9 |
| 7.00 | 6.379 | 7.407 | 6.375 | | 6.362 | 6.733 | 6.651 | 0.451 | 6.8 |
| 8.00 | | 8.383 | 7.063 | 5.813 | 7.139 | 7.265 | 7.133 | 0.912 | 12.8 |
| 23.00 | 9.511 | 11.857 | 11.418 | 11.362 | 12.032 | 12.527 | 11.451 | 1.042 | 9.1 |
| 24.00 | 8.427 | 10.946 | 12.262 | 11.032 | 11.580 | 13.086 | 11.222 | 1.588 | 14.2 |
| 25.00 | 8.154 | 12.597 | 13.814 | 10.362 | 11.782 | 13.277 | 11.664 | 2.104 | 18.0 |
| 30.25 | 8.500 | 12.543 | 13.583 | 10.957 | 12.198 | 13.888 | 11.945 | 1.986 | 16.6 |

a. Values normalized by 15mg ANT as initial amount.

Table XV. Drug release data of polymeric formulations.

c. Batch C

| | Amount released[a], mg | | | | | | |
|---|---|---|---|---|---|---|---|
| hr | 1 | 2 | 3 | 4 | Mean | S.D. | C.V.% |
| 0.25 | 1.654 | 0.935 | 2.214 | 0.967 | 1.443 | 0.612 | 42.4 |
| 0.50 | 2.102 | 1.288 | 2.924 | 1.366 | 1.920 | 0.763 | 39.8 |
| 0.75 | 2.218 | 1.579 | 3.830 | 1.535 | 2.291 | 1.073 | 46.8 |
| 1.00 | 2.363 | 1.990 | | 1.982 | 2.117 | 0.218 | 10.3 |
| 1.25 | 2.541 | 2.083 | 3.941 | 2.180 | 2.686 | 0.859 | 32.0 |
| 1.50 | 2.665 | 2.168 | 4.363 | 2.855 | 3.013 | 0.946 | 31.4 |
| 1.75 | 2.777 | 2.536 | 4.998 | 3.079 | 3.348 | 1.123 | 33.5 |
| 2.00 | 3.012 | 2.849 | | 3.425 | 3.095 | 0.297 | 9.6 |
| 3.00 | 4.139 | 3.936 | 7.474 | 3.985 | 4.884 | 1.729 | 35.4 |
| 4.00 | 4.268 | 4.407 | 7.887 | 4.989 | 5.388 | 1.695 | 29.5 |
| 5.00 | 4.742 | 5.426 | 7.857 | 5.624 | 5.912 | 1.350 | 22.8 |
| 6.00 | 5.347 | 6.021 | 9.423 | 6.721 | 6.878 | 1.787 | 26.0 |
| 7.00 | 5.751 | 6.461 | 10.967 | 6.449 | 7.398 | 2.405 | 32.5 |
| 8.00 | 6.154 | 6.783 | 11.374 | 7.728 | 8.010 | 2.334 | 29.1 |
| 23.00 | 10.399 | 10.275 | 16.000 | 12.174 | 12.212 | 2.670 | 21.9 |
| 24.00 | 10.298 | 10.325 | 16.000 | 12.077 | 12.175 | 2.682 | 22.0 |
| 25.00 | 10.071 | 10.233 | 16.101 | 12.151 | 12.139 | 2.805 | 23.1 |
| 30.25 | 10.160 | 10.155 | 16.870 | 12.567 | 12.438 | 3.165 | 25.5 |

a. Values normalized by 15mg ANT as initial amount.

Table XV. Drug release data of polymeric formulations.

d. Batch D

| | Amount released[a], mg | | | | | | |
|---|---|---|---|---|---|---|---|
| hr | 1 | 2 | 3 | 4 | Mean | S.D. | C.V.% |
| 0.25 | 1.847 | 1.411 | 1.346 | 1.625 | 1.557 | 0.227 | 14.6 |
| 0.50 | 2.365 | 1.906 | 1.751 | 2.097 | 2.030 | 0.265 | 13.0 |
| 0.75 | 2.966 | 2.350 | 2.036 | 2.291 | 2.411 | 0.394 | 16.4 |
| 1.00 | 3.257 | 2.475 | 2.063 | 2.390 | 2.546 | 0.506 | 19.9 |
| 1.25 | 3.357 | 2.638 | 2.630 | 2.741 | 2.842 | 0.347 | 12.2 |
| 1.50 | 3.532 | 2.747 | 2.895 | 2.975 | 3.037 | 0.343 | 11.3 |
| 1.75 | 3.831 | 2.843 | 3.330 | 3.285 | 3.322 | 0.404 | 12.2 |
| 2.00 | 4.195 | 4.805 | 3.302 | 3.409 | 3.928 | 0.707 | 18.0 |
| 3.00 | 4.884 | 4.246 | 3.853 | 4.254 | 4.309 | 0.426 | 9.9 |
| 4.00 | 5.473 | 4.706 | 4.939 | 5.853 | 5.243 | 0.518 | 9.9 |
| 5.00 | 6.214 | 5.069 | 4.539 | 5.787 | 5.392 | 0.751 | 13.9 |
| 6.00 | 6.360 | 5.576 | 5.803 | 6.241 | 5.995 | 0.368 | 6.1 |
| 7.00 | 6.719 | 6.022 | 5.962 | 6.756 | 6.365 | 0.431 | 6.8 |
| 8.00 | 7.216 | 6.246 | 6.990 | 6.981 | 6.858 | 0.422 | 6.2 |
| 23.00 | 12.057 | 10.832 | 10.342 | 11.394 | 11.156 | 0.738 | 6.6 |
| 24.00 | 11.738 | 11.020 | 10.688 | 11.580 | 11.257 | 0.488 | 4.3 |
| 25.00 | 11.544 | 10.988 | 10.464 | 11.117 | 11.028 | 0.445 | 4.0 |
| 30.25 | 12.193 | 10.948 | 11.258 | 12.214 | 11.653 | 0.648 | 5.6 |

a. Values normalized by 15mg ANT as initial amount.

Table XV. Drug release data of polymeric formulations.

e. Batch E

| | Amount released[a], mg | | | | | | |
|---|---|---|---|---|---|---|---|
| hr | 1 | 2 | 3 | 4 | Mean | S.D. | C.V.% |
| 0.25 | 1.995 | 0.938 | 1.276 | 1.045 | 1.314 | 0.476 | 36.2 |
| 0.50 | 2.511 | 1.287 | 1.573 | 1.389 | 1.690 | 0.560 | 33.1 |
| 0.75 | 2.765 | 1.592 | 1.807 | 1.719 | 1.971 | 0.537 | 27.2 |
| 1.00 | 2.979 | 1.599 | 2.059 | 1.992 | 2.157 | 0.584 | 27.1 |
| 1.25 | 3.254 | 1.886 | 2.244 | 2.239 | 2.406 | 0.590 | 24.5 |
| 1.50 | 3.468 | 2.012 | 2.520 | 2.383 | 2.596 | 0.620 | 23.9 |
| 1.75 | 3.381 | 2.158 | 2.435 | 2.585 | 2.640 | 0.525 | 19.9 |
| 2.00 | 3.535 | 2.458 | 2.537 | 2.922 | 2.863 | 0.492 | 17.2 |
| 3.00 | 4.385 | 2.825 | 3.514 | 3.318 | 3.511 | 0.651 | 18.5 |
| 4.00 | 4.669 | | | 4.419 | 4.544 | 0.177 | 3.9 |
| 5.00 | 5.139 | 3.690 | 4.423 | 4.578 | 4.458 | 0.597 | 13.4 |
| 6.00 | 5.216 | 4.278 | 4.999 | 5.663 | 5.038 | 0.578 | 11.5 |
| 7.00 | 5.966 | 4.663 | 5.057 | 5.973 | 5.415 | 0.660 | 12.2 |
| 8.00 | 6.178 | 4.865 | 5.582 | 6.007 | 5.658 | 0.585 | 10.3 |
| 23.00 | 11.332 | 10.291 | 11.029 | 10.215 | 10.717 | 0.550 | 5.1 |
| 24.00 | 12.370 | 10.537 | 11.997 | 11.147 | 11.513 | 0.828 | 7.2 |
| 25.00 | 11.128 | 10.127 | 10.191 | 11.524 | 10.743 | 0.693 | 6.5 |
| 30.25 | 12.006 | 9.218 | 9.604 | 12.407 | 10.809 | 1.630 | 15.1 |

a. Values normalized by 15mg ANT as initial amount.

Table XVI. Anthralin release from polymeric formulations as a function of time.

| | | Amount released[a], mg | | | | |
|---|---|---|---|---|---|---|
| hr | $hr^{1/2}$ | A[b] | B&F[c] | C[d] | D[d] | E[d] |
| 0.25 | 0.5000 | 2.304 | 1.274 | 1.443 | 1.557 | 1.314 |
| 0.50 | 0.7071 | 2.647 | 1.730 | 1.920 | 2.030 | 1.690 |
| 0.75 | 0.8660 | 3.584 | 2.107 | 2.291 | 2.411 | 1.971 |
| 1.00 | 1.0000 | 3.811 | 2.369 | 2.117 | 2.546 | 2.157 |
| 1.25 | 1.1180 | 4.765 | 2.603 | 2.686 | 2.842 | 2.406 |
| 1.50 | 1.2247 | 4.996 | 2.742 | 3.013 | 3.037 | 2.596 |
| 1.75 | 1.3229 | 5.682 | 2.943 | 3.348 | 3.322 | 2.640 |
| 2.00 | 1.4142 | 5.403 | 3.178 | 3.095 | 3.928 | 2.863 |
| 3.00 | 1.7321 | 7.098 | 3.849 | 4.884 | 4.309 | 3.511 |
| 4.00 | 2.0000 | 8.173 | 4.575 | 5.388 | 5.243 | 4.544 |
| 5.00 | 2.2360 | 9.060 | 4.897 | 5.912 | 5.392 | 4.458 |
| 6.00 | 2.4495 | 10.989 | 5.429 | 6.878 | 5.995 | 5.038 |
| 7.00 | 2.6458 | 12.045 | 6.651 | 7.398 | 6.365 | 5.415 |
| 8.00 | 2.8284 | 12.270 | 7.133 | 8.010 | 6.858 | 5.658 |
| 23.00 | 4.7958 | 13.297 | 11.451 | 12.212 | 11.156 | 10.717 |
| 24.00 | 4.8990 | 13.482 | 11.222 | 12.175 | 11.257 | 11.513 |
| 25.00 | 5.0000 | 14.371 | 11.664 | 12.139 | 11.028 | 10.743 |
| 30.25 | 5.5000 | 13.495 | 11.945 | 12.438 | 11.653 | 10.809 |

a. Average values normalized by 15 mg ANT as initial amount.

b. Average values of 5 individual run.

c. Average values of 6 individual run.

d. Average values of 4 individual run.

versus square root of time as shown in Fig. 10a to 10e. A straight line was obtained in the first eight hours for all the polymeric formulations. This suggested that drug release from the polymeric formulations was a first-order process, and the data fit well in the time-square root equation of Higuchi.

