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The Effect Of Selected Adjuvants On The In Vitro Percutaneous Penetration Of Benzocaine

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THE EFFECT OF SELECTED ADJUVANTS ON THE IN VITRO PERCUTANEOUS PENETRATION OF BENZOCAINE

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Abstract of Dissertation

This research project was designed to test whether the in vitro percutaneous penetration of benzocaine through human cadaver skin could be enhanced by dimethyl sulfoxide (DMSO), urea, polyoxyethylene (20) isohexadecyl ether and 1-dodecylazacycloheptan-2-one (Azone) in propylene glycol/water systems. Solubility and partitioning of benzocaine in propylene glycol/water systems was investigated. The adjuvant effects were studied in a 60/40 (V/V) propylene glycol/water co-solvent system.

The well known drug penetration enhancer dimethyl sulfoxide did not enhance the penetration of benzocaine at any concentration level of DMSO under the conditions of the experiment. This lack of enhancement effect was probably due to increased solubility of benzocaine in the DMSO/water system and a consequent decrease in the partitioning of drug into the skin. Urea enhanced benzocaine penetration initially but no significant steady-state penetration enhancement was noted. Polyoxyethylene (20) isohexadecyl ether appeared to retard rather than enhance the percutaneous penetration of benzocaine at concentrations below and around the critical micelle concentration. Azone showed concentration dependence for its enhancement effect on penetration of benzocaine. With 1% V/V Azone, the initial benzocaine penetration rate was higher compared to the other Azone concentrations. On the basis of comparative analysis of the steady-state rates, 5% V/V Azone was observed to be the most effective penetration enhancer for benzocaine. Azone also showed additive enhancement properties with increasing percentages of propylene glycol.

The results of this investigation emphasize the importance of in vitro skin penetration studies prior to clinical evaluation. The results also underscore the importance of a proper experimental design that will minimize variables during the study in order to properly identify cause and effect relationships.

DEDICATION

To my parents.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and deep appreciation to Dr. Ravindra C. Vasavada for his guidance, encouragement and patience throughout the course of this study. I am also indebted to Dr. Madhukar G. Chaubal, Dr. Zak T. Chowhan, Dr. Herschel Frye and Dr. Marvin H. Malone for their suggestions and counsel, as well as their assistance as dissertation committee members.

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INTRODUCTION

The study of medicaments applied topically has gained renewed interest in recent years. The skin is one of the largest organs in the body and the most readily accessible for application of drugs. However of all the tissues in the body, the skin surface is one of the least permeable. This impermeability is related to two factors: the absence of highly efficient specialized transport systems and the unique structure of the cells of the stratum corneum (1). In humans, the skin serves as a protective barrier against penetration of a variety of substances. The challenge of overcoming this barrier to promote effective drug delivery for local and systemic effects has been the subject of considerable research in both the pharmaceutical and dermatological fields (2).

Local anesthetics are widely used in a variety of OTC products intended for relief of itch and pain -- particularly when associated with thermal burns and sunburn. Since both itch and pain are subserved by the same network of cutaneous nerves, increasing the intensity of a stimulus converts the itching sensation to one of pricking or burning. Increasing the intensity still more induces true pain (3). One of the most widely used local anesthetics in OTC burn and sunburn products is benzocaine

(ethyl aminobenzoate). It is used at concentrations ranging from 5 to 20% in a variety of dosage forms such as sprays, ointments, lotions, creams and foams (3).

Many studies have been done on the action of local anesthetics on intact, abraded and burned skin, and on subcutaneous tissue. However in many of these studies, the assessment of anesthetics efficacy has been based largely upon subjective clinical impressions (4,5). It has been reported by Campbell and Adriani (6), that local anesthetics in general are not absorbed from the intact skin, but pass into the blood only if the skin is abraded or burned. This lack of efficacy of many OTC products in the relief of pain and itch has also been corrorborated by other in vivo studies of local anesthetics on intact and sunburned skin (3). There is a noticeable difference in absorption when local anesthetics are topically applied to skin and to mucous membrane. Most explanation have stressed the histological differences of these two types of epithelial coverings; but Adriani and Dalili (7) credit Herman Rein with demonstrating the presence of an electrostatic barrier effect in the skin, showing that isolated human skin behaved physiologically as a negatively charged membrane of single cell thickness. He postulated that electrostatic charges opposed the penetration of both anions and cations. It is possible that salts of local anesthetic agents do not penetrate this layer because they

are ionized.

The stratum corneum is being constantly regenerated and has a turnover period of about two weeks. The process of regeneration begins with a high level of mitotic activity in the basal layer of the epidermis followed by the complex process of maturation and migration towards the skin surface. In the process, cells flatten and dehydrate and undergo intracellular transformation. The end result is the dense, keratin-filled and metabolically inactive layer of cells. The three-dimensional protein matrix which fills the intracellular space contains at least two distinguishable forms of keratin: alpha-keratin, which is formed or woven into highly ordered filaments and constitutes about 70% of the intracellular content, and beta-keratin, which is amorphous or "noncrystalline" and approximates 10% of the total content. The residual 20% of the intracellular content is lipid, including cholesterol, fatty acids, and triglycerides. The intracellular contents are about 85% of the total stratum corneum on a dry weight basis. In its normal state the stratum corneum contains about 15% water (1). The stratum corneum or the "cornified" layer is a thin, ultradense polyphasic epidermal covering made from dehydrated, highly filamented dead cells which are fused into tight columnar arrangement held together primarily by tonofibrils and a somewhat indistinguishable substance. It has been estimated that stratum corneum

contains ten times the fibrous material in one-tenth the space as that of the viable epidermis. This forms the effective barrier of the skin. This composition of stratum corneum serves to explain the more recent view that the skin is composed of a complex number of barriers of varying degrees of penetrability.

Each penetrant may encounter a different type of barrier, and the degree of penetration is subject to the chemical and physical properties and the interaction of the drug with the tissues it attempts to traverse (7). The stratum corneum, acts a passive diffusion medium, but one which displays considerable diffusional resistance. Since the mechanism of penetration through the stratum corneum is a diffusive process, Fick's law is generally applied to describe the process of skin penetration. In general the viable epidermis and dermis are relatively permeable to most substances. The stratum corneum is interspersed with hair follicles and its associated pilosebaceous glands (the eccrine and apocrine glands). The surface openings of these structures may provide an effective pathway for penetration of benzocaine, but their relative importance is doubtful since hair follicles and pilosebaceous glands occupy respectively only 1/1,000 and 1/10,000 of the total surface of skin.

The skin also possesses an adsorptive capacity which offers a degree of protection against penetration by

retarding inward migration rather than augmenting penetration. This type of effect has not been documented with respect to benzocaine.

The stratum corneum effectively acts as a 'barrier' to the percutaneous penetration of benzocaine and thus accounts for the reported lack of efficacy of many OTC products. Relatively poor penetrability of benzocaine accounts for the use of excessive high concentrations of the drug in commercial products. The only product rated as 'effective' contains 20% of benzocaine and yet exhibits a relatively slow onset of action (3). Enhancement of rate of penetration of benzocaine topically could lower the concentration requirement for efficacy while providing a more immediate relief of pain. Incorporation of suitable adjuvants in the formulation could help to achieve this goal.

Many substances when applied to the skin can alter the permeability properties of the stratum corneum. Of major interest are those substances that have a mild or reversible effect on the skin tissue.

Polar solvents, such as dimethylsulfoxide (DMSO) and dimethylformamide (DMFA), have been shown by many investigators to be effective vehicles for increasing skin penetration (9-13). Brechner et al. (10) reported that tetracaine base dissolved in DMSO penetrated the skin readily to produce local anesthesia. In vivo anesthesia was

obtained with concentration of tetracaine from 5 to 30%. However, Kligman (11) reported a lack of anesthesia in experiments with the hydrochloride salts of lidocaine and procaine dissolved in 10 - 90% DMSO. This lack of efficacy was explained on the basis of low concentrations of the drugs used and the use of the ionized salt form of the drugs. In many other in vivo and in vitro experiments, DMSO has been shown to increase the penetration of water and a variety of drugs (12,13). This increased diffusivity of the stratum corneum may be due to the hygroscopic nature of the solvent (increasing the hydration of the tissue) or ultrastructural modification of the stratum corneum due to extraction of soluble components. DMSO does not act as a 'carrier' nor is the increased permeability due simply to a more favorable partition coefficient for the particular solute. It has been reported that the effects of DMSO are to a substantial degree reversible in that the permeability of tissue is largely restored after the DMSO is removed. But this point is still in dispute (9,11).

Azone (1-dodecylazacycloheptan-2-one) has recently received considerable interest as a penetration enhancer. Stoughton (14) reported that Azone enhances the in vivo penetration of glucocorticoids, griseofulvin and anthracene in humans. In a study with hairless mouse skin, Azone was shown to increase the total flux of ARA-A, an anti-viral agent, by 20 fold as compared to a formulation using neat

DMSO (15). Azone has been promoted to enhance penetration of active substances without producing toxicity, altering sensitivity or inducing other systemic or local side effects. The precise mechanism is unknown, but it has been postulated that Azone alters the lipid and protein structures of the stratum corneum allowing permeation of active substances into the dermis. Radioactive studies have shown that Azone is bound in the epidermis and corium and that relatively little is released from the skin (14,16).

It has been reported by various authors (17-19) that the interaction between surfactant molecules and membrane components (phospholipids, proteins and water) could lead to changes in structure and consequently in membrane permeability. Experiments with rabbit skin have demonstrated that surfactants can change the content, composition, and biosynthesis rate of epidermal phospholipid. Since phospholipids are a major component of biological membranes, these changes probably indicate changes in epidermal membrane structure. It is believed that membrane lipids constitute a barrier to water diffusion and that the proportions of individual membrane lipids or their total amount can influence water and ion transport through these membrane. The effect of surfactants on the percutaneous absorption of naproxen and benzocaine across animal and human skin have been reported (20,21). These results show that, in general, nonionic surfactants

retard the percutaneous penetration of drugs. This decrease in penetration could be due to micelle formation thus affecting the concentration of 'free' drug in the solution.

Urea has attracted interest because of its ability to moisturize the skin and also its capacity to act as mild keratolytic (22). Urea has been shown to increase the percutaneous penetration of certain topical anti-inflammatory steroids. Besides the hydrating action on the stratum corneum, urea has also been used as a solubilizer for various drugs (23). The effect of urea on solubility is due to its effect on water structure and hydrophobic bonding -- thus 'breaking up' water clusters surrounding nonpolar molecules and increasing the entropy of the system (23).

It has been shown by various authors that penetration of drug molecules can be enhanced by increasing the hydration of the skin (24,25). Both occlusion and the use of humectants have been used in attempting to accomplish this goal. Compounds having humectant properties are, for example, propylene glycol and di-isopropyl sebacate. Propylene glycol has shown promising results as a vehicle for topical delivery when used as a co-solvent with water to produce saturated solutions of the active ingredient. This would maximize the thermodynamic activity of the penetrant. Selection of an appropriate propylene glycol/water mixture with optimal solubility and partitioning profile could

provide a solvent system to facilitate topical penetration of benzocaine.

Scope of the Present Study

Incorporation of proper penetration enhancers in appropriate amounts could point the way to clinically successful formulations of topical products with an immediate effect on the relief of itch and pain. In view of this lucrative goal, this research project was designed to document whether DMSO, Arlasolve 200, urea, and Azone in a propylene glycol/water system could enhance the percutaneous penetration of benzocaine through human cadaver skin.

Propylene glycol was used as a co-solvent with water to increase the solubility of benzocaine and also for its possible penetration enhancement properties. Since DMSO has been reported to overcome the barrier properties of the stratum corneum with respect to other drugs, similar effects were expected with respect to benzocaine.

A mixture of 60/40 (V/V) propylene glycol/water was chosen as the solvent system for evaluating the effects of selected adjuvants. The effects on both benzocaine solubility and percutaneous penetration were investigated.

EXPERIMENTAL

All chemicals were used as received from the manufacturers and their respective lot numbers were logged. The solubility of the drug in a series of propylene glycol/water mixtures was determined. The partition coefficient of benzocaine in the two phase isopropyl myristate/propylene glycol/water system was also examined. Guided by the results of solubility and partition coefficient determinations, an optimum composition of a propylene glycol/water system was chosen for the in vitro skin penetration studies. The effect of selected adjuvants on skin penetration of benzocaine was examined using this vehicle.

Solubility Studies.

The solubility of benzocaine[1] was determined in a series of propylene glycol[2]-water[3] mixtures (0% to 100% in 20% increments) at 25 ± 0.5°. An excess of benzocaine was weighed and added to 25 ml of propylene glycol/water mixture in 50-ml Erlenmeyer flasks containing a Teflon

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1. 'Baker' Grade, Lot. 847340, J.T. Baker Chemical Co., Phillipsburg NJ
2. U.S.P. Grade, Lot. 9011402/727341, 8395054/727341 9124283/909349, J.T. Baker Chemical Co., Phillipsburg NJ
3. Double distilled water, Univ. of the Pacific, Stockton CA

coated magnetic stirring bar and stoppered with a ground glass stopper. The solution was then stirred magnetically[4] for a period of 48 hours. Each determination was done in triplicate and the experiment conducted in a constant temperature room. Prior to sampling, the stirring was stopped and the excess drug was allowed to settle. An aliquot of the supernatant was taken and filtered with 0.22u filter[5] using a syringe with a Swinnex adapter. The first five ml of the filtrate was discarded to avoid any error due to the possible saturation and adsorption to the filter. After appropiate dilution, the concentration was determined spectrophotometrically.

Assay Methods

Analysis of benzocaine was done on a spectophotometer[6] with a micro flow-through attachment. This allowed for small sample size and rapid sampling. The absorbance was recorded at 252nm (26). Beer's Law was obeyed throughout the concentration range studied (1.6 to 16.02 mcg/ml). A standard curve was prepared for each of the propylene glycol/water mixtures (Appendix A-F). From these curves, the saturation solubility of benzocaine in each of the systems was calculated.

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4. Vanlab DYLA-DUAL, V.W.R., San Francisco CA
5. Swinnex-25, Millipore Filter Corp., Bedford MA
6. Spectronic 710, Bausch and Lomb, Rochester, NY

Partition Coefficient

Equal volumes (100 ml) of isopropyl myristate[7] and different propylene glycol/water systems were allowed to equilibrate with respect to each other by stirring for 24 hours. After the stirring was stopped, the two phases were separated by means of a separatory funnel. Excess benzocaine was added to 25 ml of each of the propylene glycol/water fractions, stirred for 48 hours and filtered by means of millipore filter. Three ml of the saturated solution of benzocaine in propylene glycol/water system was then agitated for 4 days with 3 ml of the respective propylene glycol/water saturated isopropyl myristate solution in stoppered test tubes. The phases were separated by means of centrifugation and the propylene glycol/water phase was analyzed for loss of benzocaine to the isopropyl myristate. The partition coefficient value for each of the propylene glycol/water systems was then calculated as the ratio of benzocaine concentration in isopropyl myristate to that of propylene glycol/water.

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7. Ruger Chemical Co. Inc., Irvington NJ

In Vitro Penetration Studies

It has been reported by various authors (27) that there is great variability in penetration characteristics between human skin and animal skin. In vitro penetration studies done on excised human skin should provide a more valid representation of in vivo topical bioavailability in humans than the use of animal models. Therefore abdominal samples of human cadaver skin were used in all penetration studies.

Preparation of Skin: Abdominal skin samples obtained from autopsy were used as received. The skin sample was excised and placed in a plastic bag and stored in a freezer for periods up to but not exceeding three months. This method of storage has been reported not to damage the intergrity of the skin (28). Before the experiment the skin was removed from the freezer and allowed to thaw gradually at room temperature. When the sample was pliable, it was placed epidermal side down on a dissecting board. All connective and fatty tissues were removed by means of a scalpel. Most of the skin samples from a single donor were able to provide up to 10 - 12 cut pieces for use in the study.

Skin Cell: The skin cell consists of two parts. The bottom part is a glass chamber with a sampling port and is enclosed by a water jacket which allows for circulation of water at desired temperature. A Teflon-coated magnetic stir

bar was placed at the bottom of the chamber to ensure homogeneity in the chamber. The skin sample was placed in position on a O-ring between the two ball joints of the top and bottom chambers, using a pinch type, ground-glass joint clamp. This exposed an effective diffusion area of 2.01 cm^2. The top chamber was covered with parafilm during penetration studies to provide occlusion. Normal saline was injected into the lower chamber by means of a syringe and tubing[8]. Air bubbles that might be trapped on the dermal side of the skin were removed by tilting the skin cell and allowing them to escape via the sampling port. The cell was then mounted on top of a magnetic stirrer by means of a clamp. The temperature of the normal saline in the bottom chamber was maintained at 37 ± 0.5^{o} by circulating water from a constant temperature water bath[9] with a circulator pump. The skin sample was allowed to equilibrate in this condition for 24 hours. Before any formulation was applied to the skin, the saline in the bottom chamber was removed and replaced with fresh saline.

All experiments were carried out for at least 24 hours. Samples were withdrawn at appropriate time intervals

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8. Norton Corp., Akron OH

9. Haake Model-FE, V.W.R., San Francisco CA

and analyzed spectrophotometrically. All formulations were studied in duplicate. The data obtained from each run were computed and analyzed statistically by means of a computer[10]. The average amount penetrated per unit area per unit time was then plotted against time and the resultant steady state penetration rate was calculated.

