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EFFECT OF CATIONIC SURFACTANTS ON THE TRANSDERMAL PERMEATION OF IONIZED INDOMETHACIN

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ABSTRACT

In an attempt to enhance the skin permeation rate of ionized indomethacin by ion-pairing, a group of cationic surfactants were evaluated to determine their potential as counter-ions. Permeation rates through hairless rat skin were measured in vitro. The ion-pairing agents used were all quaternary ammonium compounds. Most enhanced the skin permeation of indomethacin to varying magnitudes and through different mechanisms. Some involved increased drug solubilization in the aqueous vehicle while others demonstrated permeability increase as a function of partitioning. One surfactant did not exert an apparent effect on any of the permeation parameters. Of Arquad^{R} 12, Arquad^{R} 16, Cetylpyridinium chloride^{R} and Benzalkonium chloride^{R}, only the last surfactant clearly indicated its ability to pair with indomethacin.

INTRODUCTION

Surface active agents may affect the permeability of a solute through biological membranes either by interacting with the solute, with the membrane, or with both. Surfactants, however, do not necessarily increase drug transfer across lipoidal barriers [1]. In some cases, they could decrease transport or exert no apparent effect at all [2].

Being charged at physiological pH, quaternary ammonium compounds could most likely participate in the membrane transport of ions by acting as counter ions to a negatively charged solute. This phenomenon would be a means of improving the absorption or permeation of ionized drug molecules. The purpose of this present investigation is to determine whether surfactants of the quaternary ammonium structure could act as potential ion-pairing agents for ionized indomethacin. In addition, their relationship to indomethacin's partition coefficient, skin permeability, and solubility are observed.

MATERIALS AND METHODS

Reagents

Crystalline indomethacin (Sigma Chemicals, St. Louis, MO) was used as received. A pH 8 citrate-phosphate (McIlvaine) buffer of 0.05M ionic strength was prepared from 2.56 gm of Na_2HPO_4 and 0.06 gm of citric acid monohydrate (Fisher Scientific, Springfield, NJ) in a liter of deionized water. Phosphate buffer (0.04M, pH 7.4) was also prepared by dissolving 2.61 gm of K_2HPO_4 and 0.89 gm of KH_2PO_4 (Fisher Scientific, Springfield, NJ) in a liter of deionized water.

Methylene chloride, HPLC grade acetonitrile and glacial acetic acid were obtained from Fisher Scientific (Springfield, NJ) and used without further purification. Benzalkonium chloride and hexadecylpyridinium chloride (*Cetylpyridinium chloride*) from Sigma Chemicals were used as received. Samples of dodecyltrimethylammonium chloride (*Arquad 12*) and hexadecyltrimethylammounium chloride (*Arquad 16*) were obtained from Akzo Chemie America (McCook, IL). 0.05M McIlvaine buffer of pH 8 was used as the medium to prepare varying strengths (2.5×10^{-4} M to 2×10^{-3} M) of the surfactant solutions.

Counter-Ion Effect on Octanol/Buffer Partitioning

Individual pH 8 citrate-phosphate buffer solutions (0.05M) were prepared to contain one of the four surfactants in increasing concentrations (0.00025, 0.0005, 0.001, and 0.002M). Each buffer was first equilibrated with 1-octanol in a 37°C shaking water bath for 12 hours. Both aqueous and organic phases were then separated. 5.5×10^{-3} M indomethacin was weighed out into the buffer phase while 2 x 10-3 M indomethacin was added to the octanol phase. The drug in the buffer solutions was equilibrated at 37°C for 24 hours . The oil phase containing the drug was allowed to equilibrate for 12 hours.

At the end of the time period, each aqueous solution was filtered, sampled, and its pH readjusted back to pH 8 using 1M KOH. Aliquots were taken and analyzed for initial drug

concentration. Aliquots were also sampled from the octanol phase and analyzed for initial drug concentration.

10 ml of each aqueous phase was measured out and mixed in with an equal volume of octanol solution. The resulting mixtures were gently shaken at 37°C for 24 hours. At the end of the time period, the buffer phase was withdrawn from each mixture. Aliquots were removed for analysis of final drug concentration. Octanol samples were also analyzed for drug content. Assays were performed by HPLC. The apparent partition coefficient was calculated as the ratio of final indomethacin concentration in the oil phase to that of the buffer phase.