Diffusion coefficients of ANT in the tested formulations were calculated in Table XVII; they ranged from 7.2 to 15.3 x $10^{-8}$ $cm^2$/sec. The within-batch variation ranged 14 to 35%, except batch C, 54%. The greater variation in batch C might result from the uneven distribution of ANT in the polymer.

One way ANOVA was done for data analysis. A significant difference between batch A and D was observed. It suggested that the 382 silicone elastomer was better than MDX-4-4210 silicone elastomer since batch A, the 382 silicone elastomer, had a relatively larger ANT diffusion coefficient. The 382 silicone elastomer is less lipophilic, and hence the affinity between the polymer and highly lipophilic ANT was smaller than that of MDX-4-4210. This may be accounted for the more favored diffusion of ANT from polymer matrix into IM in the case of using 382 silicone elastomer.

No significant difference was observed between batch A and B, batch B and C, and batch D and E. It suggested that the incorporation of glycerol and AZ in batch B and C respectively did not significantly affect ANT diffusion in 382 silicone elastomer. The incorporation of PG in batch E did not improve ANT diffusion in MDX-4-4210 silicone

Figure 10. Anthralin release profiles of silicone elastomers.

a. Batch A.

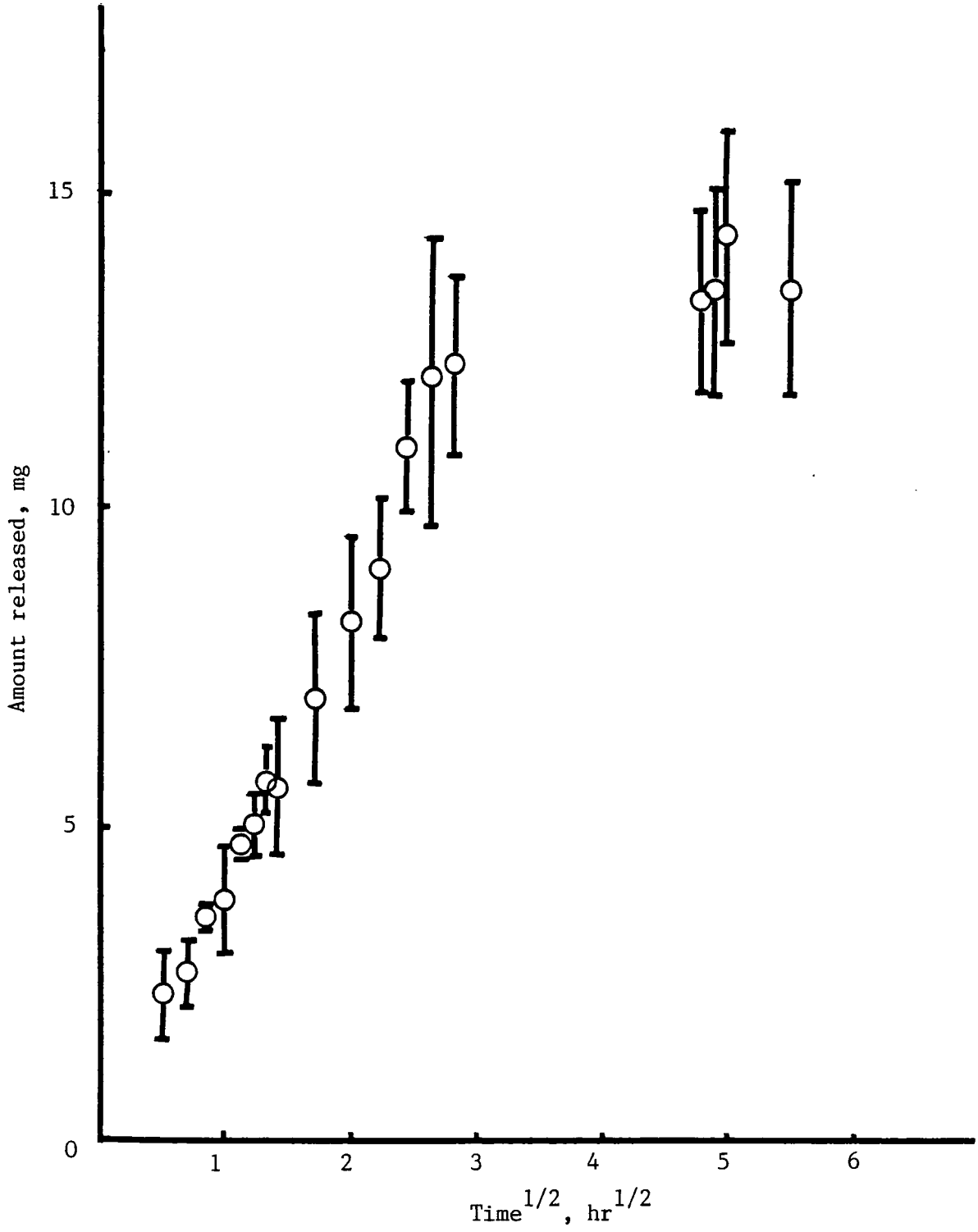


Figure 10. Anthralin release profiles of silicone elastomers.

b. Batch B & F.

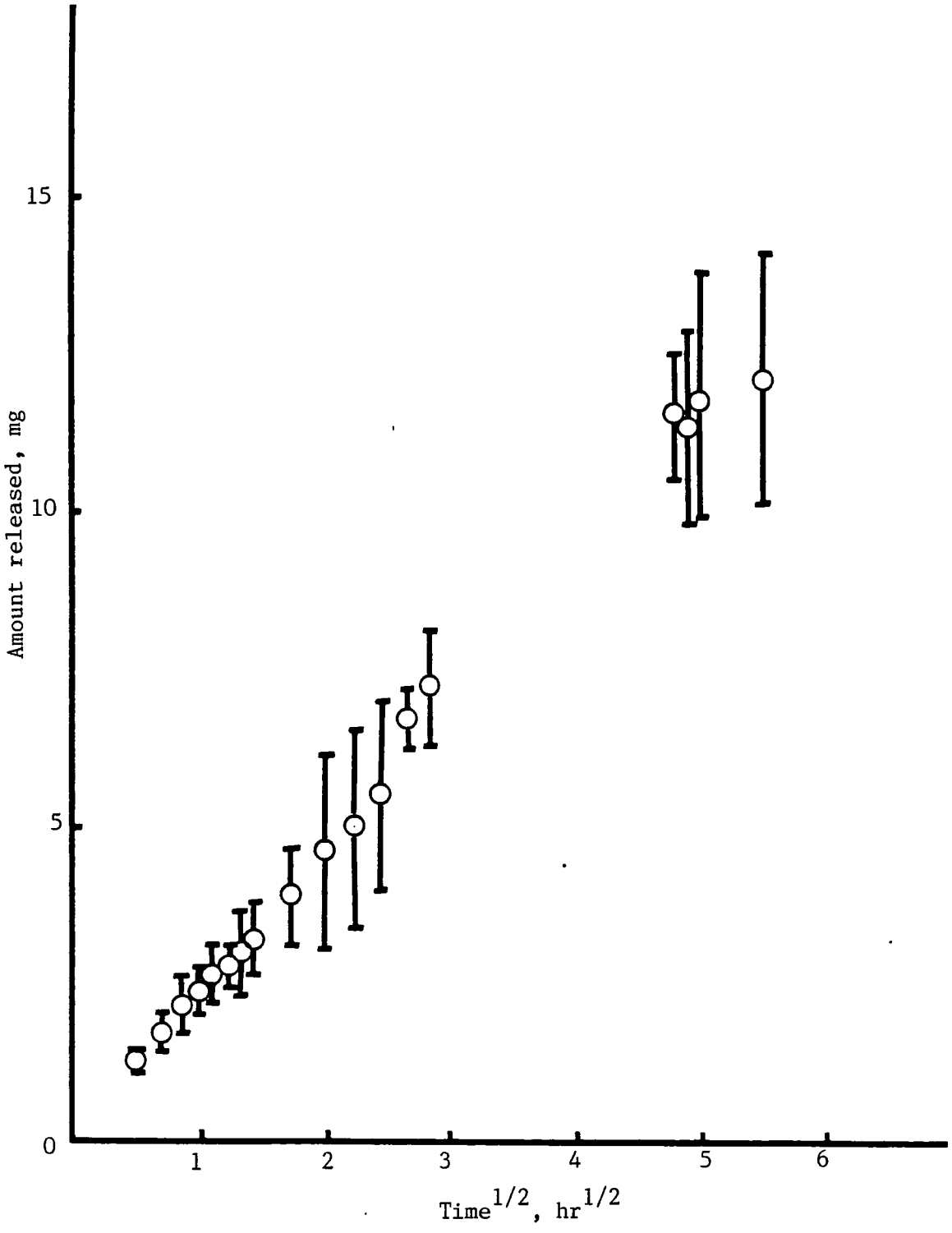


Figure 10. Anthralin release profiles of silicone elastomers.

c. Batch C.