10. Osborne 1, Osborne Computer Corp., Hayward, CA

RESULTS AND DISCUSSION

The factors which govern the passive diffusion of a solute from a vehicle into the skin are the physicochemical properties of the vehicle, the diffusant and the skin. The interaction of these three factors governs the rate of drug penetration. In vitro percutaneous absorption of benzocaine and other drugs in human cadaver skin have shown that there is considerable variation in steady state penetration rates, mostly attributable to the variability in human skin due to any number of factors such as skin age, skin condition, skin site, ethnic differences and skin metabolism. Thus the only valid comparisons are within the same skin sample and from the same general area of the body.

Propylene glycol has been reported to enhance percutaneous absorption of medicaments solubilized in it (28). Maximization of penetration rate from a vehicle requires the proper balance between the solubility of the drug in the vehicle and the skin/vehicle partition coefficient. From a plot of solubility of benzocaine in various propylene glycol/water mixtures and the partition coefficients (between propylene glycol/water mixtures and isopropyl myristate) against percent of propylene glycol, a mixture of propylene glycol and water possessing an optimum

penetration rate could be projected. This approach provided useful but empirical support to the selection of a 60/40 mixture of propylene glycol/water as the vehicle for further experimentation.

Solubility Studies

The benzocaine solubility was determined in a series of propylene glycol/water mixtures including the 60/40 mixture of propylene glycol/water containing one of the three adjuvants, namely urea[11], polyoxythylene (20) isohexadecyl ether (PIE)[12] or 1-dodecylazacycloheptan-2-one (Azone)[13].

The solubility of benzocaine in 0%, 20%, 40%, 60%, 80%, and 100% propylene glycol in water at 25±0.5° is shown in Table I. As can be seen, the solubility of benzocaine increased with increasing concentration of propylene glycol.

The solubility of benzocaine in a 60/40 mixture of propylene glycol and water together with various adjuvants was also determined. Increasing the concentration of urea from 0.11g/ml to 0.44g/ml showed a marked increase in the solubility of benzocaine (Table II). However, as can be

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11. 'Baker analyzed' Grade, Lot. 8113703, J.T. Baker Chemical Co., Phillipsburg NJ

12. ICI Americas Inc. Lot. 4526B, Wilmington DE

13. Nelson Research, Lot. 0157-132 Irvine CA

TABLE I

SOLUBILITY OF BENZOCAINE IN PROPYLENE GLYCOL/WATER SYSTEMS AT 25 ± 0.5°

PG/WATER V/V	SOLUBILITY (MG/ML)
100/0	123.75
80/20	46.40
60/40	17.00
40/60	7.75
20/80	4.25
0/100	2.00

TABLE II

BENZOCAINE SOLUBILITY IN PROPYLENE GLYCOL/WATER 60/40 SYSTEM CONTAINING UREA AT 25±0.5°

CONC. OF UREA (G/ML)	SOLUBILITY (MG/ML)
0.44	49.70
0.33	40.27
0.22	33.65
0.11	22.41
0	17.00

seen from the results given in Tables III and IV, polyoxyethylene (20) isohexadecyl ether and 1-dodecylazacycloheptan-2-one were less effective in increasing solubility. Increasing percentages of 1-dodecylazacycloheptan-2-one showed corresponding increase of 34% in benzocaine solubility was obtained. However, the solubility of benzocaine was found to fluctuate between 17-28 mg/ml as the concentration of polyoxyethylene(20) isohexadecyl ether was increased from 0 to 4 %.

TABLE III

BENZOCAINE SOLUBILITY IN PROPYLENE GLYCOL/WATER 60/40 SYSTEM CONTAINING ARLASOLVE 200 AT 25 ± 0.5°

ARLASOLVE 200 (% W/V)	SOLUBILITY (MG/ML)
4.00	24.29
3.00	28.02
2.00	22.72
1.00	19.99
0.75	20.74
0.50	25.22
0.25	18.87
0.10	20.18
0	17.00

TABLE IV

BENZOCAINE SOLUBILITY IN PROPYLENE GLYCOL/WATER 60/40 SYSTEM CONTAINING AZONE AT 25 ± 0.5°

AZONE (%V/V)	SOLUBILITY (MG/ML)
10	22.48
5	17.38
1	16.44
0.1	16.37
0	17.00

Partition Coefficient

The partition coefficient values of benzocaine in the two phase isopropyl myristate-propylene glycol/water system containing 20%, 40%, 60% and 80% propylene glycol in water are shown in Table V. These values were calculated as the ratio of benzocaine in isopropyl myristate phase to the amount of benzocaine remaining in propylene glycol phase. The solubility and partition coefficients of benzocaine as a function of percentage of propylene glycol in the solvent mixture are shown in Figs. 1-5. The relationship between solubility and the partition coefficient with the increasing percentage of propylene glycol in the system is graphically shown in Fig 1. and can be expressed by the following equations respectively:

$$\ln(\text{solubility}) = 0.041(\%\ \text{of PG}) + 0.571$$

$$\ln(\text{partition coefficient}) = -0.025(\%\ \text{of PG}) + 1.510$$

As the percentage of propylene glycol in the vehicle increased, the partition coefficient was decreased, confirming the increasing tendency of the drug molecules to remain in the vehicle with increasing fraction of propylene glycol in the vehicle. This behavior negated the benefit resulting from the increased solubility of the drug in the donor phase. The choice of using isopropyl myristate as a partitioning phase was based on the assumption that it might mimic skin lipids and the stratum corneum (30). It is possible that by using another phase system e.g. water/oil,

TABLE V

PARTITION COEFFICIENT OF BENZOCAINE IN ISOPROPYL MYRISTATE/PROPYLENE GLYCOL-WATER SYSTEM at 22 ± 0.5°

PROPYLENE GLYCOL (% V/V)	PARTITION COEFFICIENT
100	0.204
80	1.133
60	1.432
40	1.358
20	2.808
0	3.897

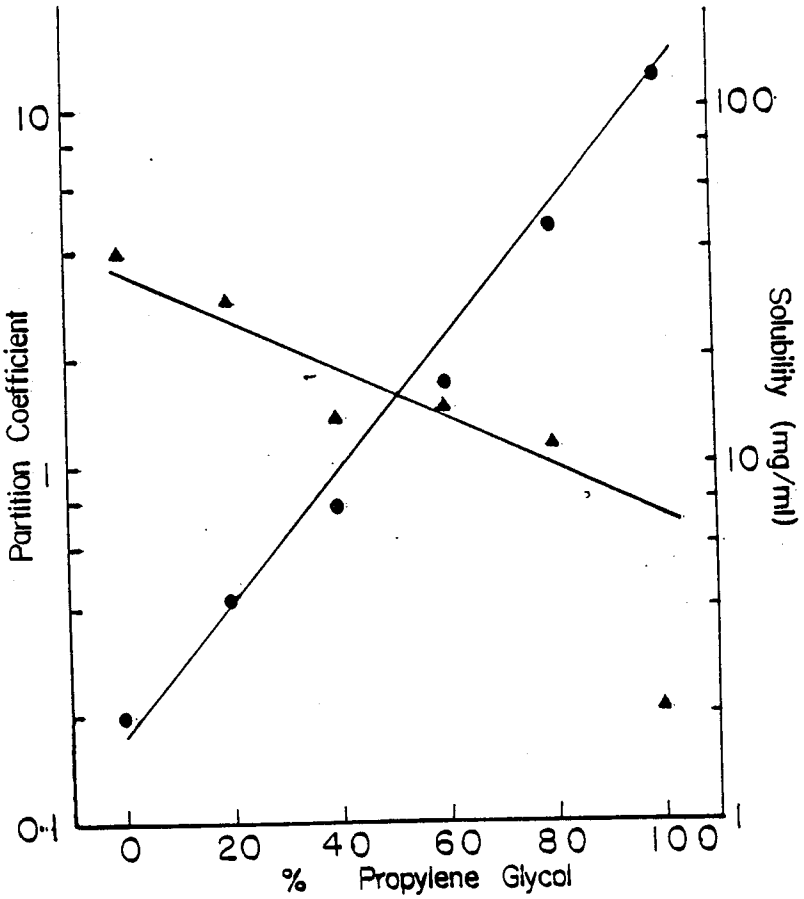

Fig. 1. Semi-log plot of solubility (●) and partition coefficient (▲) profile of benzocaine versus percent propylene glycol

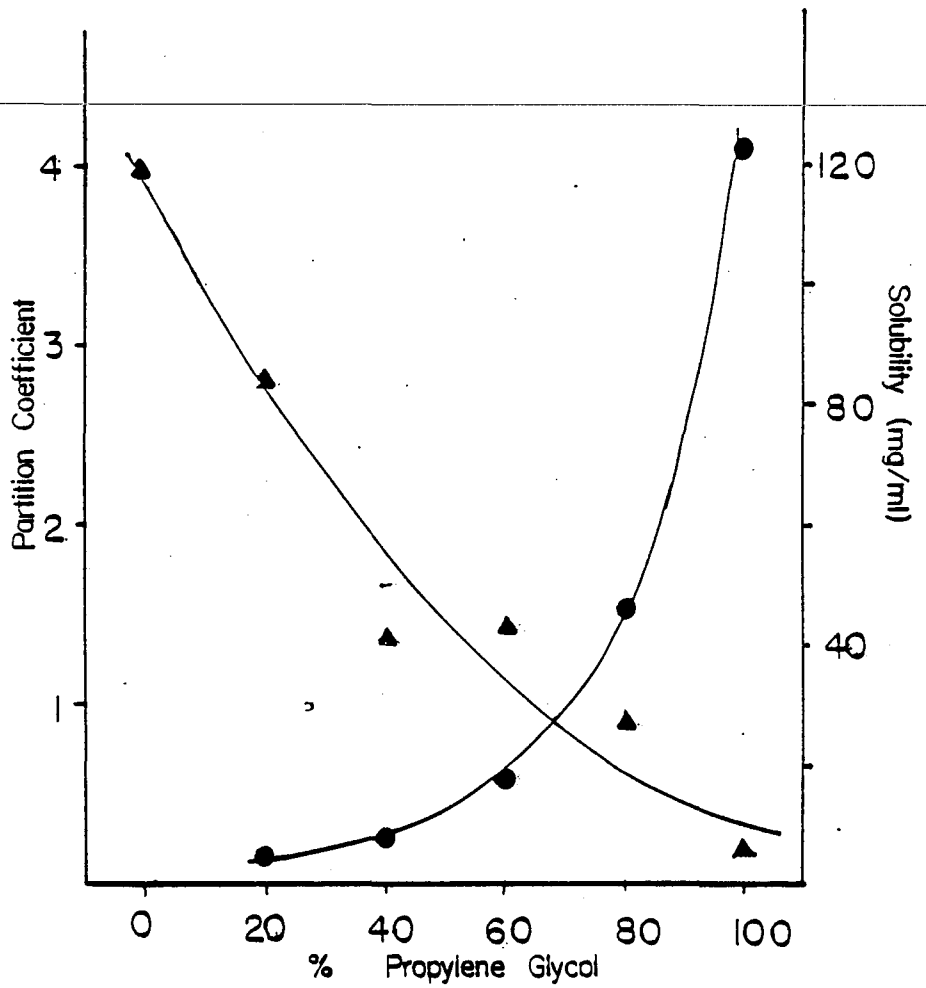

Fig. 2. Plot of solubility (●) and partition coefficient (▲) profile of benzocaine versus percent propylene glycol

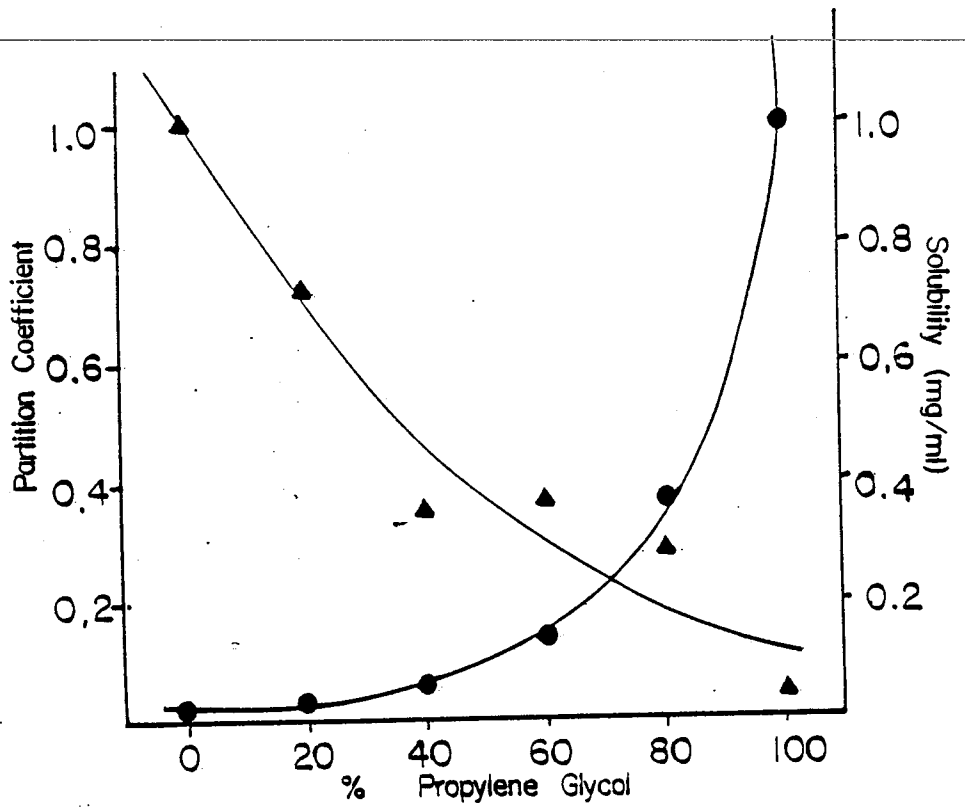

Fig. 3. Plot of fraction of maximum solubility (●) and partition (▲) coefficient profile of benzocaine versus percent propylene glycol

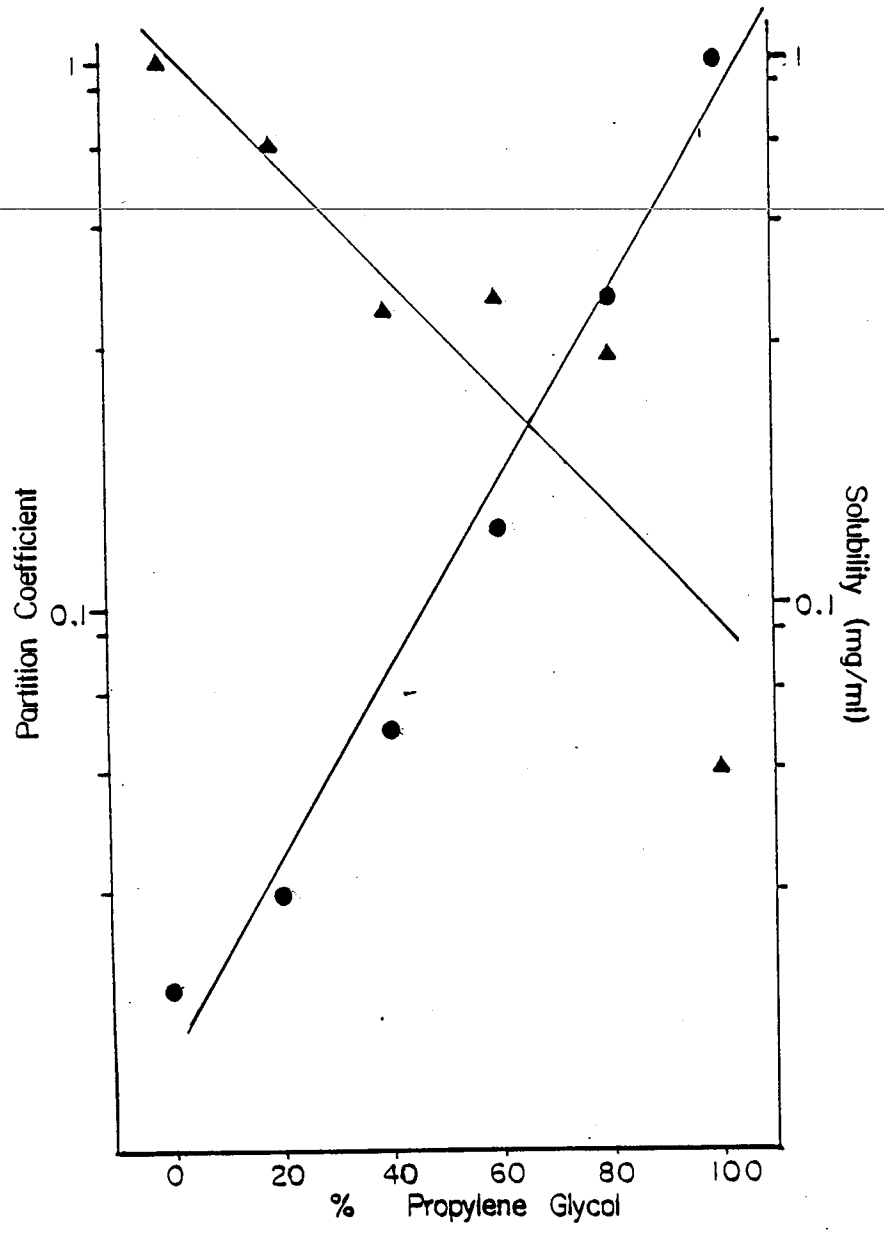

Fig. 4. Semi-log plot of fraction of maximum solubility (▲) and partition coefficient (●) profile of benzocaine versus percent propylene glycol