Effect of Counter-Ion on Drug Permeation

Excess indomethacin was equilibrated overnight at 37°C in pH 8 McIlvaine buffer (0.05M) solutions containing one of the four surfactants ----- *Arquad 12*, *Arquad 16*, *Cetylpyridinium chloride* and *Benzalkonium chloride*. Each quaternary ammonium compound was present in concentrations ranging from 2.5×10^{-4} to 2×10^{-3} M. These drug suspensions served as the different donor media for the following skin permeation studies.

Samples of freshly excised abdominal skin from female hairless rats were mounted on Valia-Chien permeation cells [3]. 3.5ml of indomethacin-surfactant solution was used as donor medium and an equal volume of pH 7.4 phosphate buffer served as receptor medium. Three replicate experiments were run under each condition.

At selected time intervals, 150 μl samples were removed from the receptor solution, immediately replaced with an equal volume of fresh pH 7.4 phosphate buffer and assayed for drug content by HPLC.

Analytical Procedure

The HPLC system (Waters) consisted of an automatic sample injection (Model 712) and solvent delivery system (Model 510). Data collection and processing were provided by a Programmable System Controller (Model 721) and a Data Module Integrator (Model 730). A reversed-phase Supelcosil C-18 column (15cm) was used together with a modified assay procedure developed by Astier and Renat [4]. The mobile phase consisted of 0.1M acetic acid:acetonitrile (53:47). The flow rate was 1.5 ml/min and indomethacin was detected at 250 nm.

RESULTS AND DISCUSSION

It has been shown that the type and magnitude of surfactant effects on drug penetration through membranes is a function of the chemical nature of a given surface active agent [5,6].

One of the goals of this study is to determine the ability of several cationic surfactants to enhance the in vitro skin permeation rate of ionized indomethacin via ion-pairing. Fig. 1 gives the chemical structures and approximate molecular weights of each counter-ion that was used in this investigation.

The concept of ion-pairing views an ion-pair to be a lipophilic complex. Theoretically, a complex made up of an ionized drug-adjunctive substance such as indomethacin-surfactant would require less activation energy to partition into a hydrophobic membrane. In this case, the skin permeation rate should be higher as compared to that of the ionized drug alone.

Fig. 2 shows the effect of the counter-ions on the skin permeation rate of indomethacin (pKa = 4.46) at pH 8. There appears to be some difference in the enhancement of drug permeation rate depending on the type of surfactant used to pair with the drug. In general, an increase in the concentration of the cationic agent gave higher permeation rates. While ionized indomethacin may exhibit increased permeation rates, it would be difficult, at this point, to determine whether any ion-pairing mechanism is responsible for the changes. The permeation rate data was further evaluated as follows.

Steady state drug diffusion from a saturated solution through a membrane and into a sink is represented as:

$$J = P.C_d = (KD/h)\, C_d$$

where **J** is rate of skin permeation and **P** the permeability coefficient which in turn is made up of the membrane/vehicle partition coefficient **K**, the effective diffusivity **D** and membrane thickness **h**. The **K** value can be determined experimentally while **P** is calculated by dividing the flux, **J**, by the drug concentration, C_d, in the donor solution. **D** was determined using the known values of **P**,**K** and **h**. An increase in skin permeation rate therefore reflects changes in any of the parameters except for **h**. The intact abdominal rat skin has an average measured thickness (**h**) of 0.08 cm.

Fig. 3 summarizes the effect of *Arquad 12* on the various permeation parameters of indomethacin. The **P** of indomethacin remained constant as a function of surfactant concentration. Diffusivity does not reflect an appreciable increase with higher *Arquad 12* concentrations either. The absence of change in the partition coefficient of indomethacin indicates a lack of interaction between the dissociated drug and the counter ion. Increasing *Arquad 12* had little effect on indomethacin solubility. In short, this particular surfactant exerted no apparent effect in transporting ionized indomethacin across the rat skin.