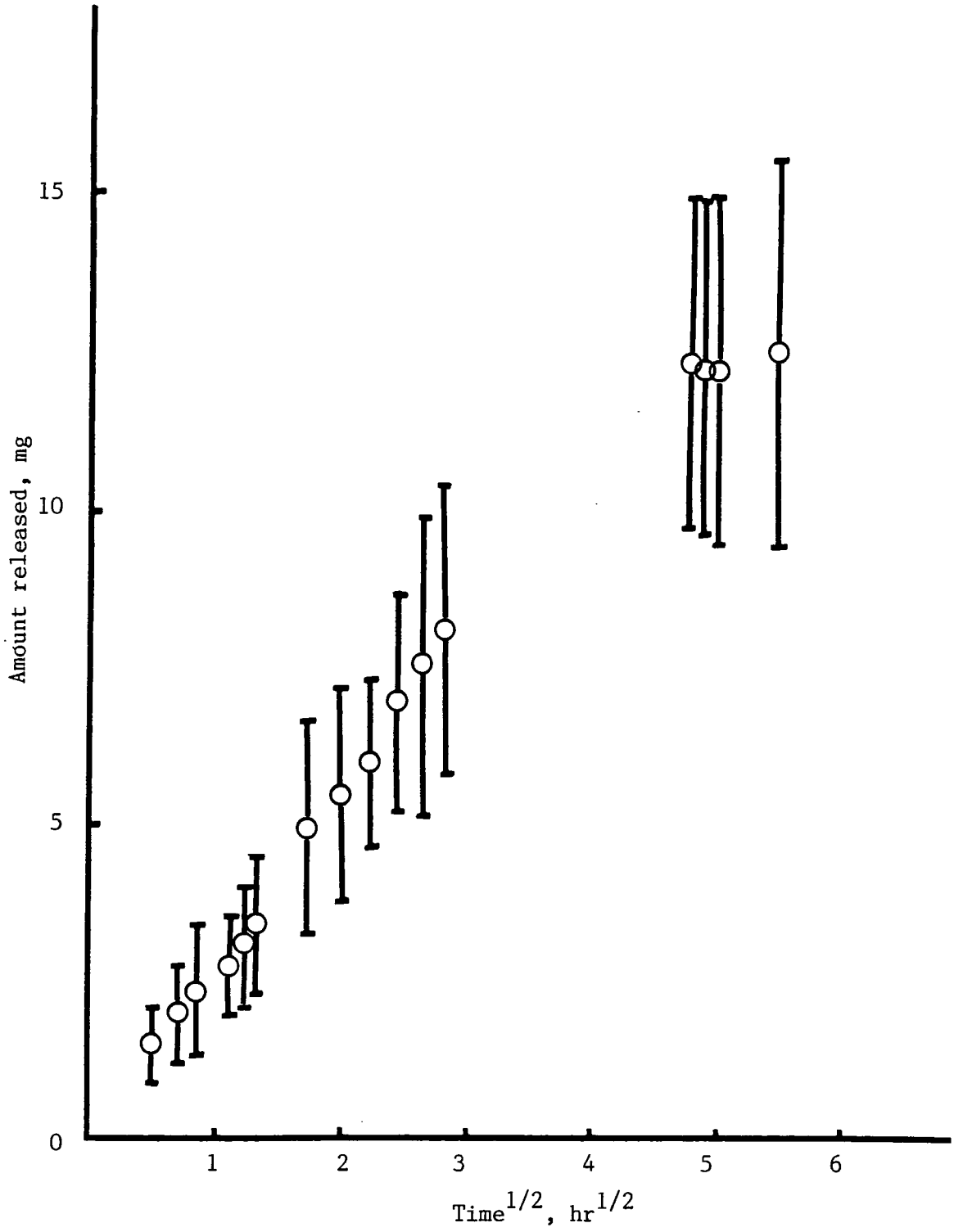


Figure 10. Anthralin release profiles of silicone elastomers.

d. Batch D.

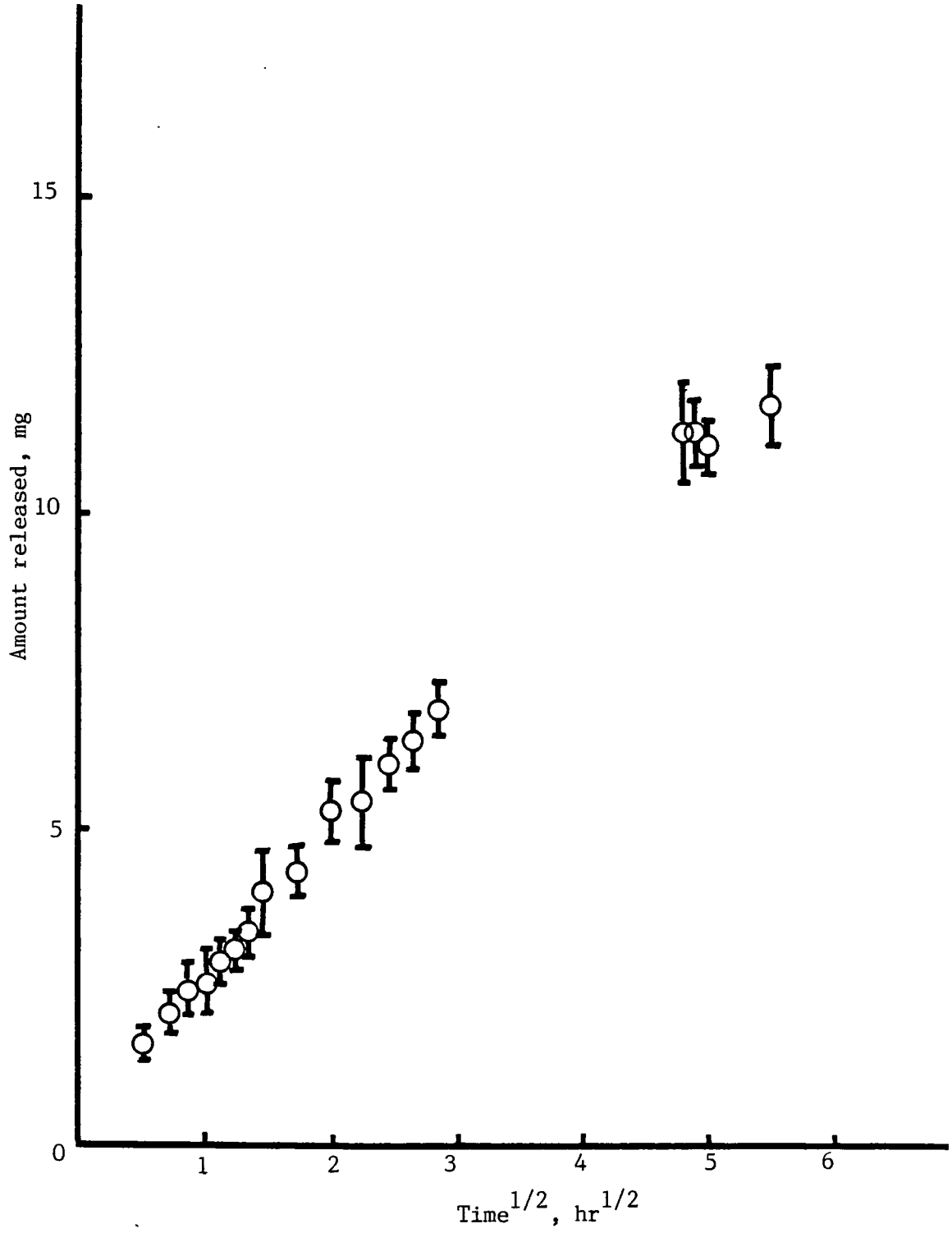


Figure 10. Anthralin release profiles of silicone elastomers.

e. Batch E.