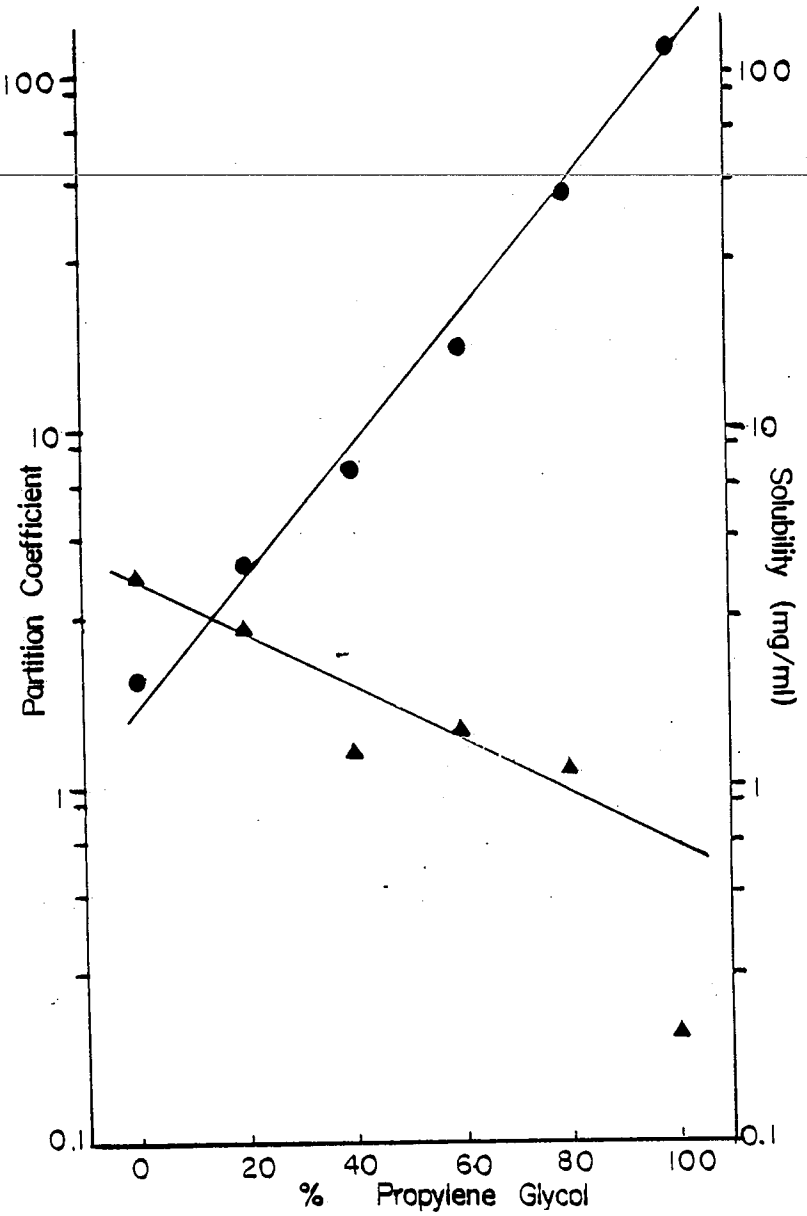

Fig. 5. Semi-log plot of solubility (●) and partition coefficient (▲) profile of benzocaine versus percent propylene glycol (equal scale)

that different values might be obtained. Moreover, determination of the one vehicle with the optimum partition and solubility characteristics based upon this approach creates a real dilemma as can be seen from the various plots (Fig 1-5). The theorectical bases of these various plots were gleaned from previous works (28-30). The majority of our plots predicted that a 60-70 percent propylene glycol/water system should possess optimal solubility and partitioning properties. Therefore, a mixture of 60/40 (V/V) propylene glycol/water was chosen for further investigations.

Penetration Studies

Unless otherwise indicated, all penetration studies were initiated using the maximum possible concentration of benzocaine in the vehicle. The penetration data were analyzed by plotting Q (amount penetrated per unit area) against time. A regression analysis of the data was performed and 95% confidence limits of the steady-state region of the penetration curve were calculated by a computer. The different slopes were then compared to the control by means of the t-test see if the lines were signifigantly parallel (statistical analysis program, Appendix F). In order to overcome the differences of skin samples from different donors and to establish a yardstick to compare the results of various experiments, a standard control with propylene glycol/water 60/40 (V/V) and 17 mg/ml of benzocaine was included in all experiments. The observed penetration rates of the control range from a low of 7.48 to a high of 16.48 mcg/cm^2/hr with an average of 11.93 mcg/cm^2/hr. These variations in control values could be explained on the basis of the variability of human skin previously discussed. The relative rates were obtained by dividing the rate for the control into the rates for the test vehicles.

In Vitro Percutaneous Penetration of Benzocaine from Propylene Glycol/Water Mixtures

The first exploratory study of skin penetration of benzocaine was done using different mixtures of propylene glycol and water (80/20, 60/40, 40/60, 20/80). It has been reported by Turi et al. (28) that propylene glycol penetrates the skin of the hairless mouse rather easily, and that skin resistence decreases as the percentage of propylene glycol is increased. From the results, as could be seen from Table VI and Fig. 6, there seems to be a general trend in agreement with this hypothesis, but as the concentration of benzocaine in each of the systems is different, a direct correlation could not be made. It should also be noted that the consistancy of the thermodynamic flux was not maintained during the penetration studies. However, there was no dramatic change in the penetration rate as the percentage of propylene glycol in the mixture was increased -- even though statistically they were different ($p<0.01$) as compared to the rate of penetration of the 60/40 (V/V) propylene glycol/water mixture. The observed rate of penetration from 60/40 propylene glycol/water system did not correlate with the empirically predicted optimum penetration rate based on the solubility and partitioning studies (Fig. 1). Realistically, the true determination of partition coefficient as it would relate to skin penetration could

TABLE VI

PENETRATION OF BENZOCAINE FROM PROPYLENE GLYCOL/WATER SYSTEMS THROUGH FULL THICKNESS HUMAN SKIN

PG/WATER SYSTEM (V/V)	RATE ($MCG/CM^2/HR$)	CORRELATION COEFFICIENT	LAG TIME (HR)
80/20	8.54 ($\pm$0.22)	0.998	0.24
60/40	7.48 ($\pm$0.20)	0.998	-0.13
40/60	6.61 ($\pm$0.23)	0.997	-0.60
20/80	8.93 ($\pm$1.23)	0.955	-0.13

Number in parentheses indicate the 95% confidence limit of the respective slope.

Benzocaine was near saturation in the above solutions.

The rates from propylene glycol/Water 80/20, 40/60, 20/80, were signifigantly different ($p<0.01$) as compared to propylene glycol/water 60/40.

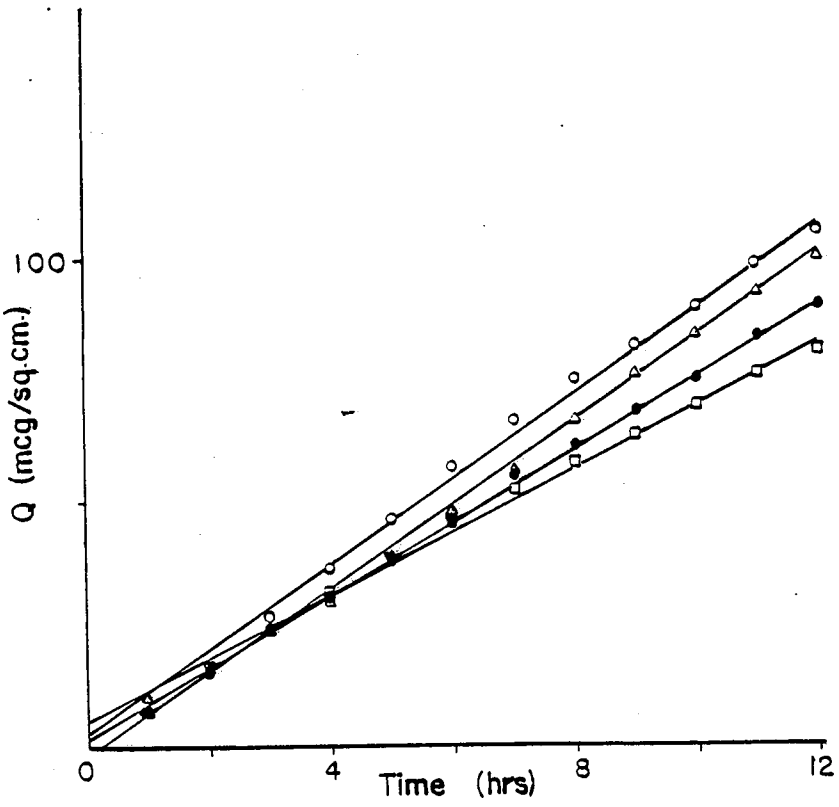

Fig. 6. Penetration of benzocaine from propylene glycol/ water systems through full thickness human skin : Q versus t plot
Key: % Propylene glycol/water (V/V) △ 80/20
● 60/40 □ 40/60 ○ 20/80

only be obtained between the vehicle and isolated stratum corneum.

In Vitro Percutaneous Penetration of Benzocaine from DMSO/Water Mixtures

DMSO[14] did not enhance the penetration of benzocaine into the stratum corneum as compared to the control of 60/40 propylene glycol/water as shown in Table VII, Fig. 7, ($p>0.05$). This could be due in part to the increased solubility of benzocaine in DMSO/water system and consequent decrease in partitioning of benzocaine into the receptor skin. While the concentration of 17mg/ml of benzocaine was at saturation level for the propylene glycol/water system and thus had a higher thermodynamic flux to penetrate into the skin, such was not the case with respect to benzocaine in DMSO/water mixture. On the other hand, it has been shown by some authors (11) that in order for DMSO to enhance penetration of certain compounds, a critical percentage of DMSO (60-80%) is necessary. The previously discussed phenomenon of increased hydration of the skin by DMSO could lead to dilution of medicaments applied on the skin thus underscoring the importance of proper experimental design based upon constant thermodynamic flux throughout the duration of release.

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14. 'Baker analyzed' grade, Lot. 8216688, J.T. Baker Chemical Co., Phillipsburg NJ

TABLE VII

PENETRATION OF BENZOCAINE FROM DMSO/WATER SYSTEMS THROUGH FULL THICKNESS HUMAN SKIN.

DMSO/WATER SYSTEM (V/V)	RATE ($MCG/CM^2/HR$)	RELATIVE RATE	CORRELATION COEFFICIENT	LAG TIME (HR)
80/20	16.06 ($\pm$3.17)	0.995	0.919	-0.36
60/40	14.39 ($\pm$3.41)	0.892	0.882	-0.93
40/60	17.28 ($\pm$2.05)	1.071	0.966	-0.19
20/80	15.58 ($\pm$0.73)	0.965	0.994	0.09
CONTROL	16.14 ($\pm$0.74)	1	0.995	-0.59

Control = propylene glycol/water 60/40.

Concentration of benzocaine in all systems was 17mg/ml.

The rates of all DMSO/Water curves did not show any signifigant difference as compared to control ($p<0.05$).

The numbers in parentheses indicate 95% confidence limits of the respective slope.

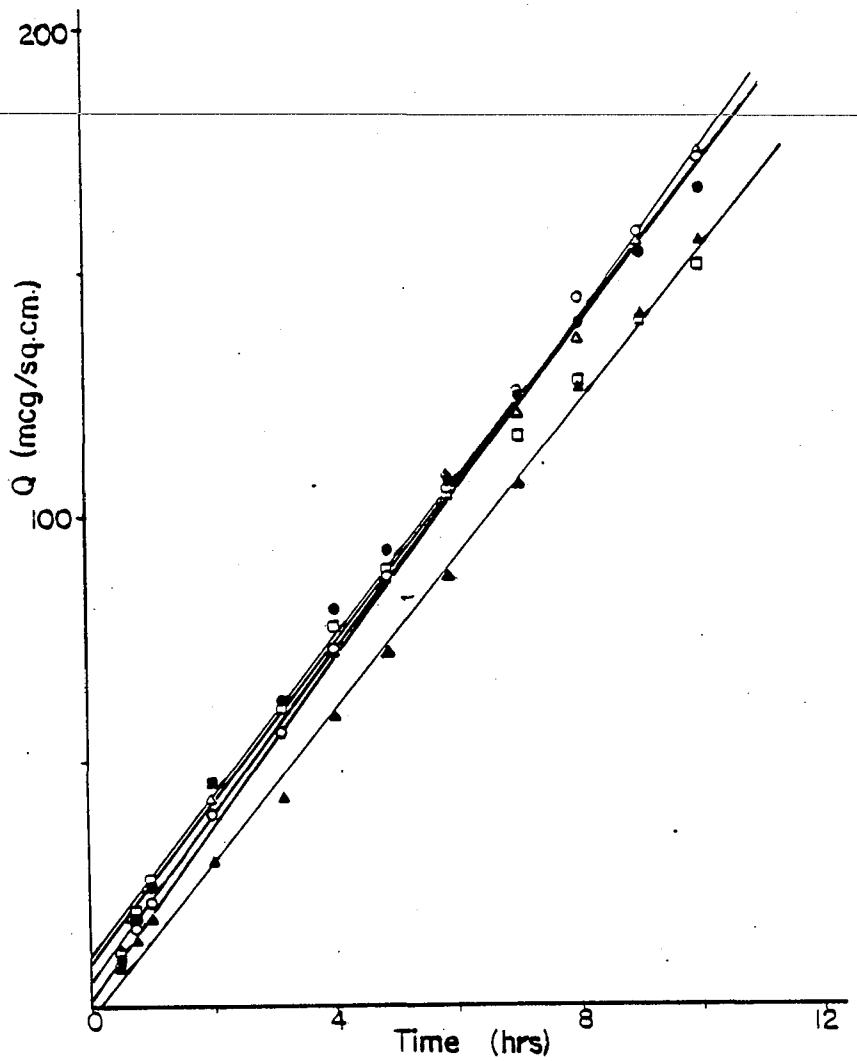


Fig. 7. Penetration of benzocaine from DMSO/water systems through full thickness human skin : Q versus t plot
Key: % DMSO/water (V/V) △ 80/20 □ 60/40 ○ 40/60 ▲ 20/80 ● Control

Effect of Urea on the Percutaneous Penetration of Benzocaine

Urea was incorporated into propylene glycol/water 60/40 mixture in the following amounts: 0.11g/ml, 0.22g/ml, 0.33g/ml, 0.44g/ml. The saturation solubility of urea in propylene glycol water 60/40 mixture was determined to be 0.44g/ml. In the initial studies as shown in Table VIII and Fig. 8, urea did not enhance the penetration of benzocaine; in fact, it appeared to retard the penetration of benzocaine. This lack of enhancement could be due to the increased affinity of benzocaine for the vehicle containing urea thus reducing the partitioning of benzocaine into the skin. Inadequate thermodynamic flux might have contributed to the findings. Feldman and Gibaldi (23) have reported that urea effectively breaks up the clusters of hydrogen bonded water surrounding the nonpolar molecules in aqueous solution resulting in an increase in the entropy of the system thus providing the energy for breaking up of hydrophobic bonds between nonpolar molecules. The net result is an increased solubility of nonpolar molecules in the aqueous medium.

In the second set of experiments with urea (Table IX, Fig. 9), there was initial saturation of benzocaine with respect to each of the solutions. In this case, the urea enhanced the initial penetration of benzocaine into the stratum corneum (Fig. 10), but after the initial hour, all

TABLE VIII

PENETRATION OF BENZOCAINE FROM PG/WATER 60/40 SYSTEM CONTAINING UREA THROUGH FULL THICKNESS HUMAN SKIN: I

CONC. OF UREA (G/ML)	RATE ($MCG/CM^2/HR$)	RELATIVE RATE	CORRELATION COEFFICIENT	LAG TIME (HR)
0.44	11.73 ($\pm$2.43)	0.878	0.996	-2.10
0.33	14.93 ($\pm$2.61)	1.118	0.997	-1.52
0.22	14.51 ($\pm$0.84)	1.087	0.999	0.20
0.11	15.98 ($\pm$2.45)	1.197	0.998	-1.25
CONTROL	13.36 ($\pm$1.13)	1	0.999	-0.52

Control = propylene glycol/water 60/40 V/V.

Concentration of benzocaine in all systems was 17 mg/ml.

Maximum solubility of urea in propylene glycol/water 60/40 was 0.44 g/ml.

The rates of benzocaine penetration from all vehicles were significantly different from control ($p<0.01$).

The numbers in parentheses indicate 95% confidence limits of the respective slopes.