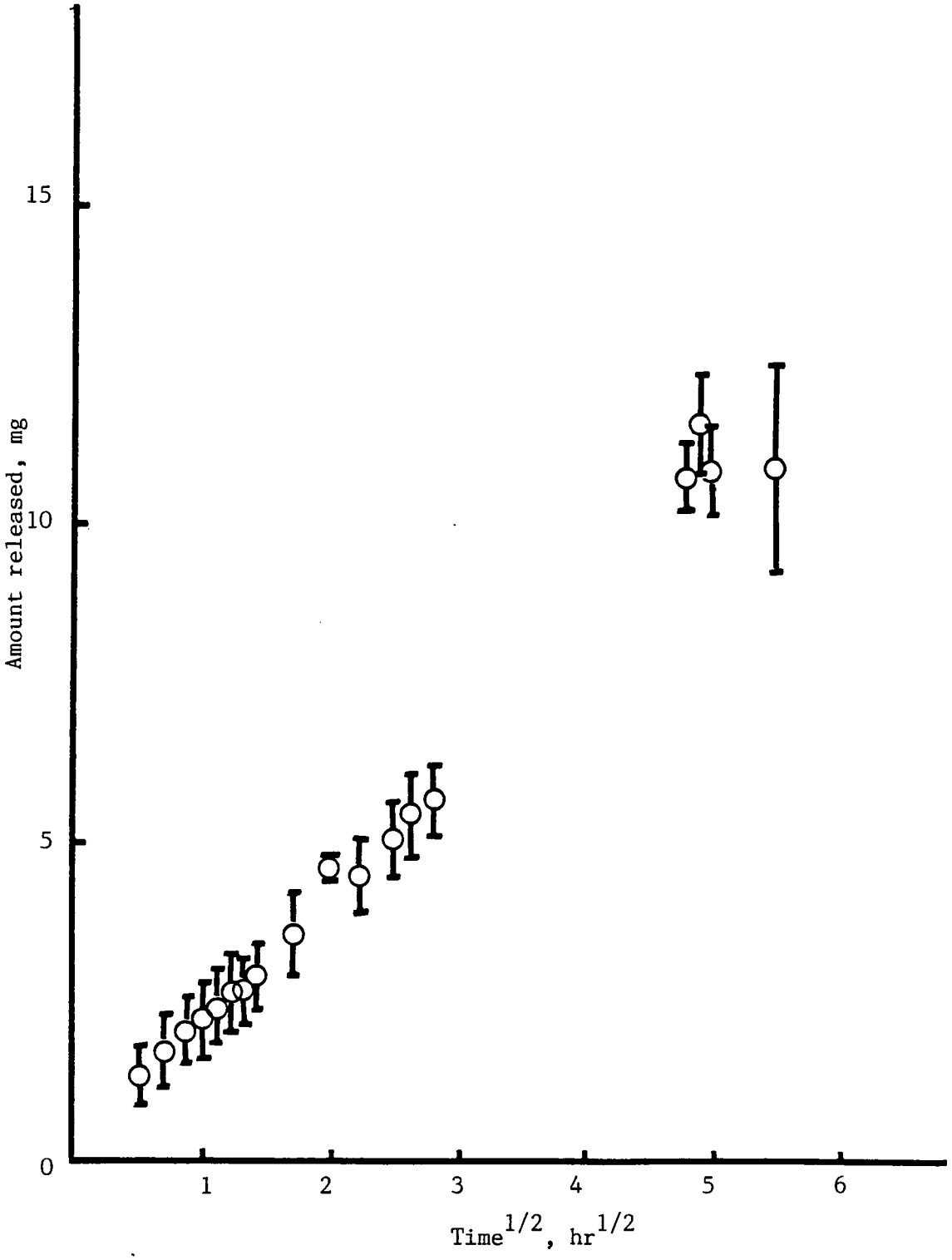


Table XVII. Diffusion coefficient of polymeric formulations.

| | Diffusion coefficients ( $\times 10^{-8}$ $cm^2/sec$ ) | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| 1 | 13.412 | 5.226 | 5.792 | 8.906 | 6.863 |
| 2 | 9.063 | 8.358 | 8.497 | 6.926 | 5.440 |
| 3 | 12.359 | 9.755 | 19.829 | 8.117 | 6.829 |
| 4 | 21.657 | 7.811 | 10.866 | 9.578 | 9.541 |
| 5 | 20.225 | 13.368 | | | |
| 6 | | 14.509 | | | |
| N | 5 | 6 | 4 | 4 | 4 |
| Mean | 15.343 | 9.845 | 11.246 | 8.382 | 7.168 |
| S.D. | 5.380 | 3.507 | 6.086 | 1.139 | 1.715 |
| C.V.% | 35.1 | 35.6 | 54.1 | 13.6 | 23.9 |
| | | a | b | a | c |
| One way ANOVA | | NS p=0.0710 | NS p=0.6515 | S p=0.0404 | NS p=0.2832 |

a. Comapred with batch A.

b. Comapred with batch B.

c. Comapred with batch D.

elastomer either.

H. In vitro percutaneous penetration study

Table XVIII lists the amount of ANT penetrated into hairless mouse skin after 48-hour treatments of polymeric formulations. The mean amount penetrated ranged from 28 to 52 nanomoles. The within-batch variation of batch C, 53%, was larger than the others, 18-35%. This tended to be consistant with the within-batch variation of diffusion coefficients, where batch C also had a relatively larger variation.

One way ANOVA was done for data analysis. The amounts of ANT penetrated into hairless mouse skin from polymer batch A and D were significantly different ($p=0.0061$). It suggested that the 382 silicone elastomer was superior to MDX-4-4210 silicone polymer in terms of percutaneous penetration of ANT.

Batch B had a significantly lower level of penetrated ANT than batch A ($p = 0.0321$). Glycerol was supposed to decrease the activation energy of drug release from polymer matrix, and facilitate the process of drug release. However, the extent of drug penetration from the glycerol-containing formulation, batch B, into the skin was observed unexpectedly lower than the glycerol-free conter part, batch A. This observation reflected that processes other than drug release might be

Table XVIII. Amount of anthralin penetrated into hairless mouse skin after 48-hour treatment with polymeric formulations.

| | Amount penetrated, nanomoles | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| 1 | 73.48 | 17.94 | 49.20 | 28.35 | 53.14 |
| 2 | 61.73 | 20.40 | 50.29 | 30.81 | 51.38 |
| 3 | 42.46 | 35.13 | 12.72 | 22.56 | 50.88 |
| 4 | 34.07 | 42.05 | 20.13 | 31.86 | 48.30 |
| 5 | 48.71 | 21.68 | 58.33 | 33.85 | 29.88 |
| 6 | | 41.61 | | 21.31 | 27.60 |
| 7 | | 41.44 | | | |
| 8 | | 47.83 | | | |
| N | 5 | 8 | 5 | 6 | 6 |
| Mean | 52.09 | 33.51 | 38.13 | 28.12 | 43.53 |
| S.D. | 15.65 | 11.73 | 20.30 | 5.13 | 11.59 |
| C.V.% | 30.04 | 35.01 | 53.23 | 18.23 | 26.61 |
| | | a | b | a | c |
| One way ANOVA | | S p=0.0321 | NS p=0.6091 | S p=0.0061 | S p=0.0138 |

a. Mean value was compared with batch A.

b. Mean value was compared with batch B.

c. Mean value was compared with batch D.

the rate limiting steps in the over all percutaneous penetration of the drug, and glycerol exerted a detrimental effect on such processes.

The difference between batch B and C was statistically insignificant ($p=0.6091$). AZ coexistence with ANT in batch C did not affect the ANT percutaneous penetration, which is not surprising since 382 silicone elastomer is claimed a lipophilic polymer, and AZ has been known to have no effect on drug percutaneous penetration when incorporated in conventional lipophilic vehicle.

On the other hand, batch E had a significantly higher level ($p=0.0138$) of penetrated ANT than batch D, and it suggested that the incorporation of PG promoted the extent of ANT penetration from MDX-4-4210 silicone elastomer into hairless mouse skin.

The 382 silicone elastomer was superior to MDX-4-4210 silicone elastomer in terms of both ANT diffusion and percutaneous penetration. However, the effects of various additives on the drug release process were less evident than those on the percutaneous penetration process. It might suggest that drug release study alone was insufficient in predicting the extent of drug percutaneous penetration from a given formulation. More factors than release process may be involved in governing drug penetration. In addition, the sink of IM may not perfectly represent the skin characteristics, in terms of drug partition between the polymer and the skin.

In order to verify that AZ exerting no effect on ANT penetration

as incorporated in polymer batch C was not due to the inactivation of AZ by polymer or other ingredients, saturated ANT in PG solutions with or without 2% AZ were used as penetrant, and no significant difference in level of penetrated ANT was observed (Table XIX). It suggested that the coexistence of AZ with ANT did not improve percutaneous penetration of ANT from conventional vehicle as well.

To exclude the possibility of AZ inactivation by ANT as coexisted in the PG solution, a study of AZ pretreatment was conducted. When hairless mouse skin was pretreated with PG or 2% AZ/PG solution for 8 hours followed by the application of saturated ANT/PG, the 8-hour AZ pretreatment significantly promoted ANT percutaneous penetration from conventional vehicle, where the limiting step of percutaneous absorption process resides in stratum corneum of the skin.

Similar studies with or without AZ pretreatment were carried out for polymeric formulations. The effects of 8-hour pretreatments with PG, 1% AZ, and 2% AZ were compared while product B was used as a penetrant (Table XX). The extents of penetrated ANT from polymeric dosage form into hairless mouse skin, with or without AZ pretreatment, were not significantly different. Therefore, it strongly suggested that the rate-limiting step of ANT percutaneous penetration for polymeric formulation was built in the polymeric device, which is the characteristic of using transdermal controlled-release mechanism, and no longer in stratum corneum.

Table XIX. Amount of anthralin penetrated after 48hr of various treatments with conventional dosage forms.

| Amount penetrated, nanomoles | | | | | | |
|---|---|---|---|---|---|---|
| Sat. ANT/PG | Sat. ANT/PG with 2% AZ | Sat.ANT/PG[a] | | Sat. DAN/PG[b] | |
| no pretreatment | | PG | 2% AZ | PG | 2% AZ |
| 33.60 | 16.97 | 11.39 | 19.99 | 8.60 | 26.16 |
| 15.78 | 20.59 | 12.80 | 15.79 | 7.02 | 21.78 |
| 17.05 | 15.78 | 11.59 | 28.98 | 13.85 | 10.42 |
| 20.09 | 11.80 | 13.97 | 21.18 | | 12.98 |
| | | 10.78 | 16.72 | | 26.30 |
| | | 19.14 | | | 13.91 |
| N 4 | 4 | 6 | 5 | 3 | 6 |
| Mean 21.63 | 16.29 | 13.28 | 20.53 | 9.82 | 18.59 |
| S.D. 8.18 | 3.62 | 3.09 | 5.22 | 3.57 | 7.03 |
| C.V.% 37.83 | 22.23 | 23.26 | 25.43 | 31.36 | 37.82 |
| Students' t-test | [c] NS 0.2<p<0.5 | | [c] S 0.02<p<0.05 | | [c] NS 0.1<p<0.2 |

* Two-tenth ml of conventional dosage forms were given in all cases.

a. Concentration of saturated ANT in PG solutions was 1.0808 mg/ml.

b. Concentration of saturated DAN in PG solutions was 45 ug/ml.

c. Mean value was compared with cooresponding treatment without AZ.

Table XX. Amount of anthralin penetrated into hairless mouse skin after 48hr treatment of polymer batch C or polymer batch B with various pretreatments.

| | Amount penetrated, nanomoles | | | | |
|---|---|---|---|---|---|
| | Batch with 8hr pretreatment | | | | Batch C |
| | none | PG | 2% AZ | 1% AZ | |
| | 17.94 | 75.44 | 37.12 | 63.92 | 49.20 |
| | 20.40 | 64.57 | 41.73 | 63.09 | 50.29 |
| | 35.13 | 43.67 | 27.22 | 67.27 | 12.72 |
| | 42.05 | 55.86 | 50.56 | 51.45 | 20.13 |
| | 21.68 | 42.89 | | | 58.33 |
| | 41.61 | 44.01 | | | |
| | 41.44 | 40.09 | | | |
| | 47.83 | 44.02 | | | |
| N | 8 | 8 | 4 | 4 | 5 |
| Mean | 33.51 | 51.32 | 39.16 | 61.43 | 38.13 |
| S.D. | 11.73 | 12.76 | 9.72 | 6.90 | 20.30 |
| C.V.% | 35.01 | 24.87 | 24.82 | 11.23 | 53.23 |
| | | a | b | b | a |
| Students' t-test | | S | NS | NS | NS |
| | | 0.01<p<0.02 | 0.1<p<0.2 | 0.2<p<0.5 | p>0.5 |

a. Mean value was compared with that of the first column.

b. Mean value was compared with that of the second column.

I. Qualitative characterization of unknown metabolite

ANT, DAN and one unidentified compound were recovered from the extract of ANT-treated skin. The retention time of unidentified compound was 8.7 minutes, between those of DAN and ANT, 7.5 and 9.0 minutes, respectively using HPLC condition-A.

The unknown compound was not from the other ingredients of the polymer, because it was observed as well when ANT/PG or DAN/PG as a penetrant. Therefore it was associated with both ANT and DAN. According to the literature information, ANT is rapidly oxidized to DAN in the skin, followed by the protein binding of DAN that stabilizes DAN from further degradation.

In this study, the unknown metabolite was isolated from the HPLC eluent of ANT treated-skin extract. The prelimnary data of structure elucidation with 300 MHz NMR (Fig. 11a-11c) suggested the unknown metabolite was an aromatic compound, having more than one ring, and might have more oxygen than DAN and ANT, and it was different from ANT and DAN in chemical structure.

The data would imply that the unknown metabolite might be an oxidized compound of DAN; therefore, we tentatively quantitate the amount of the unknown compound using the calibration curve of DAN to estimate the total penetrated ANT.

Figure 11. NMR spectra

a. Anthralin

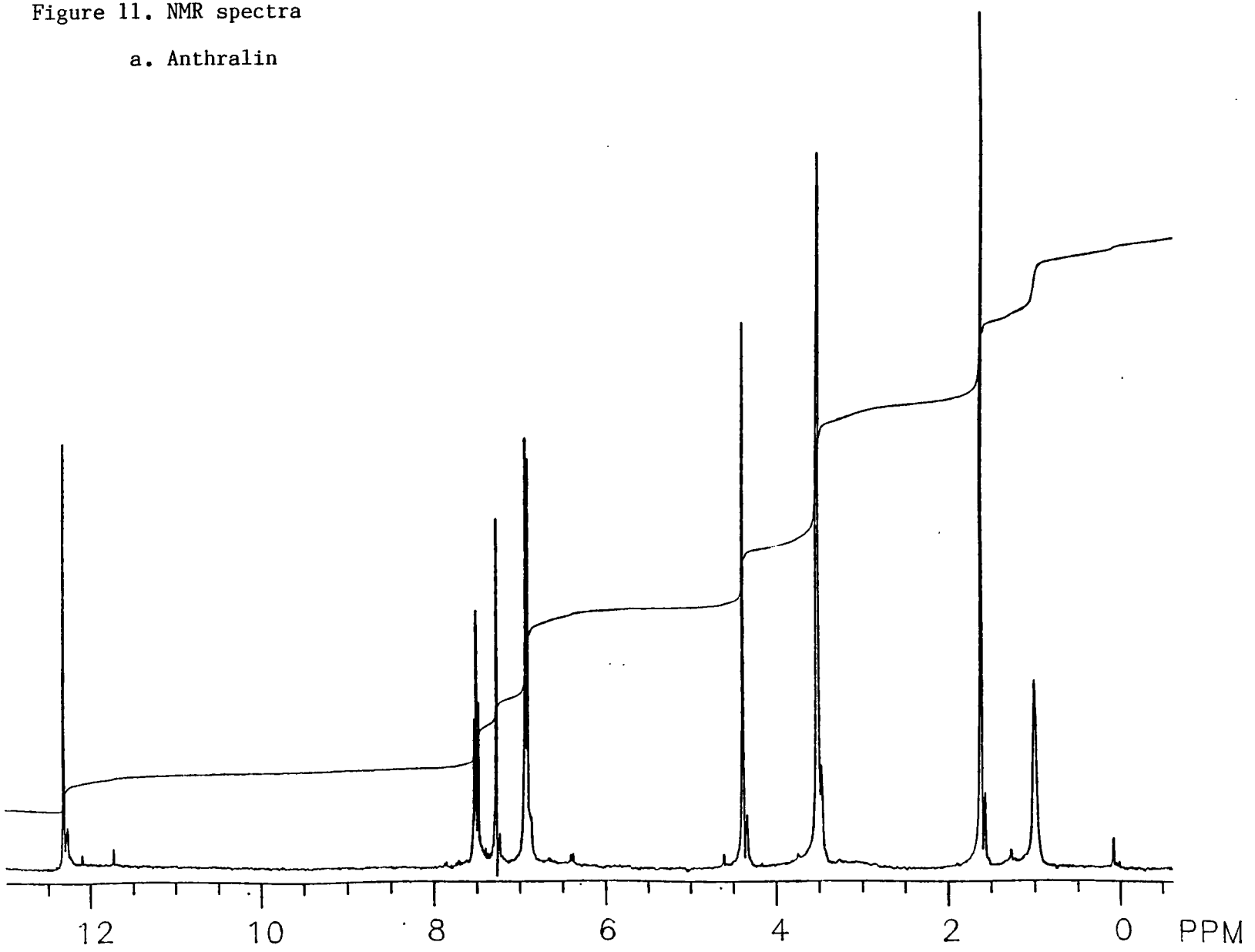


Figure 11. NMR spectra

b. Danthron

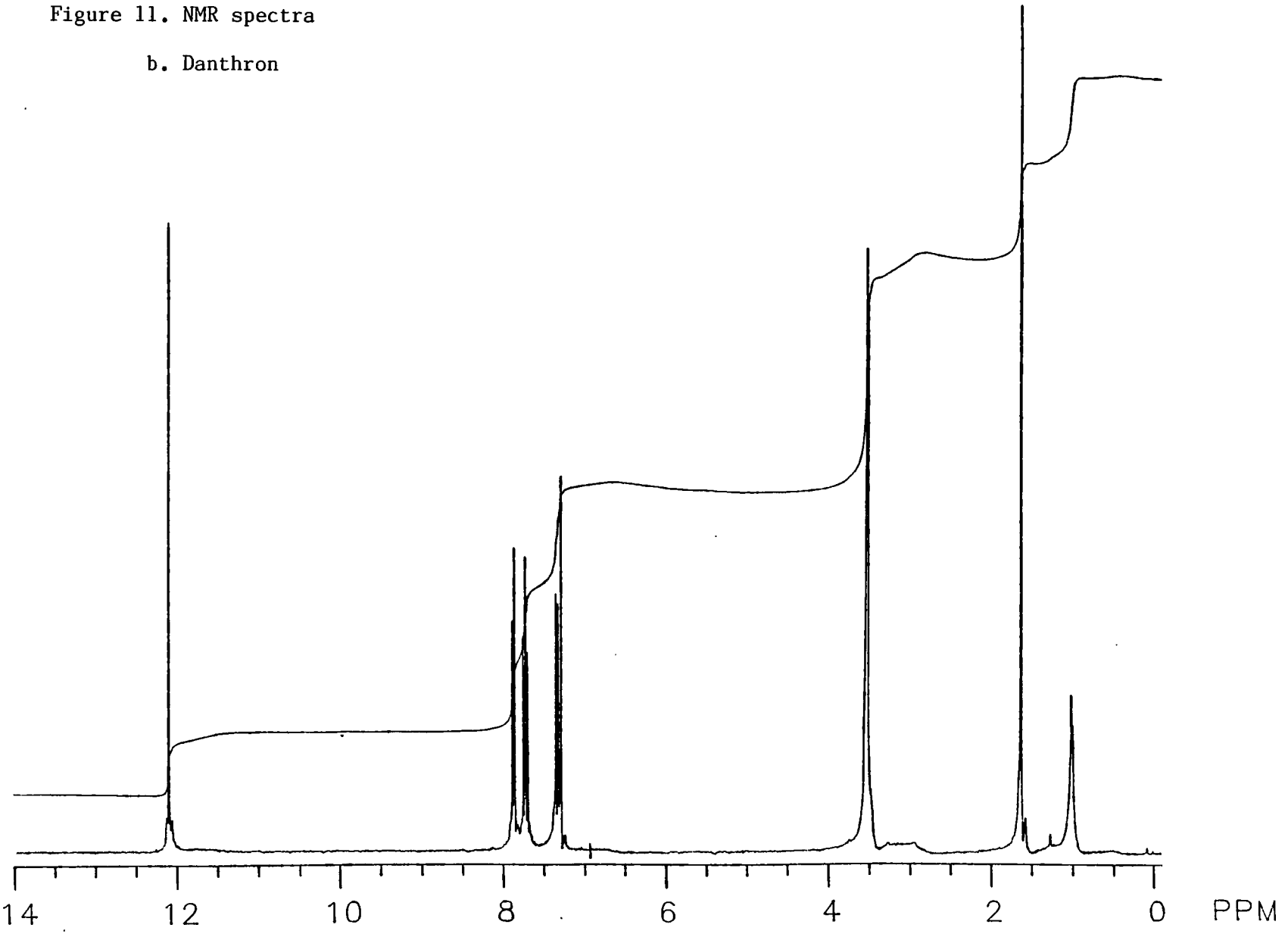
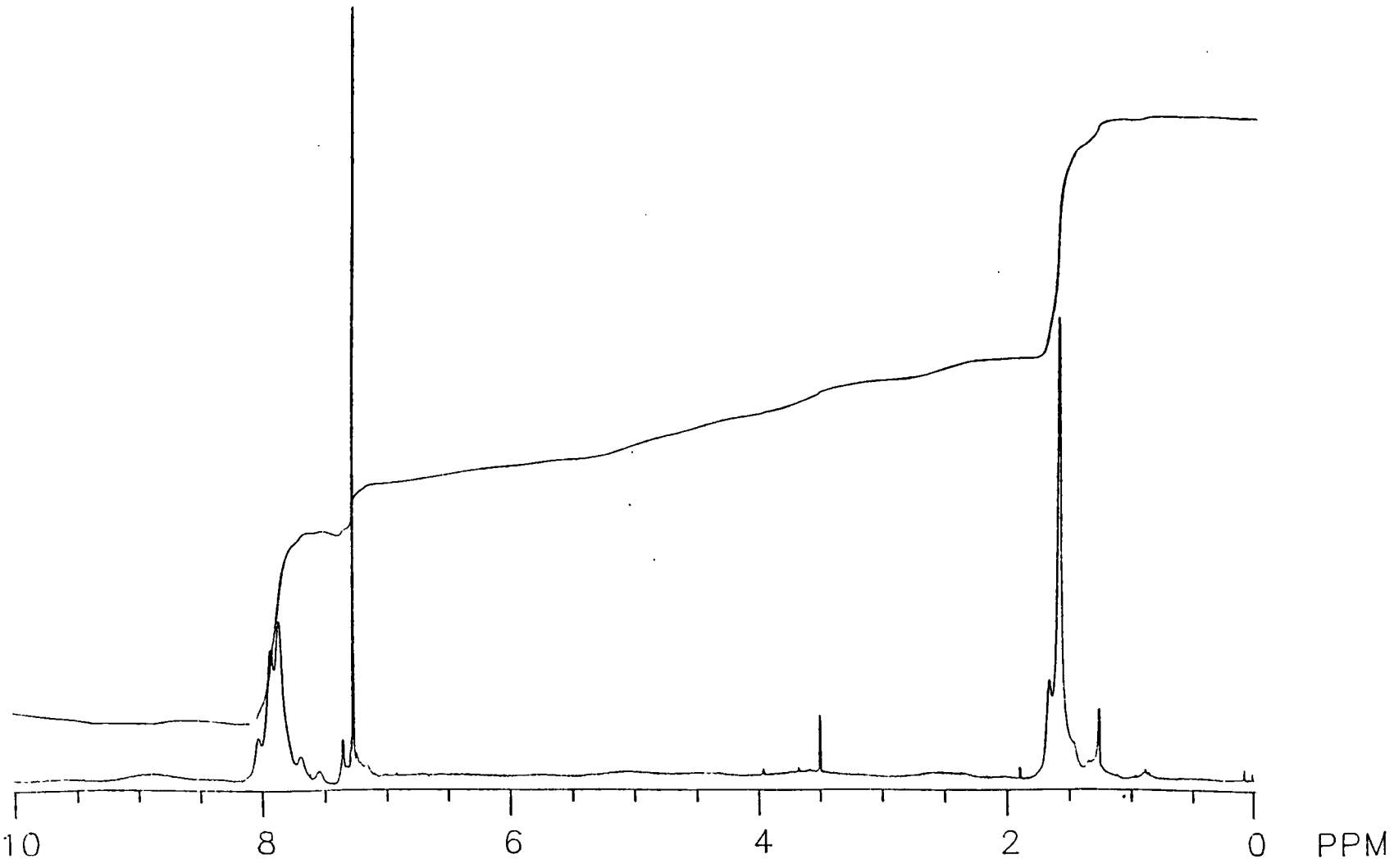