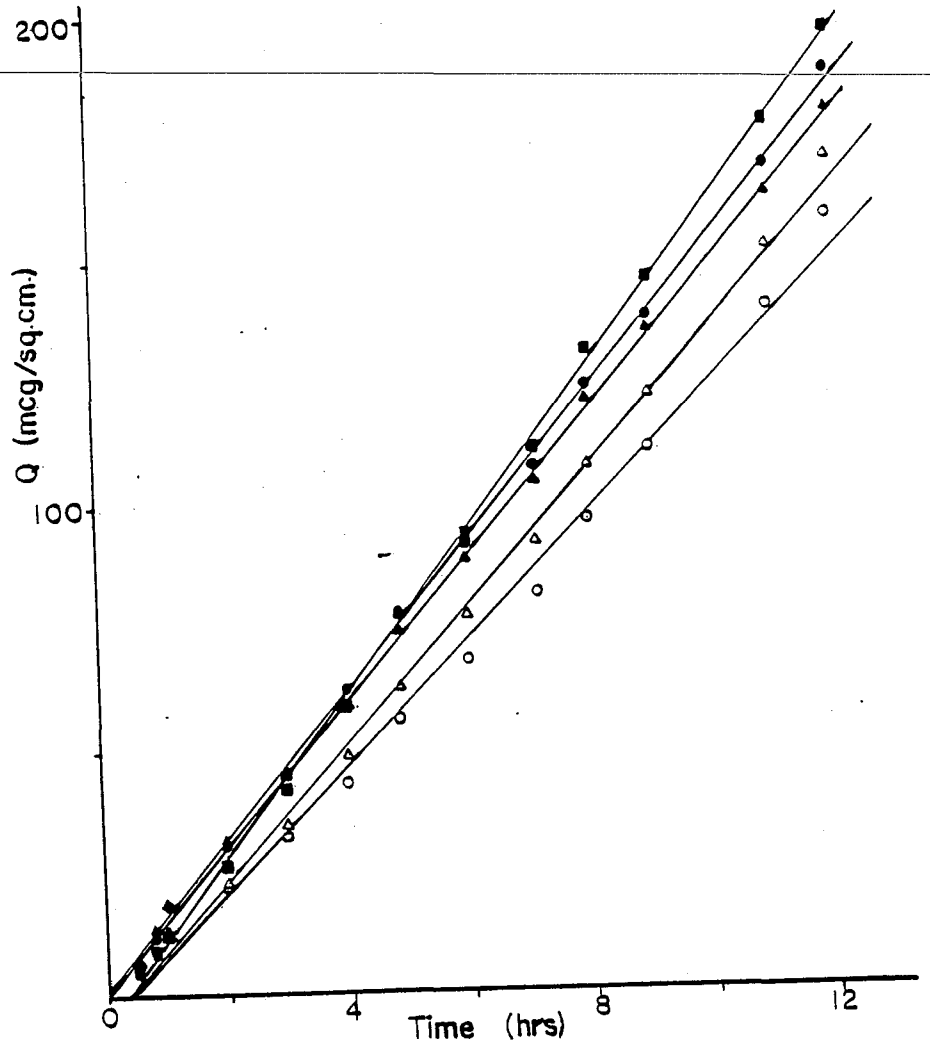


Fig. 8. Penetration of benzocaine from propylene glycol/water 60/40 system containing urea through full thickness human skin: I - Q versus t plot
Key: Conc. of urea (g/ml) ▲ 0.44 ○ 0.33
■ 0.22 △ 0.11 ● Control

TABLE IX

PENETRATION OF BENZOCAINE FROM PROPYLENE GLYCOL/WATER 60/40 SYSTEM CONTAINING UREA THROUGH FULL THICKNESS HUMAN SKIN: II

CONC. OF UREA (G/ML)	RATE ($MCG/CM^2/HR$)	RELATIVE RATE	CORRELATION COEFFICIENT	LAG TIME (HR)
0.44	14.72 ($\pm$0.71)	0.958	0.994	-0.13
0.33	12.88 ($\pm$2.37)	0.838	0.916	0.35
0.22	16.68 ($\pm$0.79)	1.085	0.994	0.31
0.11	14.09 ($\pm$0.44)	0.917	0.997	0.33
CONTROL	15.37 ($\pm$0.90)	1	0.991	-0.04

Control = propylene glycol/water 60/40 V/V.

Concentration of benzocaine in all system were near saturation with respect to the solution.

Maximum solubility of urea in propylene glycol/water 60/40 was 0.44 g/ml.

The rates of benzocaine penetration from all vehicles were significantly different from control ($p<0.01$) except the vehicle containing 0.44gm/ml urea which was not signifigantly different ($p>0.05$).

The numbers in parentheses indicate 95% confidence limits of the respective slopes.

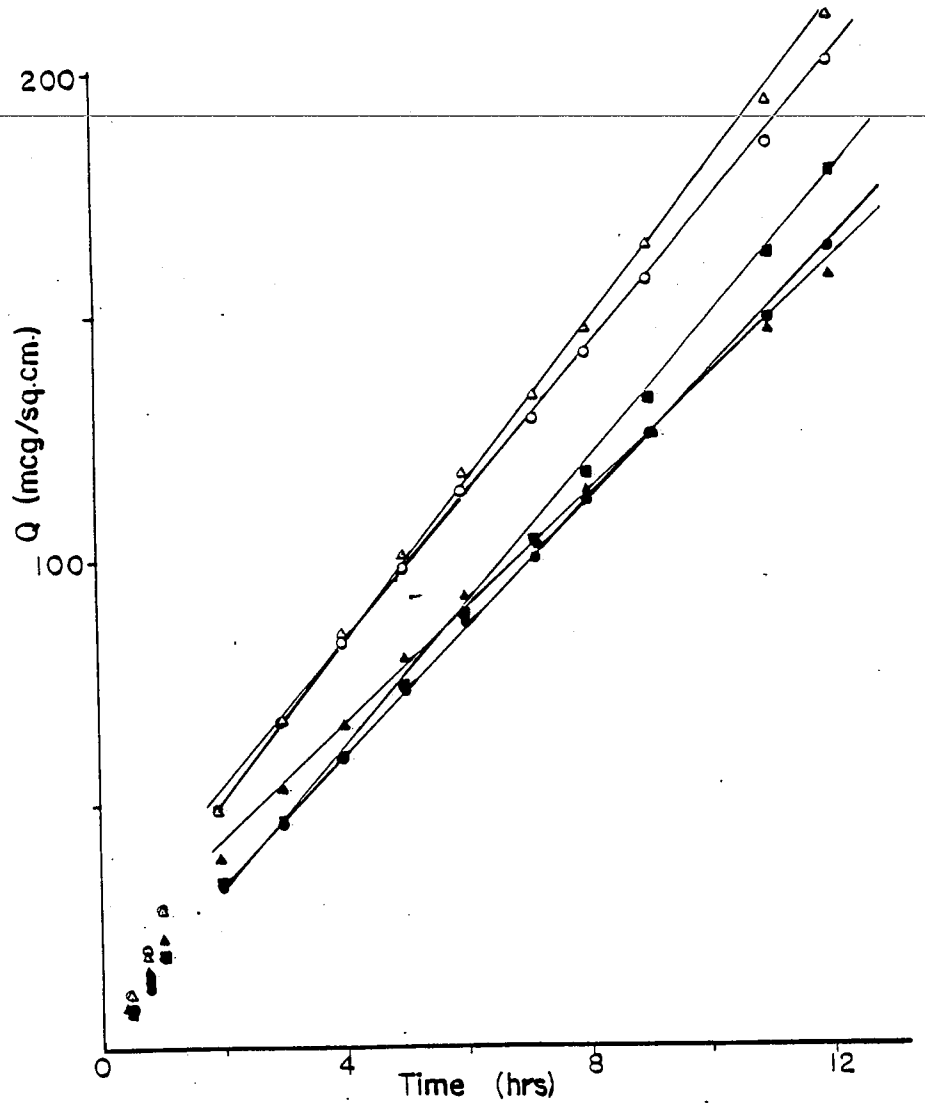


Fig. 9. Penetration of benzocaine from propylene glycol/water 60/40 system containing urea through full thickness human skin: II - Q versus t plot
Key: Conc. of urea (g/ml) ▲ 0.44 o 0.33 ■ 0.22 △ 0.11 ● Control

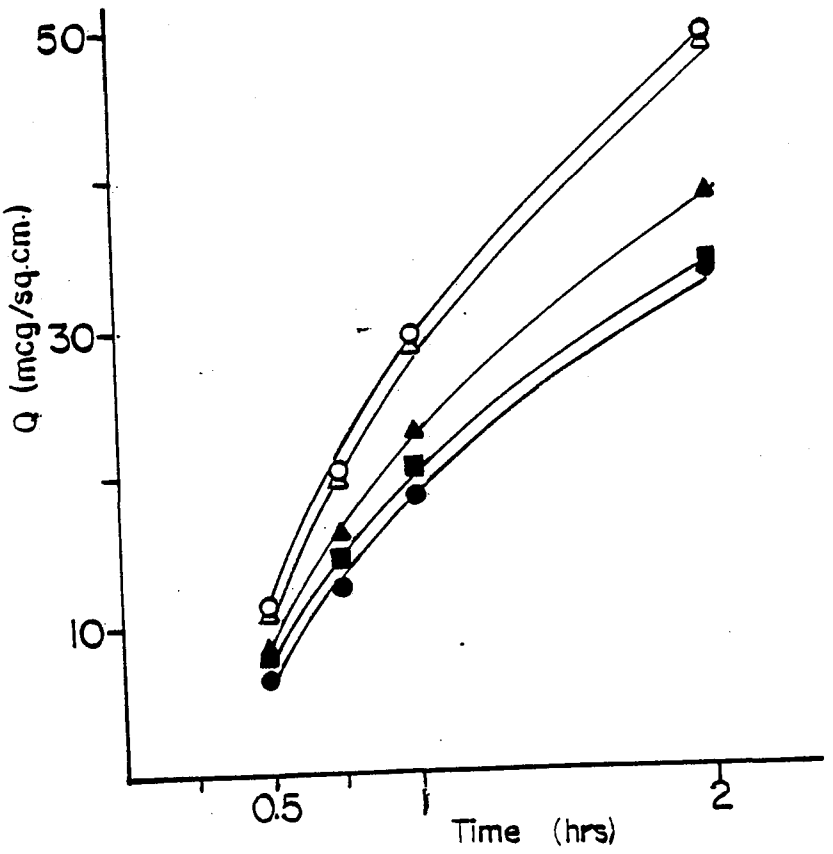


Fig. 10. Initial penetration of benzocaine from propylene glycol/water 60/40 system containing urea through full thickness human skin: II - Q versus t plot
Key: Conc. of urea (g/ml) ▲ 0.44 ○ 0.33
■ 0.22 △ 0.11 ● Control

the rates were essentially the same. The observed biphasic nature of the penetration curve could be due to drug loss from the donor solution. The hydration effect of urea on stratum corneum could be a time-related phenomenon. Thus if the skin was pretreated with urea solution and the penetration experiment conducted, the enhancement effect of urea might be more pronounced. However, urea did not exert its potential penetration enhancing effect even with increased concentration of benzocaine.

Effect of Polyoxyethelene (20) Isohexadecyl Ether on the Percutaneous Penetration of Benzocaine

Polyoxythylene (20) isohexadecyl ether (PIE), a nonionic surfactant has been marketed as a solubilizer (31). The critical micelle concentration (CMC) of PIE was found to be between 0.005-0.009% (W/V). This was in agreement with the reported CMC value provided by the supplier (0.005-0.01%) (31).

The results shown in Table X and Fig. 11 revealed that PIE retarded, rather than enhanced, the penetration of benzocaine through full thickness human skin in the concentration range studied. This could be due in part to micellar solubilization of the drug. The resulting decrease in partitioning of benzocaine into the skin coupled with decreasing thermodynamic flux could account for the observed effect. The penetration rate of benzocaine was found to decrease as PIE concentration approached the CMC and increased as it exceeded the CMC. Above the CMC, less surfactants are available for interaction with the skin, provided that PIE does interact with the skin. This effect in conjunction with the previouly discussed phenomenon of micelle formation, might be used to explain the change in penetration rate of benzocaine around the CMC. The penetration rate of benzocaine from the PIE formulations was signifigantly ($p<0.01$) lower than from the control. Dalvi and Zatz (21) while studying nonionic surfactant

TABLE X

PENETRATION OF BENZOCAINE FROM PROPYLENE GLYCOL/WATER 60/40 SYSTEM WITH ARLASOLVE 200 THROUGH FULL THICKNESS HUMAN SKIN

ARLASOLVE 200 CONC. (% W/V)	RATE ($MCG/CM^2/HR$)	RELATIVE RATE	CORRELATION COEFFICIENT	LAG TIME (HR)
0.0008	15.87 (±0.99)	0.967	0.990	0.37
0.005	14.18 (±1.17)	0.882	0.983	-0.11
0.007	10.60 (±1.95)	0.646	0.924	-0.14
0.009	14.63 (±1.43)	0.892	0.979	-0.07
0.07	15.23 (±3.59)	0.934	0.883	-0.02
CONTROL	16.41 (±5.78)	1	0.799	-0.47

Control = propylene glycol/water 60/40 (V/V).

Concentration of benzocaine in all system was 17 mg/ml.

CMC of Arlasolve 200 was within the range 0.005 - 0.009%.

The rates of benzocaine penetration of all vehicles were signifigantly different from the control ($p<0.01$).

The numbers in parentheses indicate 95% confidence limits of the respective slopes.

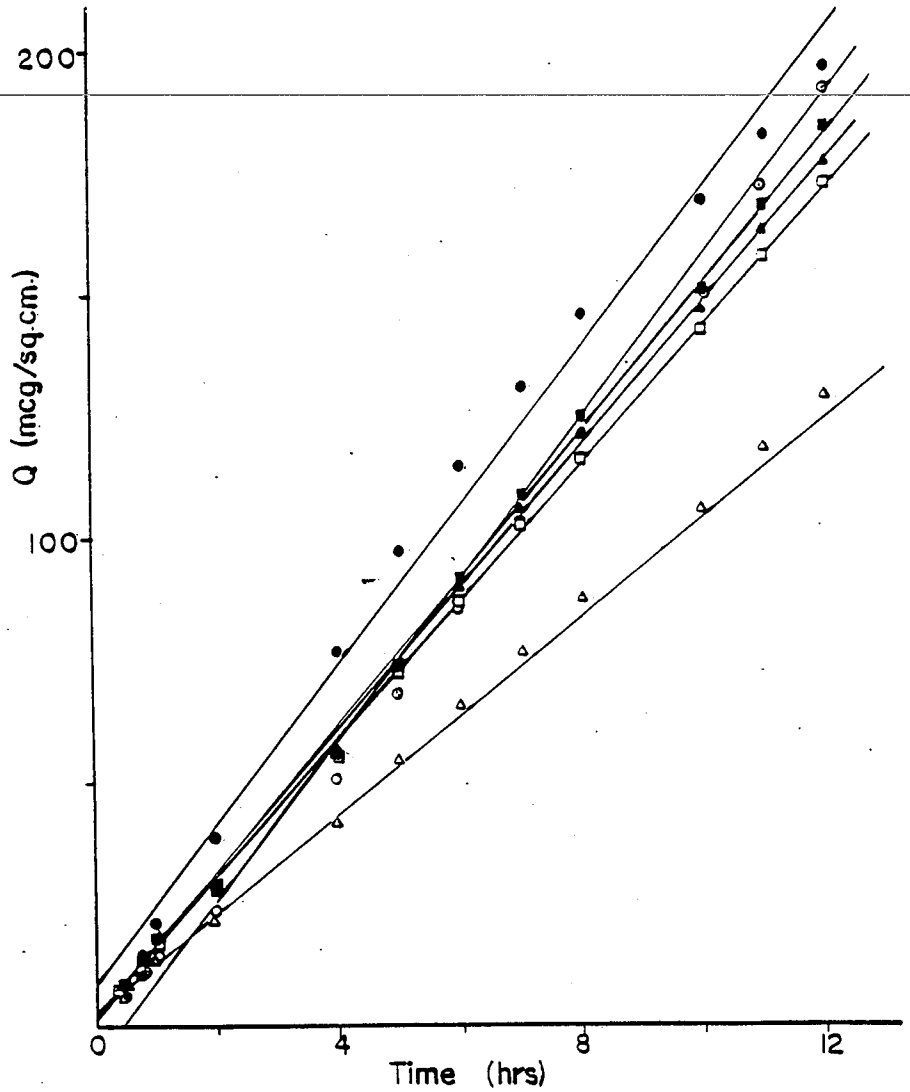


Fig. 11. Penetration of benzocaine from propylene glycol/water 60/40 system with PIE through full thickness human skin : Q versus t plot
Key: Conc. of Arlasolve 200 (W/V)
○ 0.0008% □ 0.005% △ 0.007%
▲ 0.009% ■ 0.07% ● Control

effects on the penetration of dissolved benzocaine through hairless mouse skin observed that penetration flux from solutions containing a fixed benzocaine concentration was inversely related to surfactant concentration. For solutions saturated with benzocaine, surfactant concentration did not influence the benzocaine flux. The penetration of benzocaine was proportional to the free benzocaine concentration. Benzocaine solubilized by micellar entrapment would not be available for skin penetration. The foregoing interpretation by Dalvi and Zatz could not be applied directly to the PIE data in view of widely different levels of surfactant concentrations employed in the two studies and the difference in permeability of hairless mouse skin as compared to human skin. Their studies also employed significantly higher levels of surfactant concentration (1-5% W/V). It would appear that surfactant effects on penetration rate at and around CMC are also related to free drug concentration. Additional studies covering a wider range of PIE concentrations with varying drug concentration are warranted in order to fully understand the nonionic surfactant effects of PIE on drug penetration.