Figure 11. NMR spectra

c. Unknown metabolite



# V. SUMMARY AND CONCLUSIONS

The product assesment of five polymeric formulations were conducted. Weight of polymer ranged 153-192 mg per 0.143 $cm^3$ while thickness of six batches of formulations varied 0.9483 to 1.4161 mm. However, the thickness of polymer was taken into account in calculating the diffusion coefficient, and the variation in thickness among batches would not affect the determination of diffusion coefficient of ANT in polymeric formulations.

Polymer batch B, C, and D contained more than 90% of claimed ANT, other batches contained 75-82% labeled amount. Because six batches of polymer carried different percentage of claimed content, the actual amount of ANT was used in all calculations for the drug release study and percutaneous penetration study.

ANT degradation might not be a simple first-order process where oxygen might play a role. AZ facilitated ANT oxidation in conventional vehicle which might result from its surface activity. On the other hand, Vit. C significantly stabilized ANT in conventional vehicle. However, when ANT was incorporated in silicone elastomer, the degradation process was even further slowed down to a negligible level than when ANT was spiked with Vit. C in conventional vehicle. All formulated polymers served the purpose of preventing ANT from decomposition.

ANT release from the polymer was a first-order process, which is the characteristic of matrix-type of controlled-release device. Diffusion coefficient of ANT in 382 silicone elastomer was significantly larger than that in MDX-4-4210 silicone elastomer. The incorporation of glycerol and AZ did not affect ANT diffusion in 382 silicone elastomer. The incorporation of PG did not improve ANT diffusion in MDX-4-4210 silicone elastomer either.

The extent of ANT penetration from batch A, the 382 silicone elastomer, was significantly higher than that from batch D, the MDX-4-4210 silicone elastomer. Glycerol in batch B exerted a detrimental effect on percutaneous penetration of ANT unexpectedly. The incorporation of AZ in batch C did not improve percutaneous penetration of ANT from 382 silicone elastomer, while PG in batch E increased the level of ANT penetration from MDX-4-4210 silicone elastomer significantly.

More factors than release process may be involved in governing drug penetration. Processes other than drug release might be the rate limiting steps in the over all percutaneous penetration of the drug. In addition, the sink of IM may not perfactly represent the skin characteristics, in terms of drug partition between the polymer and the skin.

Eight-hour pretreatment with 2% AZ significantly promoted percutaneous penetration of ANT from conventional vehicle where the rate-limiting step of percutaneous absorption process resides in stratum

corneum of the skin. The penetration of ANT from polymeric formula into hairless mouse skin was not affected by AZ pretreatment. It strongly suggested that the rate-limiting step of ANT percutaneous pentration was built in the polymeric device and no longer in stratum corneum, which is the characteristic of using transdermal controlled-release mechanism.

## VI. REFERENCES


1. Wm.D. Stewart et al, Dermatology Diagnosis and Treatment of Cutaneous Disorders, 3rd Edition, The C.V. Mosby Company, Saint Louis, p. 406-417 (1974).
2. R.A. Hardie and J.A.A. Hunter, Psoriasis, British Journal of Hospital Medicine, July, 1978, p13-23.
3. G. Lomholt, Psoriasis: Prevalence, Spontaneous Course and Genetics. GAD, Copenhagen (1963).
4. W. Kimberling and R.L. Dobson, The inheritance of psoriasis, The Journal of Investigative Dermatology 60:538-540 (1973).
5. W. Watson, H.M. Cann, E.M. Farber, and M.L. Nall, The genetics of psoriasis, Archives of Dermatology 105:197-207 (1972).
6. J.C. Woodrow, V.K.Dave, N Usher, and J. Anderson, The HL-A system and psoriasis, British Journal of Dermatology 92:427-436 (1975).
7. L. Krulig, E.M. Farber, F.C. Grumet, and R.O. Payne, Histocompatibility (HL-A) antigens in psoriasis, Archives of Dermatology 111:857-860 (1975).
8. I. Jajic, A. Kastelan, A. Brnobic, V. Kerhin, and L. Brkljacic, HLA antigens in psoriatic arthritis and psoriasis, Archives of Dermatology 113:1724-1725 (1977).
9. L. Beckman, K. Bergdahl, B. Cedergren and S. Liden, Genetic markers in psoriasis. Acta Dermatovener 57:247-251 (1977).
10. A.J. McMichael, V. Morhenn, R. Payne, T. Sasazuki, and E.M. Farber, HLA C and D antigens associated with psoriasis, British Journal of

Dermatology 98:287-292 (1978).

11. R.H. Seville, Psoriasis nad stress, British Journal of Dermatology 97:297-302 (1977).
12. R.H. Seville, Psoriasis and stress II, British Journal of Dermatology 98:151-154 (1978).
13. S. Shuster, Research into psoriasis-the last decade, British Medical Journal iii:236-239 (1971).
14. G.D. Weinstein and P. Frost, Abnormal cell proliferation in psoriasis, Journal of Invstigative Dermatology 50:254-259 (1968).
15. G.D. Weinstein and J.L. McCullough, Cytokinetics in diseases of epidermal hyperplasia, Annual Review of Medicine 24:345-351 (1975).
16. F.W. Bauer and R.M.De Grood, Impulse cytophotometry in psoriasis, British Journal of Dermatology 93:225-227 (1975).
17. M. Duffill, N. Wright, and S. Shuster, The cell proliferation kinetics of psoriasis examined by three in vivo techniques, British Journal of Dermatology 94:355-362 (1976).
18. K. Thomsen and H. Schmidt, PUVA-induced bullous pemphigoid, British Journal of Dermatology, 95:568-569 (1976).
19. C.E. Orfanos, G.S. Lever, G. Mahrle, and W.F. Lever, Alterations of cell surfaces as a pathogenetic factor in psoriasis, Archives of Dermatology 107:38-46 (1973).
20. J.J. Voorhees, M. Stawiski, and E.A. Duell, Increased cyclic GMP and decreased cyclic AMP levels in the hyperplastic, abnormally differentiated epidermis of psoriasis, Life Sciences 13:639-653 (1973).
21. K. M. Halprin, Cyclic nucleotides and epidermal cell proliferation. Journal of Investigative Dermatology 66:339-343 (1976).

22. K.M. Halprin, K. Adachi, K. Yoshikawa, V. Levine, M.M. Mui, and S.L. Hsia, Cyclic AMP and psoriasis, Jounnal of Investigative Dermatology 65:170-178 (1975).

23. N.D. Goldburg, M.K. Haddox, C.E. Zeilig, S.E. Nicol, T.S. Acott, and D.B. Glass, Cyclic GMP, cyclic AMP, and the Yin Yang hypothesis of biologic regulation, Journal of Investigative Dermatology 67:641-645 (1976).