Effect of Azone on the Percutaneous Penetration of Benzocaine

Azone (1-dodecylazacycloheptan-2-one) has been reported to enhance the percutaneous absorption of a variety of drugs (14-16). Azone was found to be immiscible with water or other polar solvents or their mixtures. In order to facilitate its incorporation into our system of propylene glycol/water 60/40, an emulsion and a gel were formulated. Since PIE was used previously and its percutaneous penetration characteristics were known, a formulation using it as a surfactant/emulsifier was prepared. The gel system was prepared with Klucel LF (hydroxypropyl cellulose)[15], as it provided the desired viscosity at the concentration chosen. The four concentrations of Azone studied were 0.1%, 1%, 5%, and 10% (V/V).

1. Azone in an emulsion vehicle with PIE

As could be seen from Table XI and Fig. 12, Azone appreciably enhanced the penetration of benzocaine through full thickness human skin. The rate of penetration in the steady state region was greatest with 5% Azone (5% > 1% > 10% > 0.1% > control). However in the initial two-hour period (Fig. 13), the rate was greatest with 1% Azone (1% > 5% = 0.1% > 10% > control). It is interesting to note that

15. Grade MF, Lot. 2981, Hercules Inc., Wilmington DE

TABLE XI

EFFECT OF EMULSIFIED AZONE ON PERCUTANEOUS PENETRATION OF BENZOCAINE FROM PROPYLENE GLYCOL/WATER 60/40 SYSTEM

CONC OF AZONE (% V/V)	RATE ($MCG/CM^2/HR$)	RELATIVE RATE	CORRELATION COEFFICIENT	LAG TIME (HR)
10%	13.60 (±1.84)	1.296	0.968	1.17
5%	21.63 (±2.21)	2.062	0.987	1.77
1%	16.71 (±1.25)	1.593	0.987	-1.71
0.1%	12.80 (±0.50)	1.220	0.997	-0.26
CONTROL	10.49 (±0.31)	1	0.997	-0.23

Control = propylene glycol/water 60/40 (V/V).

Concentration of benzocaine in all system was 17 mg/ml.

0.5% (W/V) Arlasolve 200 was used in all systems to emulsify azone.

The rates of all the Azone curves were signifigantly different from the control curve ($p<0.01$).

The numbers in parentheses indicate 95% confidence limits of the respective slope.

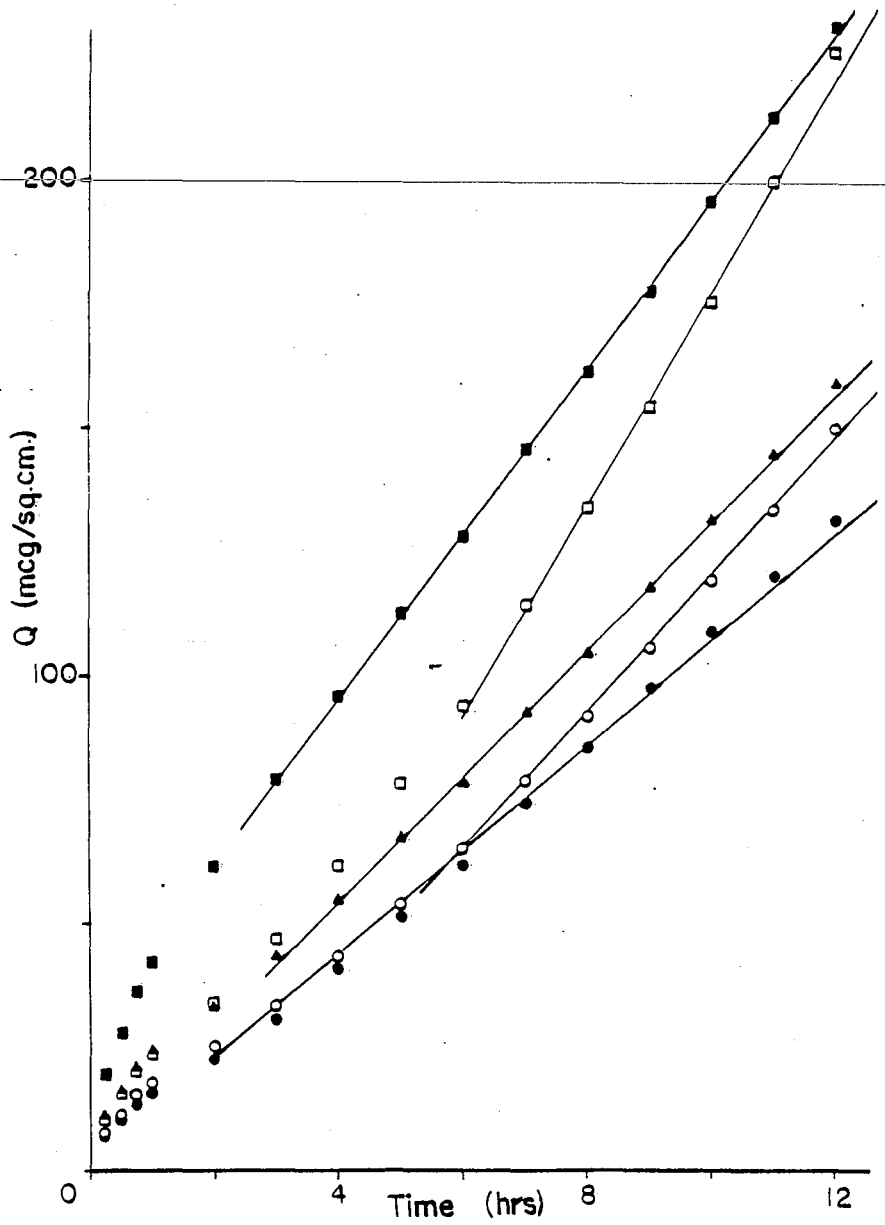


Fig. 12. Effect of emulsified Azone on percutaneous penetration of benzocaine from propylene glycol/water 60/40 system : Q versus t plot
Key: Conc. of Azone (V/V) o 10% □ 5%
■ 1% ▲ 0.1% ● Control

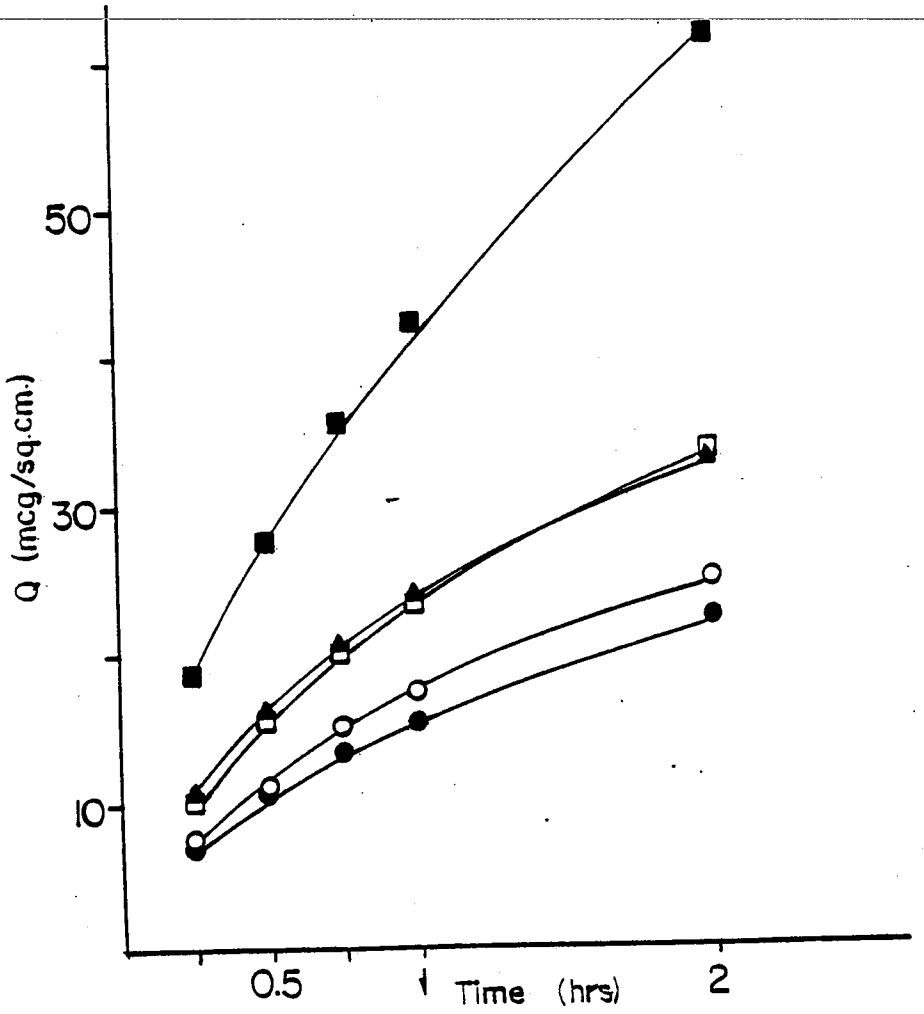


Fig. 13. Initial effect of emulsified Azone on percutaneous penetration of benzocaine from propylene glycol/water 60/40 system : Q versus t plot
Key: Conc. of Azone (V/V) ○ 10% □ 5%
■ 1% ▲ 0.1% ● Control

Azone also overcame the mildly inhibiting effect of PIE on the penetration rate of benzocaine.

2. Azone in a gel system with 4%W/V Klucel LF

Azone was found to enhance the percutaneous penetration of benzocaine. The rate of enhancement in the steady state region was greatest with 5% Azone (5% > 1% > 0.1% > 10% > control). Again, similar results were obtained in the initial two-hour period as obtained from the emulsion system (Table XII, Fig 14), i.e. Azone was the most effective enhancer at 1% concentration during the early stages (Fig. 15) of drug penetration (1% > 0.1% > 5% > 10% > control).

3. Gel system with excess of benzocaine.

In view of the promising potential of Azone as a penetration enhancer for benzocaine as determined from the previous two sets of experiments, a formulation of propylene glycol/water and azone with excess benzocaine was prepared. The excess benzocaine in the system provided maximal thermodynamic flux throughout the penetration period. The resultant penetration curve was biphasic with two distinct steady state regions (Table XIII, Fig. 16-17). This could be due to some artifact of the skin sample used or to the enhancement mechanism of Azone at work. The concentration of Azone that seems to provide the maximal penetration rate was 5%.

TABLE XII

EFFECT OF GELLED AZONE ON PERCUTANEOUS PENETRATION OF BENZOCAINE FROM PROPYLENE GLYCOL/WATER 60/40 SYSTEM

CONC. OF AZONE (%V/V)	RATE ($MCG/CM^2/HR$)	RELATIVE RATE	CORRELATION COEFFICIENT	LAG TIME (HR)
10%	9.11 (±0.17)	1.205	0.998	-0.77
5%	17.04 (±1.06)	2.254	0.998	1.20
1%	16.36 (±0.97)	2.164	0.997	-1.10
0.1%	11.42 (±0.54)	1.511	0.998	0.29
CONTROL	7.56 (±0.19)	1	0.999	-0.72

Control = propylene glycol/water 60/40 (V/V) with 4% Klucel LF.

Concentration of benzocaine in all system was 17 mg/ml.

The rates for all azone containing system were signifigantly different from the control ($p<0.01$).

The numbers in parentheses indicate 95% confidence limits of the relative slopes.

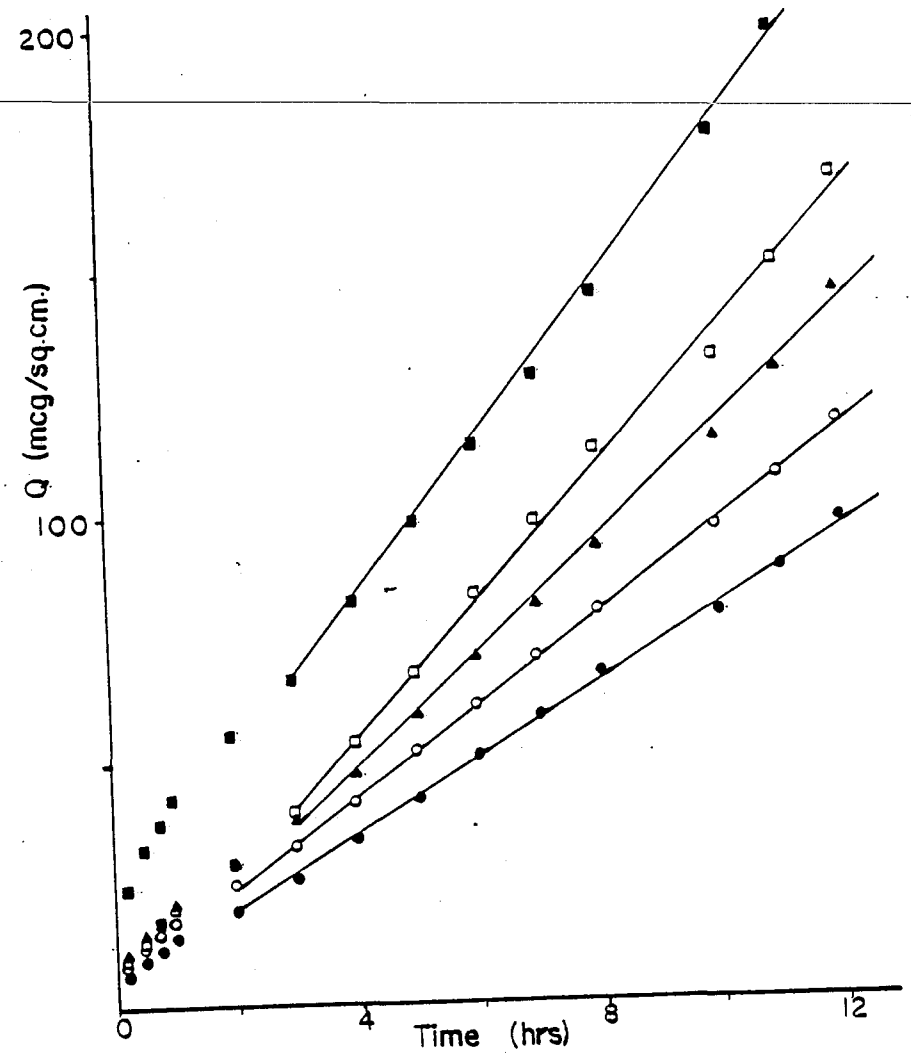


Fig. 14. Effect of gelled Azone on percutaneous penetration of benzocaine from propylene glycol/water 60/40 system : Q versus t plot
Key: Conc. of Azone (V/V) ○ 10% □ 5%
■ 1% ▲ 0.1% ● Control

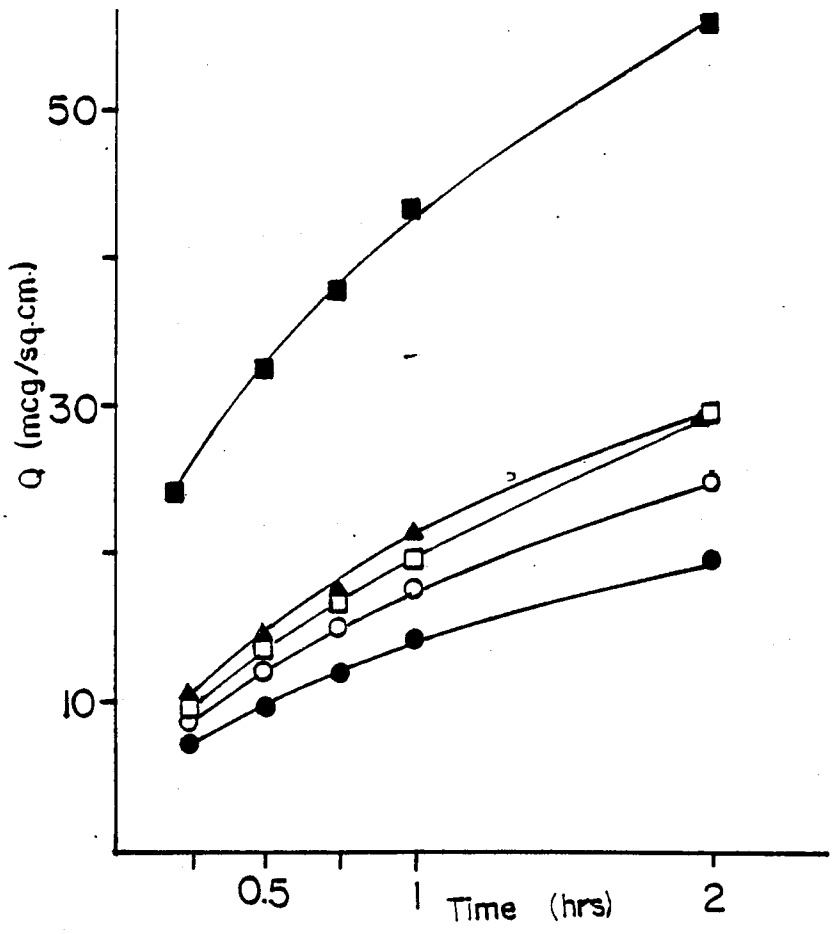


Fig. 15. Initial effect of gelled Azone on percutaneous penetration of benzocaine from propylene glycol/water 60/40 system : Q versus t plot
Key: Conc. of Azone (V/V) ○ 10% □ 5%
■ 1% ▲ 0.1% ● Control

TABLE XIII

EFFECT OF GELLED AZONE ON PERCUTANEOUS PENETRATION OF BENZOCAINE FROM PROPYLENE GLYCOL/WATER 60/40 SYSTEM WITH EXCESS BENZOCAINE

CONC. OF AZONE (% V/V)	RATES ($MCG/CM^2/HR$)		CORRELATION COEFFICIENT	
	I	II	I	II
10%	27.63 (±7.06)	14.24 (±1.15)	0.969	0.987
5%	19.29 (±0.37)		0.999	
1%	15.50 (±1.73)	19.25 (±9.74)	0.979	0.850
0.1%	9.11 (±0.53)	17.11 (±3.06)	0.995	0.969
CONTROL	9.99 (±0.54)	16.09 (±0.61)	0.996	0.999

Control = propylene glycol/water 60/40 (V/V) with 4% Klucel LF.

Concentration of Benzocaine in all formulations was 50 mg/ml.

The numbers in parentheses indicate 95% confidence limits of the respective slopes.

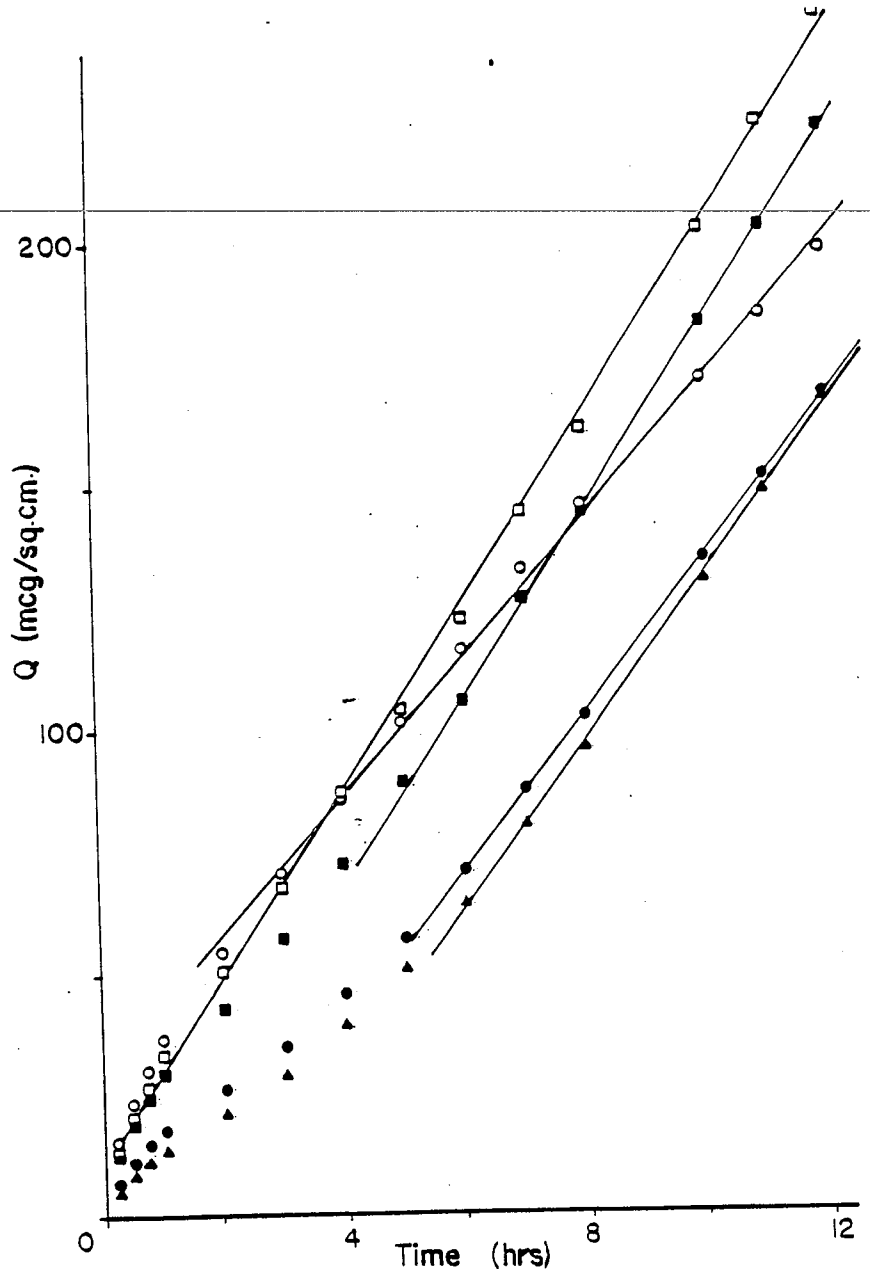


Fig. 16. Effect of gelled Azone on percutaneous penetration of benzocaine from propylene glycol/water 60/40 system with excess benzocaine : Q versus t plot
Key: Conc. of Azone (V/V) ○ 10% □ 5%
■ 1% ▲ 0.1% ● Control

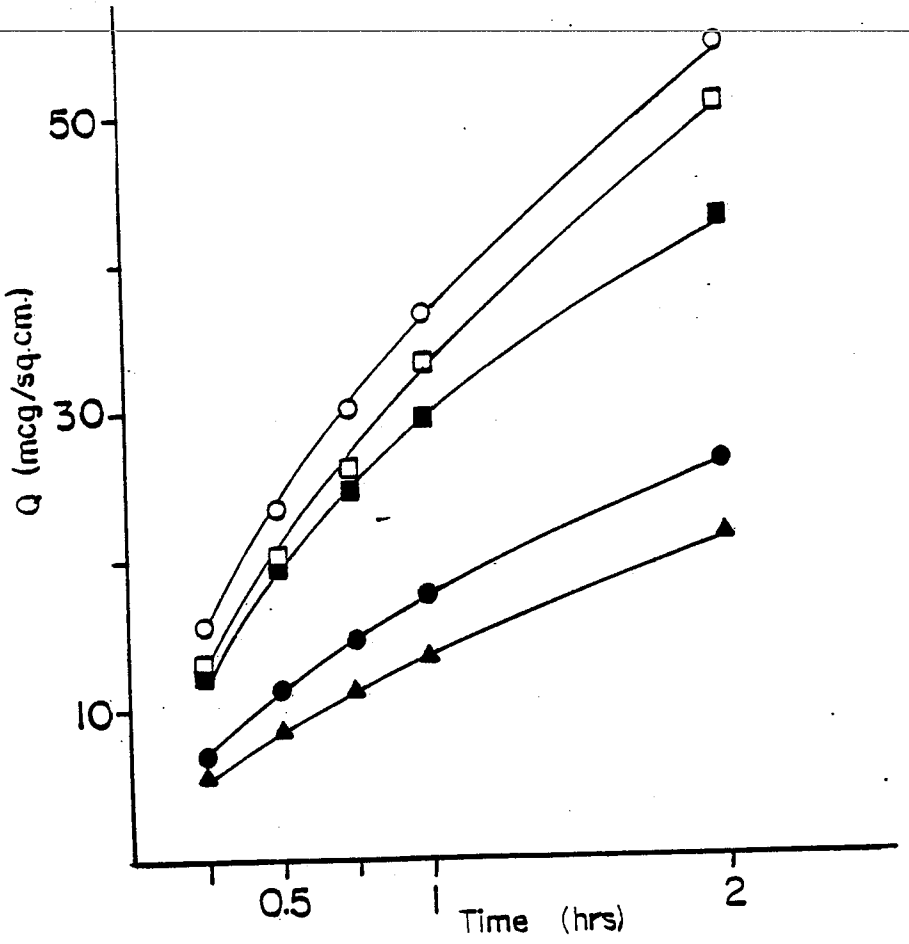


Fig. 17. Initial effect of gelled Azone on percutaneous penetration of benzocaine from propylene glycol/water 60/40 system with excess benzocaine : Q versus t plot
Key: Conc. of Azone (V/V) ○ 10% □ 5%
■ 1% ▲ 0.1% ● Control

4. Azone in a gel system with excess benzocaine with various percentage of propylene glycol.

These sets of experiments were conducted since propylene glycol has also been reported to possess some concentration dependent percutaneous enhancement effects of its own (28). Since 5% Azone was the concentration that exerted this maximal penetration enhancement effect, a series of gelled formulations with increasing percentages of propylene glycol were prepared with 5% Azone, and percutaneous penetration of benzocaine investigated to find the optimal propylene glycol concentration. The concentration of benzocaine employed was in excess so as to provide for maximum thermodynamic force. The data presented in Table XIV and Fig. 18 reveal that rate of penetration of benzocaine is favored by increasing the concentration of propylene glycol.

From these experiments, it can be concluded that Azone exhibits a concentration-dependent enhancement of percutaneous penetration of benzocaine. At 10% Azone in the formulation when benzocaine level was not at saturation, Azone was able to overcome this thermodynamic deficit and still enhance the penetration. The biphasic nature of the penetration curves may be suggestive of but not conclusive of a change in mechanism of penetration of benzocaine in the presence of Azone. In each of the experiments with

TABLE XIV

PENETRATION OF BENZOCAINE FROM GELLED PROPYLENE GLYCOL/WATER SYSTEMS WITH 4% KLUCEL LF AND 5% AZONE THROUGH FULL THICKNESS HUMAN SKIN

PG/WATER SYSTEM (V/V)	RATE ($MCG/CM^2/HR$)	RELATIVE RATE	CORRELATION COEFFICIENT	LAG TIME (HR)
80/20	55.37 ($\pm$2.89)	2.496	0.999	3.67
60/40	38.37 ($\pm$2.31)	1.730	0.999	3.49
40/60	45.26 ($\pm$1.49)	2.041	0.999	2.39
20/80	40.00 ($\pm$1.05)	1.803	0.999	2.89
0/100	34.98 ($\pm$0.79)	1.577	0.997	2.65
0/100[NO AZONE]	22.18 ($\pm$0.94)	1	0.999	2.08

All of the above formulations contain 60 mg/ml of benzocaine.

All the rates were signifigantly different from the propylene glycol/water 0/100 control with no Azone.

The numbers in parentheses indicate 95% confidence limits of the respective slopes.

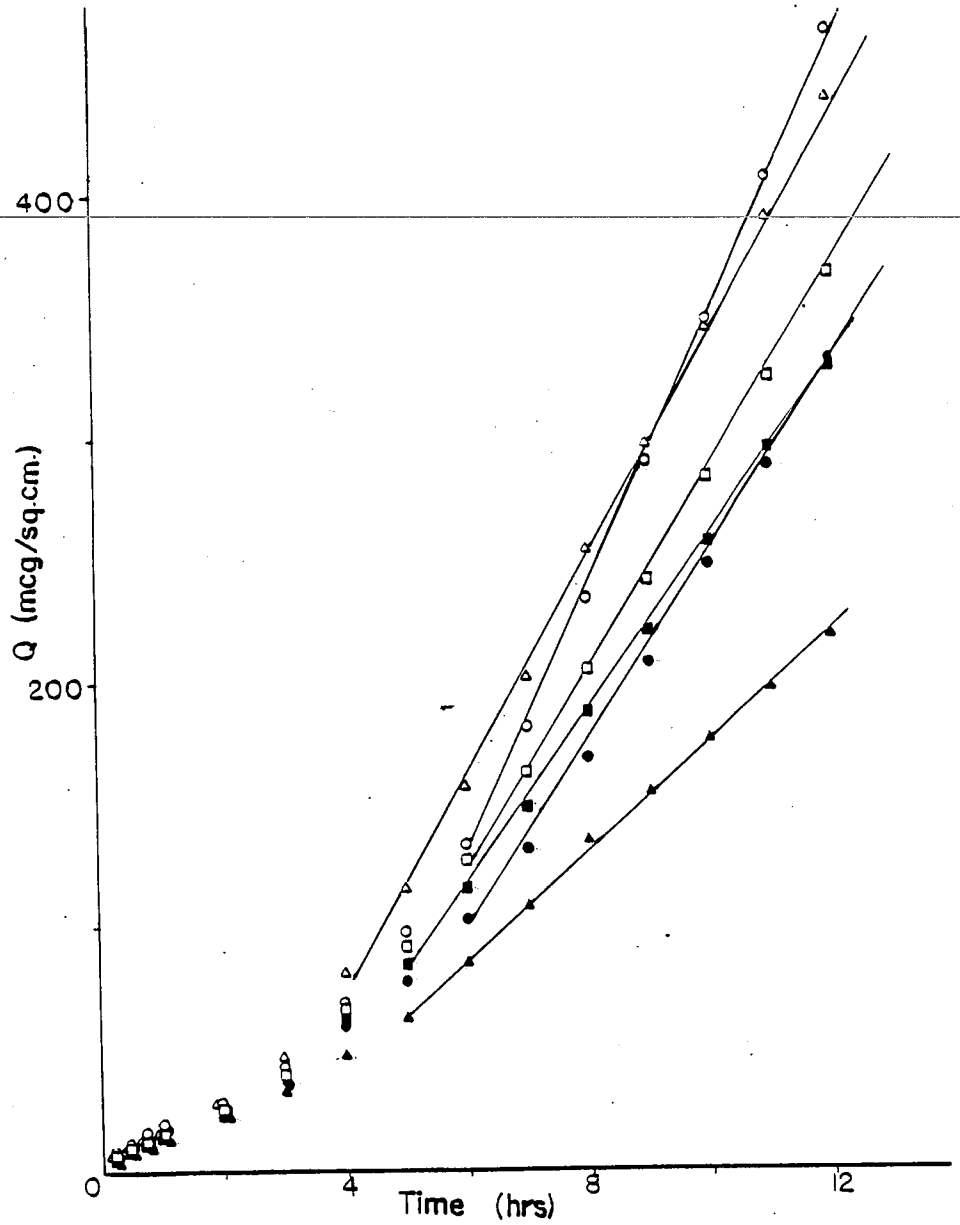


Fig. 18. Penetration of benzocaine from gelled propylene glycol/water systems with 4% Klucel LF and 5% Azone through full thickness human skin : Q versus t plot
Key: % Propylene glycol/water (V/V) ○ 80/20
● 60/40 △ 40/60 □ 20/80 ■ 0/100
▲ Control

Azone, there was definite enhancement in the initial two hour period before steady state was reached. The pattern of high initial rates followed by slightly lower steady-state rates later suggests that Azone produces significant structural changes in the stratum corneum, lowering its resistance to drug penetration. However, these changes are possibly reversible due to dilution during subsequent equilibrium. If Azone is bound to the epidermis, it may create channels in between the flattened cells of the stratum corneum and allow for the passage of solubilized drugs. Through gross examination of the stratum corneum at the end of each set of runs, the stratum corneum was found to be intact with no visible changes. While our findings corraborate the results of other workers (14,15) as to the enhancement effect of Azone, they have failed to provide any additional insights into the mechanism of action of Azone. Further research mainly directed towards this goal is strongly recommended.

CONCLUSIONS

The *in vivo* lack of efficacy of benzocaine during clinical trials of many OTC products has been reported by various authors (3,7,33). This is in part due to the fact that benzocaine does not effectively penetrate the stratum corneum. In the present study, the effects of a number of dosage form-related parameters were investigated with a view to enhance penetration of benzocaine topically in order to achieve rapid relief from itch and pain.

1. Benzocaine solubility and partitioning characteristics in propylene glycol/water solvent systems were studied to enable identification of optimum levels. The *in vitro* findings with human cadaver skin did not support the empirical prediction of the partitioning study. This lack of predictability demonstrates the limitations of a simple two-phase system. However, if partition studies were performed using isolated stratum corneum, better predictive capability might be expected.
2. The *in vitro* penetration studies strongly suggested that thermodynamic flux should be maintained at a constant during the penetration period to achieve optimal results and to allow one to draw meaningful conclusions regarding penetration enhancement effects.
3. Of the various adjuvants investigated, Azone was

found to be the most effective penetration enhancer for topical benzocaine. It appears to have its maximal effect on steady-state rates at a 5% V/V concentration and on initial rates of penetration at even lower Azone levels (1% V/V).

4. Under the conditions of the experiment, DMSO did not enhance topical penetration of benzocaine. A definitive conclusion must await further studies under conditions of constant thermodynamic flux together with higher percentages of DMSO.

5. Interestingly, urea was found to enhance benzocaine penetration initially during the first hour only. The lack of any significant steady-state rate enhancement by urea even at saturated levels of benzocaine could be explained on the basis of a shift of thermodynamic flux as the experiment progressed.