24. J.J. Voorhees, D.A. Chambers, E.A. Duell, C.L. Marcelo, and G.G. Krueger, Molecular mechanisms in proliferative skin disease, Journal of Investigative Dermatology 67:442-450 (1976).

25. G. Mahrle and C.E. Orfanos, The plasma unit membrane: membrane mediated growth control and its failure in psoriasis, British Journal of Dermatology 96:215-223 (1977).

26. R.H. Cormane, J. Hunyadi, et F. Hamerlinck, Mecanismes immunologiques du psoriasis, Annales de Dermatologie et de Syphiligraphie 103:567-572 (1976).

27. J.J. Guilhou, and J. Meynadier, Immunological aspects of psoriasis II: Dissociated impairment of thymus-dependent lymphocytes. British Journal of Dermatology 95:295-301 (1976).

28. J.J. Guilhou, J. Clot, and J. Meynadier, T cell defect in psoriasis: further studies on membrane markers and T cell functions from 60 patients, Archives for dermatological research 260:163-166 (1977).

29. S. Obalek, M. Haftek, and W. Glinski, Immunological studies in psoriasis, the quantitative evalution of cell-mediated immunity in patients with psoriasis by experimental sensitization to 2,4-dinitrochlorobenzene. Dermatologica 155:13-23 (1977).

30. J.J. Guilhou, J. Clot, J, Meynadier, and H. Lapinski, Immunological

aspects of psoriasis I: Immunoglobulins and anti-IgG factors. British Journal of Dermatology 94:501-507 (1976).

31. E.H. Beutner, S. Jablonska, M.J. Chorzelska, Studies in immunodermatology: Studies of autoantibodies to the stratum corneum and of in vivo fixed IgG in stratum corneum of psoriastic lesions, International Archives of Allergy and Applied Immunology 48:301-323 (1975).
32. R. H. Cormane et al, Journal of Dermatology 3:247 (1976).
33. W. Watson, Psoriasis: topical and systemic therapy, Rational Drug Therapy 13:1-7 (1979).
34. L. Hodge and J.S. Comaish, Psoriasis: current concepts in management, Drugs 13:288-296 (1977).
35. K.A. Arndt, Manual of Dermatologic Therapeutics with Essentials of Diagnosis, 2nd Edition, Little, Brown and Company, Boston, p. 319-320 (1981).
36. E.M. Farber, E.A. Abel, and A. Charuworn, Recent advances in the treatment of psoriasis, Journal of the American Academy of Dermatology 8:311-321 (1983).
37. R.H. Seville, Advances in the use of anthralin, Journal of the American Academy of Dermatology 5:319-321 (1981).
38. G.P. Stoehr, Plaque psoriasis: Etiology and treatment. U.S. Pharmacist, February 1983, p.31-52.
39. Galewsky, Dermatol Wchnschr 6:113 (1916).
40. G. Swanbeck and S. Liden, The inhibitory effect of dithranol (anthralin) on DNA synthesis, Acta Dermato-Venereologica 46:228-230 (1966).
41. D.L. Baxter and R. B. Stoughton, Mitotic index of psoriatic lesions

treated with anthralin, glucocorticosteroid and occlusion only. The Journal of Investigative Dermatology 54:410-412 (1970).

42. L.B. Fisher and H.I. Maibach, Proceedings of the International Symposium on Psoriasis, pp. 335-345, Stanford University Press, Stanford.

43. D. Gaudin, R.S. Gregg, and K.L. Yielding, Inhibition of DNA repair by cocarcinogens, Biochemical and Biophysical Research Communications 48:945-949 (1972).

44. M.C. Poirier, B.T. De Cicco, and M.W. Lieberman, Nonspecific inhibition of DNA repair synthesis by tumor promoters in human diploid fibroblasts damaged with N-acetoxy-2-acetylaminofluorene, Cancer Research 35:1392-1397 (1975).

45. G. Swanbeck and N. Thyresson, Interaction between dithranol and nucleic acids. A possible mechanism of the effect of dithranol on psoriasis, Acta Dermato-Venereologica 45:344-348 (1965).

46. J.F. Walter, R.B. Stoughton, and P.R. DeQuoy, Suppression of epidermal proliferation by ultraviolet light, coal tar and anthralin, British Journal of Dermatology 99:89-96 (1978).

47. L.B. Fisher and H.I. Maibach, The effect of anthralin and its derivatives on epidermal cell kinetics, The Journal of Investigative Dermatology 64:338-341 (1975).

48. M. Patel, B. Schaarschmidt, and U. Reichert, Calorimetric and manometric measurements on human skin fibroblasts in culture. British Journal of Dermatology 105, Supplement 20:60-61 (1981).

49. E.B. Klem,Effects of antipsoriasis drugs and metabolic inhibitors on the growth of epidermal cells in culture, The Journal of Investigative Dermatology 70:27-32 (1978).

50. Y. Jacques and U. Reichert, Effect of anthralin and analogues on growth and [3H] thymidine incorporation in human skin fibroblasts. British Journal of Dermatology 105, Supplement 20:45-48 (1981).

51. F. D. Dean, The action of zinc oxide on dithranol, British Journal of Dermatology 85:494 (1975).

52. M.P. Waelsch and H.J. Hulsebosch, Further studies on the interaction between anthralin salicylic acid and zinc oxide in pastes, Arch. Derm. Forsch 249:141-152 (1974).

53. J. Martinmaa, L. Vanhala, and K.K. Mustakallio, Free radical intermediates produced by autoxidation of 1,8-dihydroxy-9-anthrone (dithranol) in pyridine. Experimentia 34:872-873 (1978).

54. H.J. Hulsebosch and M.P. Waelsch, The interaction of anthralin, salicylic acid and zinc oxide in pastes, Dermatologica 144:287-293 (1972).

55. W.P, Raab and B.M. Gmeiner, Influence of ultraviolet light, various temperatures, and zinc ions on anthralin (dithranol), Dermatologica 150:267-276 (1975).

56. S.Comaish, J. Smith, and R.H. Seville, Factors affecting the clearance of psoriasis with dithranol (anthralin), British Journal of Dermatology 84:282-289 (1971).

57. M. Whitefield, Pharmaceutical formulations of anthrlain. British Journal of Dermatology 105, Supplement 20:28-32 (1981).

58. B. Kammerau, A. Zesch, and H. Schaefer, Absolute concentrations of dithranol and triacetyl-dithranol in the skin layers after local treatment: in vivo investigations with four different types of pharmaceutical vehicles, The Journal of Investigative Dermatology 64:145-149 (1975).

59. I. Brody and A. Johansson, A topical treatment program for psoriasis with low anthralin concentrations. Journal of Cutaneous Pathology 4:233-243 (1977).

60. L.F. Montes, W.H. Wilborn, and I. Brody, Low strength anthrlin in psoriasis, Journal of Cutaneous Pathology 6:445-456 (1979).

61. I. Brody, Treatment of psoriasis vulgaris; gentleness to the entire psoriatic skin and the use of low concentrations of anthralin. British Journal of Dermatology 105, Supplement 20:109-110 (1981).

62. H. Schaefer, E.M. Farber, L. Goldberg, and W. Schalla, Limited application period for dithranol in psoriasis: Preliminary report on penetration and clinical efficacy. British Journal of Dermatology 102:571-573 (1980).

63. U. Runne and J. Kunze, Short-duration ('minutes') therapy with dithranol for psoriasis: a new out-patient regimen. British Journal of Dermatology 106:135-139 (1982).

64. N.J. Lowe, R.E. Ashton, H. Koudsi, M. Verschoore, and H. Schaefer, Anthralin for psoriasis: short-contact anthralin therapy compared with topical steroid and conventional anthralin. Journal of the American Academy of Dermatology 10:69-72 (1984).

65. P. Hindryckx and J.De Bersaques, Short-duration dithranol therapy for psoriasis. Dermatologica 167:304-306 (1983).

66. R.J. Chalmers, J.M. Marks, and S. Shuster, A nove, wax stick preparation of anthralin, Acta-Derm-Venereol, 62: 181-182 (1982).

67. K.K. Mustakallio, Irritation, staining and antipsoriatic activity of 10-acyl analogues of anthralin. British Journal of Dermatology 105, Supplement 20:23-27 (1981).

68. M. Colin, J. Maignan, G. Lang, and B. Shroot, Anthralin oxidation

products: role of the C -methylene and phenol groups. British Journal of Dermatology 105, Supplement 20:59 (1981).

69. K.K. Mustakallio and H. Brandt, Irritation, staining and antipsoriatic activity of some 10-acyl analogues of anthralin. British Journal of Dermatology 105, Supplement 20:51 (1981).

70. M. Szycher. Biocompatible Polymers, Metals and Composites. Technomic Publishing Co. Inc., Lancaster, Pa. (1983).

71. R.L. Juliano, Drug Delivery Systems Characteristics and Biomedical Applications. New York Oxford, Oxford University Press (1980)

72. P.J. Dzuik and B. Cook, Passage of steroids through silicone rubber. Endocrinology 78:208-211 (1966).

73. H.W. Rudel, J.M. Manauton, and M.M. Topete, The role of progestogens in the hormonal control of fertility. Fertility and Sterility, 16:158-169 (1965).

74. F.A. Kincl, G. Benagiano, and I. Angee, Sustained release hormronal preparations, Steroids 11:673-680 (1968).

75. A.F. Pi, A. Oriol, L.H. Lasso, M. Maqueo, R.I. Dorfman and F.A. Kincl, Inhibition of fertility in mice by steroid implants, Acta Endocrinologica 48:602-608 (1965).

76. A. Scommegna, G.N. Pandya, M. Christ, A.W. Lee, and M.R. Cohen, Intrauterine administration of progesterone by a slow releasing device, Fertility and Sterility, 21:201-210 (1970).

77. A. Scommegna et al, Obstetrics and Gynecology 43:769 (1974).

78. D.R. Mishell and M.E. Lumkin, Contraceptive effect of varying dosages of progestogen in silastic vaginal rings, Fertility and Sterility, 21:99-103 (1970).