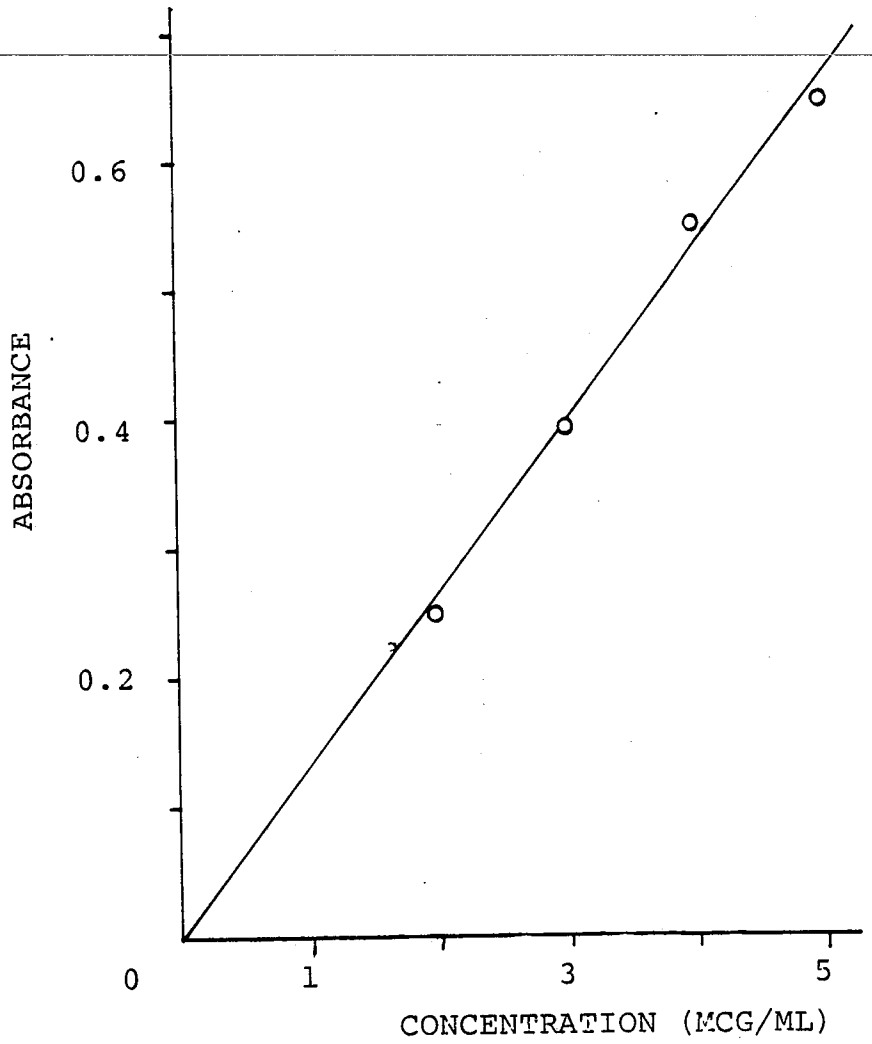
6. The results with polyoxyethylene (20) isohexadecyl ether are inconclusive since study was conducted over a narrow range of surfactant concentration. A proper understanding of its observed effects requires a more rigorous investigation of its effects on solubility and partitioning of benzocaine.

7. Propylene glycol did exhibit some penetration enhancement effect. This effect was additive in the experiments with azone.

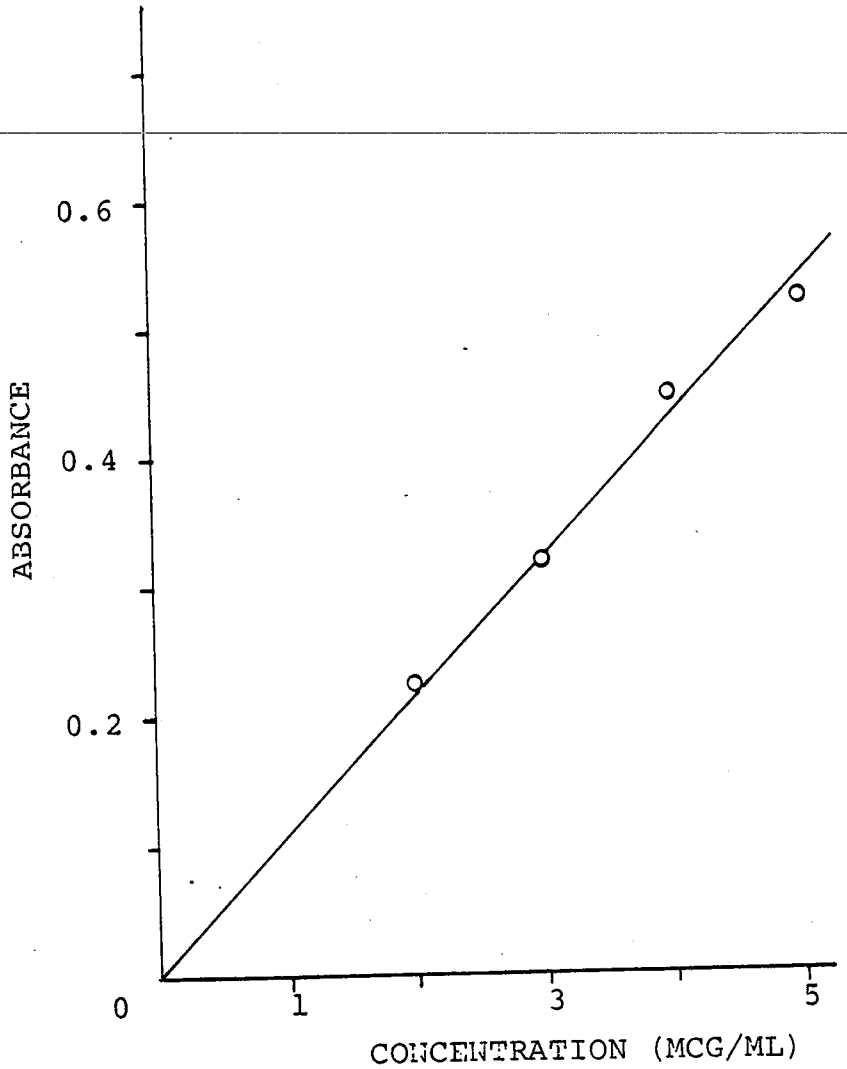
In vitro penetration studies have intrinsic limitations and the results of this investigation presently serve only as guidelines for further *in vitro* studies. The variability of the skin samples and even the variability within one skin sample and the chosen experimental design preclude drawing definitive conclusions at this time. Nevertheless it is generally agreed that *in vitro* experiment on human cadaver skin are far more reliable indicators of clinical action than *in vitro* models utilizing animal skins.

A propylene glycol/water mixture has been shown to be a suitable vehicle for topical application of benzocaine. Results indicated that favorable solubility, partitioning and skin penetration could be obtained from such a system. The classical approach of adjusting the physico-chemical properties of the solvent system to that of the drug and the skin in order to enhance percutaneous absorption has been validated by this study.

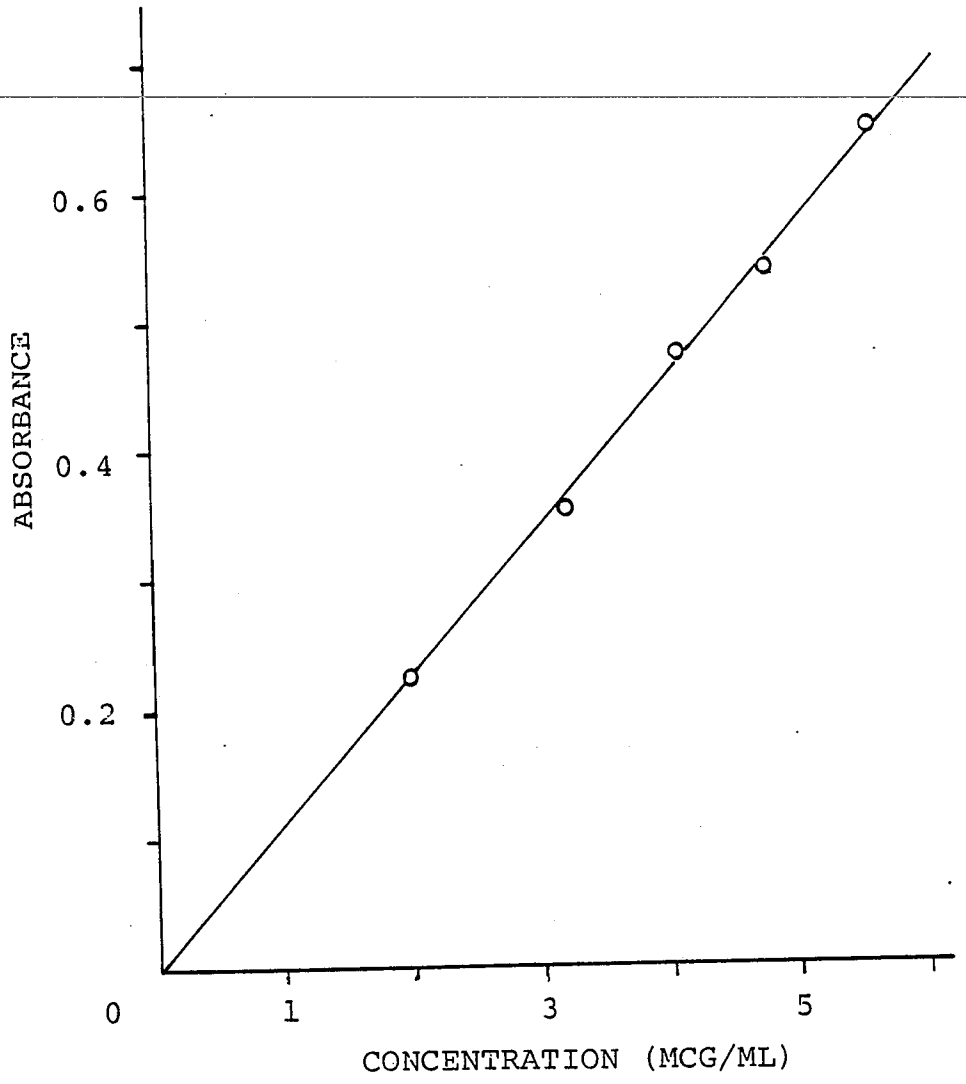
Significantly high initial rates of penetration of benzocaine with Azone strongly warrant further *in vitro* and *in vivo* examination . If the observed findings are corroborated *in vivo*, a successful commercial product is a real possibility.



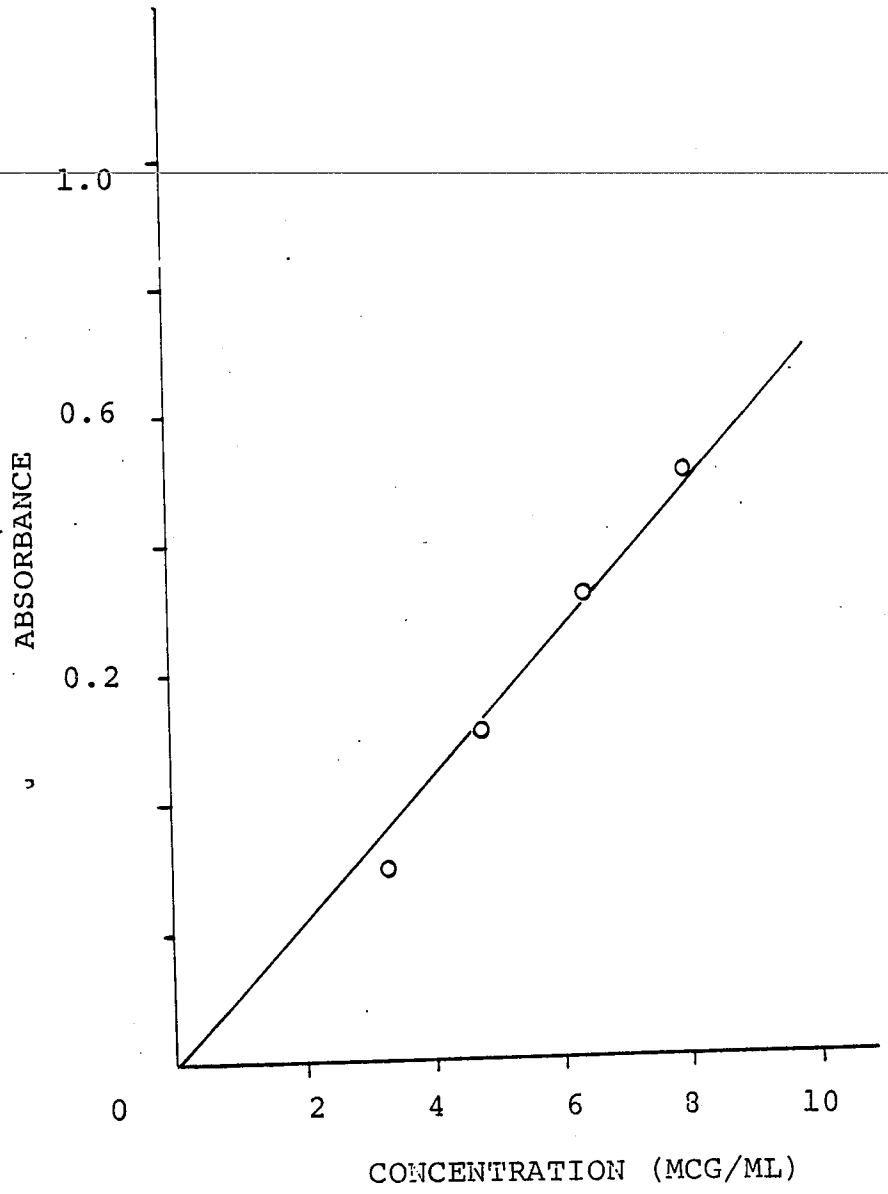
APPENDIX A: Beer's law plot of benzocaine in propylene glycol/water 100/0 (V/V); each point represents the average of three determinations.



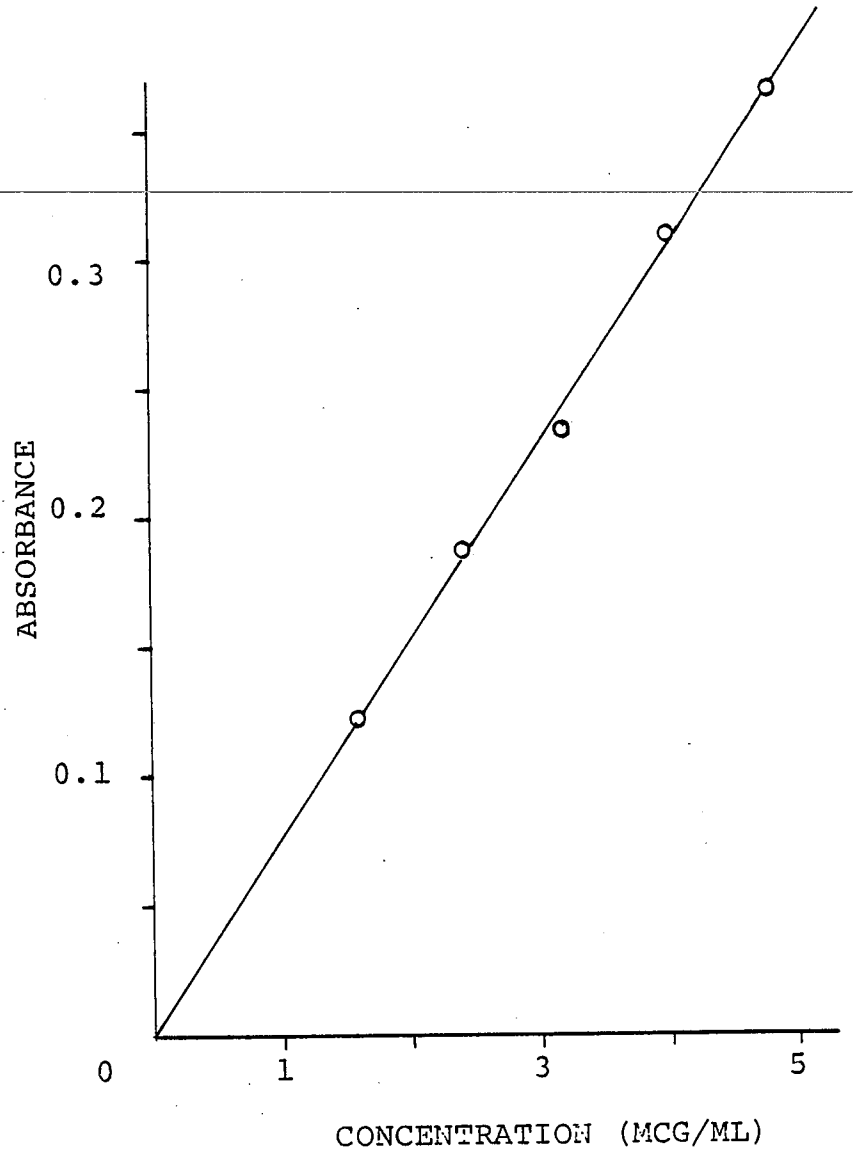
APPENDIX B: Beer's law plot of benzocaine in propylene glycol/water 80/20 (V/V); each point represents the average of three determinations.



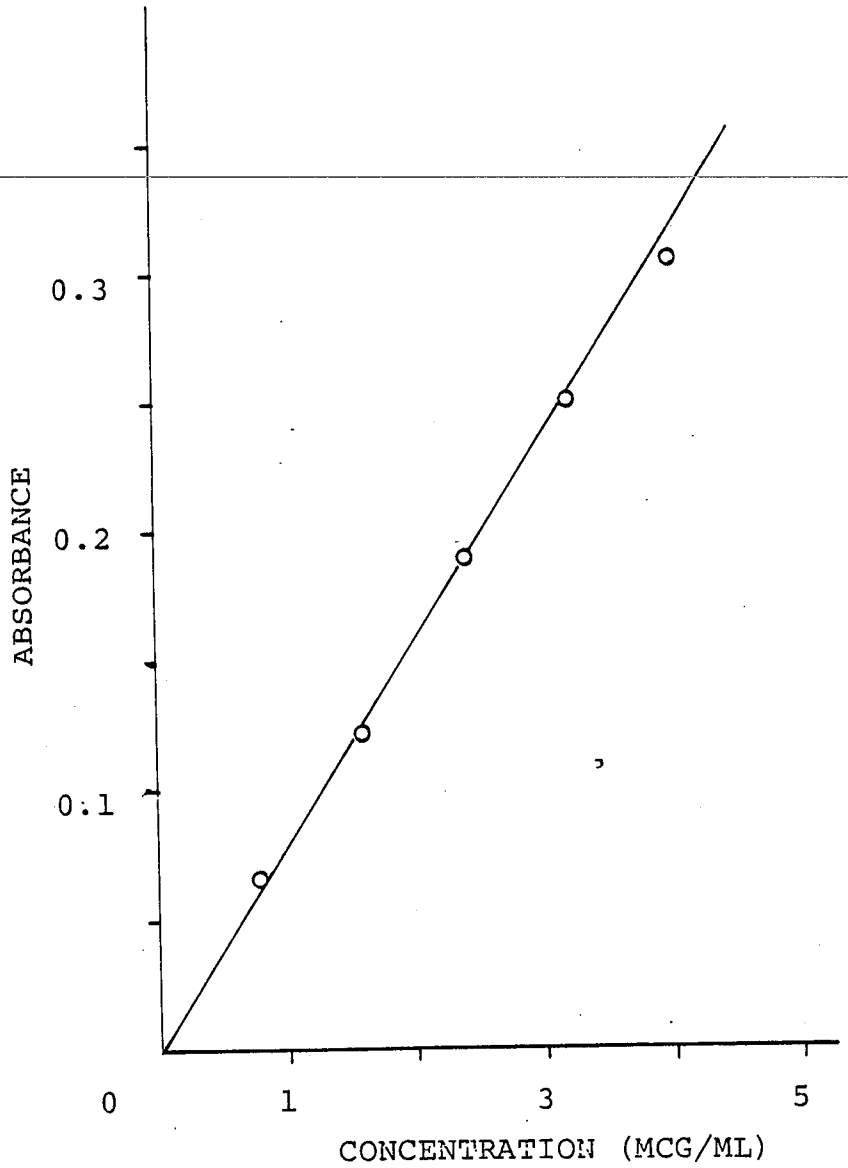
APPENDIX C: Beer's law plot of benzocaine in propylene glycol/water 60/40 (V/V); each point represents the average of three determinations.



APPENDIX D: Beer's law plot of benzocaine in propylene glycol/water 40/60 (V/V); each point represents the average of three determinations.



APPENDIX E: Beer's law plot of benzocaine in propylene glycol/water 20/80 (V/V); each point represents the average of three determinations.



APPENDIX F: Beer's law plot of benzocaine in propylene glycol/water 0/100 (V/V); each point represents the average of three determinations.

APPENDIX G

Modified computer analysis program from "Manual of Pharmacological Calculations with Computer Programs"
by R. J. Tallarida and R. B. Murray (35)

```
20 REM     -------MAIN ROUTINE--------
30 CLEAR 500
40 DEFINT I,J,L,N
50 DIM DA(40,20), SR$(34), X(40), Y(40), TB(101)
60 ON ERROR GOTO 50000
70 SC$="((PHARMACOLOGIC CACULATION PROGRAM))"+STRING$(63,"-")
80 PRINT CHR$(26):PRINT SC$;"    INITIALIZING PROGRAM"
90 LS=12:REM              LINES/SCREEN
100 ID=0:REM                DATA FLAG
110 RESTORE
120 IL=I:READ I,SR$(I):IF I<>0 THEN 120
130 SR$(IL+1)="END":REM           LAST SR$
140 I=1:LC=0
150 PRINT CHR$(26):PRINT SC$
160 IF SR$(I)="END" THEN I=1:LC=0:GOTO 200
170 IF SR$(I)<>"" THEN PRINT I;"- ";SR$(I):LC=LC+1
180 I=I+1
190 IF (LC/LS=INT(LC/LS)) AND LC<>0 THEN 200 ELSE 160
200 JP=2:U=255:PRINT
210 L$="":PN$="":PX$="INDEPENDENT VARIABLE":
    PY$="DEPENDENT VARIABLE"
220 PRINT:PRINT "ENTER PROCEDURE # OR <RETURN> FOR NEXT PAGE ==>";
230 U=0:INPUT U:REM                          ENTER SR#
240 IF U>0 AND U<34 THEN 260
250 GOTO 150
260 PRINT CHR$(26):TT=(54-LEN(SR$(U)))/2
270 PRINT TAB(TT);"< #";U;"- ";SR$(U);" >":PRINT
280 ON U GOSUB 1000,2000,3000,4000,5000,6000,7000,8000,9000,
    10000,11000,12000,13000,14000,15000,16000,17000,18000,
    19000,20000
290 IF U<20 GOTO 310
300 ON U-20 GOSUB 21000,22000,23000,24000,25000,26000,27000,
    28000,29000,30000,31000,32000,33000
310 I=1
```

```
320 GOTO 200
330 REM          (COMMON SUBROUTINES)
500 PRINT:INPUT"PRESS (ENTER) TO CONTINUE";E$:RETURN
510 REM          (CONVERT TO LOG(10))
520 FOR I=1 TO N(J):DA(I,J)=LOG(DA(I,J))/LOG(10):NEXTI:ID=0:
    RETURN
530 REM          (PRINT CALC VALUES ? )
540 PRINT:A$="":INPUT"PRINT CALCULATED VALUES (Y/N) ";
    A$:A$=LEFT$(A$,1)
550 PRINT:RETURN
560 REM          (CALC NEW VALUES ? )
570 PRINT:PRINT"ENTER NUMBER TO CALCULATE NEW VALUES OR
    (999) TO CONTINUE."
580 RETURN
590 INPUT X:RETURN
600 REM          (PRINT X Y MESSAGE)
610 IF PX$<>XL$ OR PY$<>YL$ THEN PRINT:
   PRINT"NOTE: X = ";PX$;:PRINT TAB(36);"Y = ";PY$:PRINT
620 XL$=PX$:YL$=PY$:RETURN
700 REM           (INPUT DATA)
710 L=0
720 ID=0
730 XL$="":YL$=""
740 J=0:N(1)=0:I=0
750 GOSUB 610
780 IF L<>0 THEN 800 ELSE PRINT"ENTER NUMBER OF";
785 IF L$<>"" THEN PRINT L$;:ELSE
    IF JP=1 THEN PRINT"GROUPS OF DATA  ";:ELSE
    IF JP=2 THEN PRINT"LINES OR CURVES ";
790 INPUT L:L=L*JP
800 FOR J = 1 TO L STEP JP
805 PRINT"ENTER NUMBER OF "
810 IF PN$<>"" THEN PRINT PN$;:ELSE
    IF JP=1 THEN PRINT"OBSERVATION OF GROUP #";J;:ELSE
    IF JP=2 THEN PRINT"OBSERVATION OF LINE OR CURVE #";(J+1)/2;
840 INPUT N:IF N<=0 THEN 805
850 IF N>100 THEN PRINT"N MUST BE LESS THAN 100.":GOTO 805
860 N(J)=N:IF JP=2 THEN N(J+1)=N
870 FOR I = 1 TO N(J)
880 IF JP=2 THEN PRINT"ENTER X,Y PAIR #";I;
    :INPUT DA(I,J),DA(I,J+1):GOTO 910
890 PRINT "ENTER OBSERVATION #";I;
900 INPUT DA(I,J)
910 NEXT I,J
```

```
920 IF L=0 THEN RETURN:REM        DATA ?
930 FOR J=1 TO L
940 PRINT:PRINT"GROUP #";J;": ";
950 FOR I=1 TO N(J):PRINT DA(I,J);:NEXT I
960 NEXT J
970 ID=1:REM       SET DATA FLAG
980 PRINT:PRINT
990 RETURN

2000 DATA 2,"MEAN, STANDARD DEVIATION & CONFIDENCE LIMITS"
2030 GOSUB 2100
2050 GOSUB 2600
2060 RETURN
2100 JP=1:PX$="":PY$="":GOSUB 710
2200 FOR J=1 TO L
2210 GOSUB 2660
2220 NEXT
2230 RETURN
2600 N=N(1)-1:GOSUB 2960
2605 GOSUB 2790
2610 FOR J=1 TO L
2620 GOSUB 2810
2630 NEXT
2640 RETURN
2650 J1=J:GOSUB 2660:J=J+1:GOSUB 2660:J=J1:RETURN
2660 N=N(J):X=0:SX=0:ME=0:S2=0:DE=0:DS=0:SE=0:VA=0
2670 FOR I=1 TO N
2680 X=DA(I,J):SX=SX+X:S2=S2+X*X
2690 NEXT
2700 ME=SX/N
2710 FOR I=1 TO N
2720 X=DA(I,J):DS=DS+(X-ME)*(X-ME)
2730 NEXT
2740 VA=DS/(N-1)
2750 DE=SQR(VA)
2760 SE=DE/SQR(N)
2770 SX(J)=SX:S2(J)=S2:DE(J)=DE:DS(J)=DS:VA(J)=VA:ME(J)=
     ME:SE(J)=SE
2780 RETURN
2790 PRINT CHR$(27)+"1";" #   N   SUM       MEAN      T VALUE   +/-
     STD DEV   STD ERR ";CHR$(27)+"m"
2800 RETURN
```

```
2810 N=N(J):DE=DE(J)
2860 N=N-1:GOSUB 2960
2870 PRINT J;TAB(3)N(J);
2880 CL=TV*SE(J)
2890 PRINT TAB(5);SX(J);TAB(15);ME(J);TAB(24);TV;
2900 PRINT TAB(33);CL;
2910 PRINT TAB(42);DE(J);TAB(51);SE(J)
2920 RETURN
2950 REM FI$ MAY BE CHANGED TO A T TABLE FILE FOR P=99
2960 IF N>30 THEN TV=1.96 ELSE FI$="TTABLE/P95":
     GOSUB 41000:TV=TB(N)
2999 RETURN

3000 DATA 3,"LINEAR REGRESSION I"
3030 GOSUB 710:REM INPUT
3040 GOSUB 3200:REM PROCESS
3050 GOSUB 3800:REM OUTPUT
3060 RETURN
3200 GOSUB 2200
3210 FOR J=1 TO L STEP 2:GOSUB 3230:NEXT J
3220 RETURN
3230 N=N(J):X=0:SX=SX(J):XM=ME(J):X2=S2(J):Y=0:SY=SX(J+1):
     YM=ME(J+1):Y2=S2(J+1):XY=0:M=0:B=0
3240 FOR I=1 TO N(J)
3250 X=DA(I,J)
3260 Y=DA(I,J+1)
3270 XY=XY+(X*Y)
3280 NEXT I
3290 M=((SX*SY/N)-XY)/((SX^2/N)-X2)
3300 M(J)=M
3310 B=YM-(M*XM)
3320 B(J)=B
3330 XY(J)=XY
3350 RETURN
3800 FOR J=1 TO L STEP 2:GOSUB 3830:NEXT J
3810 GOSUB 3860
3820 RETURN
3830 GOSUB 610:PRINT"REGRESSION LINE #";(J+1)/2;": ";
3840 PRINT" Y = ";M(J);"* X + ";B(J)
3850 RETURN
```

```
3860 GOSUB 540:IF A$<>"Y" THEN RETURN
3870 FOR J=1 TO L STEP 2:GOSUB 3880:NEXT J:RETURN
3880 PRINT"LINE #";(J+1)/2,"OBSERVED X","OBSERVED Y",
     "CALCULATED Y"
3890 FOR I=1 TO N(J)
3900 YC=M(J)*DA(I,J)+B(J)
3910 PRINT "",DA(I,J),DA(I,J+1),YC
3920 NEXT I
3930 GOSUB 570
3940 PRINT"",:GOSUB 590:IF X=999 THEN PRINT:GOTO 3999
3950 YC=M(J)*X+B(J):PRINT"",X,"",YC
3960 GOTO 3940
3999 RETURN

4000 DATA 4,"LINEAR REGRESSION II: LINES THROUGH ORIGIN"
4030 GOSUB 710:REM INPUT
4040 GOSUB 3200:REM PROCESS
4050 GOSUB 4800:REM OUTPUT
4060 RETURN
4800 FOR J=1 TO L STEP 2
4810 PRINT"LINE #";(J+1)/2;": ";
4820 SL=XY(J)/S2(J)
4830 GOSUB 610
4840 PRINT"REGRESSION THROUGH ORIGIN : Y = ";SL;"* X "
4850 GOSUB 540:IF A$="Y" THEN GOSUB 4880
4860 NEXT J
4870 RETURN
4880 PRINT"OBSERVED X","OBSERVED Y","CALCULATED Y"
4890 FOR I=1 TO N(J)
4900 YC=SL*DA(I,J)
4910 PRINT DA(I,J),DA(I,J+1),YC
4920 NEXT I
4930 GOSUB 570
4940 GOSUB 590:IF X=999 THEN PRINT:RETURN
4950 YC=SL*X:PRINT X,"",YC
4960 GOTO 4940
4999 RETURN
```

```
5000 DATA 5,"ANALYSIS OF THE REGRESSION LINE"
5030 GOSUB 710:REM-INPUT
5040 GOSUB 5200:REM-OUTPUT
5050 GOSUB 5400:REM-PROCESS
5060 RETURN
5200 GOSUB 2200
5210 FOR J=1 TO L STEP 2:GOSUB 5220:NEXT J:RETURN
5220 GOSUB 3230
5230 XX=0:SS=0:R=0:EM=0:EB=0:EX=0
5240 FOR I=1 TO N
5250 X=DA(I,J)
5260 XX=XX+(X-XM)*(X-XM)
5270 Y=DA(I,J+1)
5280 CY=M*X+B
5290 SS=SS+((Y-CY)*(Y-CY))
5300 NEXT I
5310 SS(J)=SS
5320 XX(J)=XX
5330 R(J)=(XY-(N*XM*YM))/SQR((X2-(N*(XM*XM)))*(Y2-(N*(YM*YM))))
5340 S=SQR(SS/(N-2)):S(J)=S
5350 EM(J)=S*SQR(1/XX)
5360 EY(J)=S*SQR((1/N)+(XM*XM/XX))
5370 EX(J)=ABS(S/M)*SQR((1/N)+(YM/M)*(YM/M)/XX)
5380 RETURN
5400 FOR J=1 TO L STEP 2:GOSUB 5410:NEXT J:GOSUB 3860:RETURN
5410 GOSUB 3830
5420 N=N(J)-2:XI$="X-INTERCEPT"
5430 PRINT"CORRELATION COEFFICIENT = ";R(J)
5440 PRINT:GOSUB 5600:T=TB(N):PRINT"(STAND. ERROR)"
5460 P1$="SLOPE":P2=M(J):P3=EM(J)*T:P4=EM(J):GOSUB 5550
5470 P1$="Y-INTERCEPT":P2=B(J):P3=EY(J)*T:P4=EY(J):GOSUB 5550
5490 P1$=XI$:P2=-B(J)/M(J):P3=EX(J)*T:P4=EX(J):GOSUB 5550
5510 RETURN
5550 REM HOLD POINT
5555 PRINT P1$;" = ";STR$(P2);" +/- ";STR$(P3);" (";STR$(P4);")"
5560 RETURN
5600 GOSUB 2960
5610 PRINT"T TABLE VALUE = ";TV;TAB(29);"DF =";N;" P = 95%",
5620 RETURN
5700 GOSUB 5600:PRINT:PRINT"T CALCULATED = ";ABS(T);
5720 PRINT TAB(29);:IF ABS(T)<TV THEN PRINT "NOT ";
5740 PRINT"SIGNIFICANT"
5999 RETURN
```

```
6000 DATA 6,"PARALLEL LINES I:TEST FOR PARALLELISM"
6010 REM
6020 REM  SUBROUTINES: 2,3,5
6030 GOSUB 6100:REM INPUT
6040 GOSUB 5200:REM PROCESS
6050 GOSUB 6800:REM OUTPUT
6060 RETURN
6100 GOSUB 710
6110 IF L<4 THEN PRINT"YOU MUST HAVE MORE THAN ONE LINE!":
     ID=0:GOTO 6100
6120 RETURN
6800 GOSUB 3050
6810 FOR J=3 TO L STEP 2
6820 T=0:SP=0
6830 SP=SQR(((N(1)-2)*S(1)^2+(N(J)-2)*S(J)^2)/(N(1)+N(J)-4))
6840 T=ABS((M(1)-M(J))/(SP*SQR((1/XX(1))+(1/XX(J)))))
6850 PRINT:PRINT"LINE # 1 VS. LINE #";(J+1)/2;":"
6860 N=N(1)+N(J)-4:GOSUB 5700
6870 NEXT J
6999 RETURN

40000 DATA 0,"END"
41000 REM     READ TABLE DATA
42010 IF FI$=FL$ AND TB(N)<>0 THEN RETURN
42020 PRINT:PRINT"ENTER ";FI$;"TABLE VALUE FOR";N;"D.F.";
42040 NL=N:FL$=FI$:IN$="":INPUT IN$:TB(N)=VAL(IN$)
42050 PRINT
42100 RETURN
50000 PRINT:REM     ERROR TRAP ROUTINES
50030 PRINT TAB(5)*******ERROR - ";
50040 IF ERR=11 THEN PRINT"DIVISION BY ZERO, CHECK DATA!":
      RESUME 50090
50050 IF ERR=5 THEN PRINT"ILLEGAL FUNCTION, CHECK DATA!":
      RESUME 50090
50060 IF ERR=8 THEN PRINT"SUBROUTINE DOES NOT EXIST ****":
      RESUME 50090
50065 IF ERR=7 THEN PRINT"OUT OF MEMORY (RE-DIMENSION OR RE-RUN)":
      RESUME 50999
50070 PRINT"ON LINE #";ERL;".  TYPE #";ERR;".
50080 RESUME 50100
50090 GOSUB 310
50100 GOSUB 500:RUN
50999 END
```

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