79. D.R. Mishell, M. Lumkin, and S. Stone, Inhibition of ovulation with

cyclic use of progestogen-impregnated intravaginal devices, American Journal of Obstetrics and Genecology 113:927-932 (1972).

80. M.R. Henzl, D.R. Mishell, J.G. Velazquez, and W.E. Leitch, Basic studies for prolonged progestogen administration by vaginal devices, American Journal of Obstetrics and Genecology, 117:101-106 (1973).

81. E.D.B. Johansson, T. Luukkainen, E. Vartiainen, and A. Victor, The effect of progestin R 2323 released from vaginal rings on ovarian function. Contraception 12:299-307 (1975).

82. A. Victor, H.A. Nash, T.M. Jackanicz and E.D.B. Johansson, Collagen bands: a new vaginal delivery system for contraceptive steroids, Contraception 16:125-135 (1977).

83. D.R. Mishell, D.E. Moore, S. Roy, P.F. Brenner, and M.A. Page, Clinical performance and endocrine profiles with contraceptive vaginal rings containing a combination of estradiol and d-norgestrel, American Journal of obstetrics and Genecology 130:55-62 (1978).

84. J. Urquhart, Controlled-release pharmaceuticals. Academy of Pharmaceutical Sciences (1979).

85. A.F. Kudonieus, Controlled release technologies: Methods, theory, and applications. CRC Press Inc. (1980).

86. T.J. Franz. Percutaneous absorption on the relevance of in vitro data. Journal of Investigative Dermatology. 64:190-195 (1975).

87. G.L. Flynn, H. Durrheim and W.I. Higuchi. Permeation of hairless mouse skin II: Membrane sectioning techniques and influence on alkanol permeabilities. Jounal of Pharmaceutical Science 70:52-56 (1981).

88. N. Barker and J. Hadgraft. Facilitated percutaneous absorption: A

model system. Int. J. Pharmaceutics 8:193-202 (1981).

89. T.R. Bates, M. Gibaldi and J.L. Kanig. Rate of dissolution of griseofulvin and hexoestrol in bile salt solutions. Nature 210: 1331-1332 (1966).

90. W.I. Higuchi. Analysis of data on the medicament release from ointments. Journal of Pharmaceutical Science. 51:802-804 (1962).

# VII. APPENDIX

## A. Program for correcting drug release data

```
      PROGRAM MODIFY
      IMPLICIT DOUBLE PRECISION(A-H,O-Z)
C
C     ------------------------------------------------------
C     1                                                    1
C     1     PURPOSE : TRANSFER THE RAW DATA INTO A NEW     1
C     1               DATA FILE                            1
C     1     DATE    : SEPTEMBER 25, 1984                   1
C     1     AUTHOR  : TSUI-I WANG                          1
C     1                                                    1
C     ------------------------------------------------------
C
      PARAMETER (I=50)
      DIMENSION TIME(I),AMOUNT(I),CORAMT(I),CORNOM(I)
C
C   DATA REQUIRED :
C
C          ITER   : NUMBER OF ITERATION (INTEGER, ----)
C          NBATCH : BATCH NUMBER (INTEGER, ----)
C          FACTOR : FACTOR (REAL, ----)
```

```
C          QOANT  : INITIAL AMOUNT OF ANT (REAL, MG)
C          NODATA : NUMBER OF DATA (INTEGER, ----)
C          TIME   : TIME (REAL, HR)
C          AMOUNT : AMOUNT (REAL, MG)
C
      ZA=1.D00
      READ(5,*)ITER
      WRITE(7,3010)ITER
      DO 800 N=1,ITER
      READ(5,*)NBATCH
      READ(5,*)FACTOR
      READ(5,*)QOANT
      READ(5,*)NODATA
      WRITE(7,3010)NBATCH
      WRITE(7,3010)NODATA
      AUX=ZA/FACTOR
      SUM=0.D00
      PREVOS=0.D00
      DO 700 J=1,NODATA
      READ(5,*)TIME(J),AMOUNT(J)
      SUM=SUM+PREVOS
      PREVOS=AMOUNT(J)
      CORAMT(J)=AMOUNT(J)+AUX*SUM
      CORNOM(J)=CORAMT(J)/QOANT*1.5D01
  700 CONTINUE
      WRITE(6,2010)NBATCH
      WRITE(6,2020)(TIME(K),AMOUNT(K),CORAMT(K),CORNOM(K),K=1,NODATA)
```

```
      WRITE(7,3020)(TIME(K),CORAMT(K),K=1,NODATA)
  800 CONTINUE
 2010 FORMAT(////T10,'BATCH NUMBER :',I5///)
 2020 FORMAT(T12,'SAMPLE TIME',T32,'AMOUNT RELEASED',
     1       T53,'CORRECTED AMOUNT',T74,'NORMALIZED AMOUNT'//
     2       T15,'(HR)',T37,'(MG)',T58,'(MG)',T79,'(MG)'/(4D22.5/))
 3010 FORMAT(I3)
 3020 FORMAT(2D16.6)
      CALL EXIT
      END
```

B. Program for calculating diffusion coefficient

```
      PROGRAM EXPER
      IMPLICIT DOUBLE PRECISION(A-H,O-Z)
      REAL*8 INTCP
      PARAMETER (I=50)
      DIMENSION TIME(I),YTIME(I),ZTIME(I),AMOUNT(I),
     1          PCTREL(I),AMTNOM(I)
C
C     DATA REQUIRED :
C
C          ITER   : NUMBER OF ITERATION (INTEGER, ----)
C          NBATCH : BATCH NUMBER (INTEGER, ----)
```

```
C          WTPOLY : WEIGHT OF POLYMER (REAL, G)
C          ANT    : WEIGHT OF ANT (REAL, G)
C          TOTALW : TOTAL WEIGHT (REAL, G)
C          PCT    : PERCENTAGE (REAL,%)
C          NODATA : NUMBER OF DATA (INTEGER, ----)
C          THICK  : THICKNESS (REAL, CM)
C          TIME   : TIME (REAL, HR)
C          AMOUNT : AMOUNT (REAL, MG)
C
      READ(5,*)ITER
      DO 800 N=1,ITER
      READ(5,*)NBATCH
      READ(5,*)WTPOLY,ANT,TOTALW,PCT
      QOANT=WTPOLY*ANT*PCT*1.D-2/TOTALW
      READ(5,*)NODATA
      NOREGR=NODATA
      READ(5,*)THICK
      DO 700 J=1,NODATA
      READ(5,*)TIME(J),AMOUNT(J)
      YTIME(J)=DSQRT(TIME(J))
      ZTIME(J)=6.D01*YTIME(J)
      PCTREL(J)=AMOUNT(J)/QOANT
      AMTNOM(J)=1.5D01*PCTREL(J)
  700 CONTINUE
      CALL REGRES(ZTIME,AMOUNT,NODATA,NOREGR,SLOPE,INTCP,R,RSQ)
      AUX=DSQRT(0.785398144D00)*THICK*SLOPE/QOANT
      D=AUX*AUX
```

```
      WRITE(6,1000)NBATCH,THICK,PCT,ANT,WTPOLY,TOTALW,QOANT
      WRITE(6,1010)(TIME(K),YTIME(K),ZTIME(K),AMOUNT(K),
     1              PCTREL(K),AMTNOM(K),K=1,NODATA)
      WRITE(6,1020)SLOPE,INTCP,R,RSQ,D
  800 CONTINUE
 1000 FORMAT(1H1///T10,'BATCH NUMBER :',I5//
     1       T10,'THICKNESS :',D13.5,' (CM)',
     2       T60,'PERCENTAGE  :',D13.5,' (%)'/
     3       T10,'A.N.T.    :',D13.5,' (G)',
     4       T60,'WT. POLYMER :',D13.5,' (MG)'/
     5       T10,'TOTAL WT. :',D13.5,' (G)',
     6       T60,'INITIAL ANT :',D13.5,' (MG)'//5X,100(1H-)//
     7       T10,'SAMPLE TIME',T27,'TIME**1/2',T43,'TIME**1/2',
     8       T56,'AMOUNT RELEASED',T73,'PCT. RELEASED',
     9       T88,'NORMALIZED AMOUNT'//
     A       T13,'(HR)',T28,'(HR1/2)',T44,'(SEC1/2)',
     B       T61,'(MG)',T78,'(--)',T94,'(MG)'/)
 1010 FORMAT(4X,6D16.5/)
 1020 FORMAT(5X,100(1H-)//15X,'SLOPE :',D20.5//15X,'INTERCEPT :',D16.5
     1       //15X,'CORR. COEFF. :',D13.5//15X,'R*R :',D22.5//
     2       15X,'D :',D24.5)
      CALL EXIT
      END
      SUBROUTINE REGRES(XVEC,YVEC,NODATA,NOREGR,SLOPE,INTCP,R,RSQ)
      IMPLICIT DOUBLE PRECISION(A-H,O-Z)
      REAL*8 INTCP
      PARAMETER (I=50)
```

```
      DIMENSION XVEC(I),YVEC(I)
      RNDATA=NOREGR
      XSUM=0.D00
      YSUM=0.D00
      XYSUM=0.D00
      XSQ=0.D00
      YSQ=0.D00
      DO 700 K=1,NOREGR
      X=XVEC(K)
      Y=YVEC(K)
      XSUM=XSUM+X
      YSUM=YSUM+Y
      XYSUM=XYSUM+X*Y
      XSQ=XSQ+X*X
      YSQ=YSQ+Y*Y
  700 CONTINUE
      XAV=XSUM/RNDATA
      YAV=YSUM/RNDATA
      SLOPE=(XAV*YSUM-XYSUM)/(XAV*XSUM-XSQ)
      INTCP=YAV-SLOPE*XAV
      XVAR=XSQ/RNDATA-XAV*XAV
      YVAR=YSQ/RNDATA-YAV*YAV
      R=SLOPE*DSQRT(XVAR/YVAR)
      RSQ=R*R
      RETURN
      END
```