Fig. 4 suggests that *Arquad 16* has a slightly reduced effect on the aqueous solubility of indomethacin. On the other hand, the permeability and partition coefficients are slightly enhanced as a function of *Arquad 16* concentration. The partitioning tendency of indomethacin gradually increased as more surfactant was added. This means that the

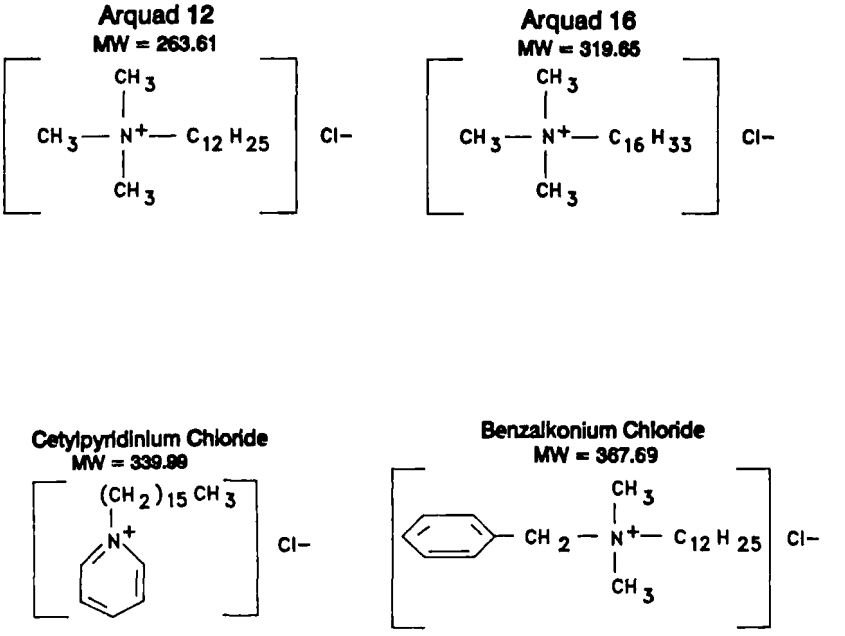


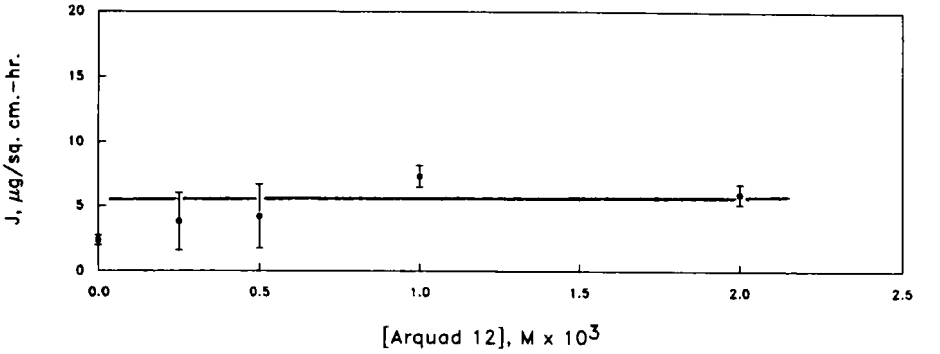
FIGURE 1

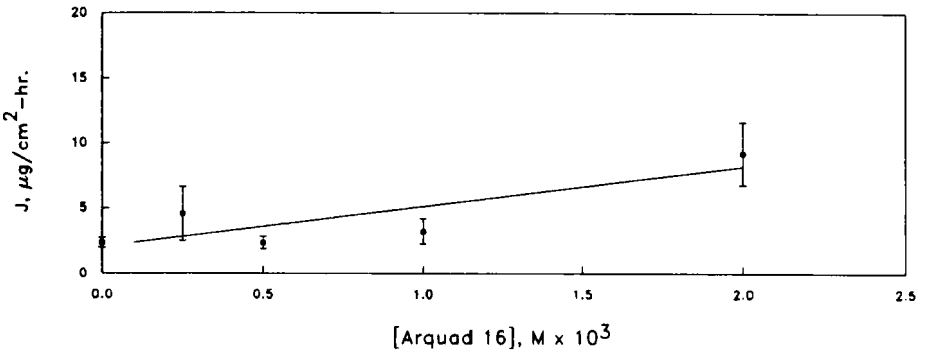
Chemical Structures and Molecular Weights of Quaternary Ammonium Compounds

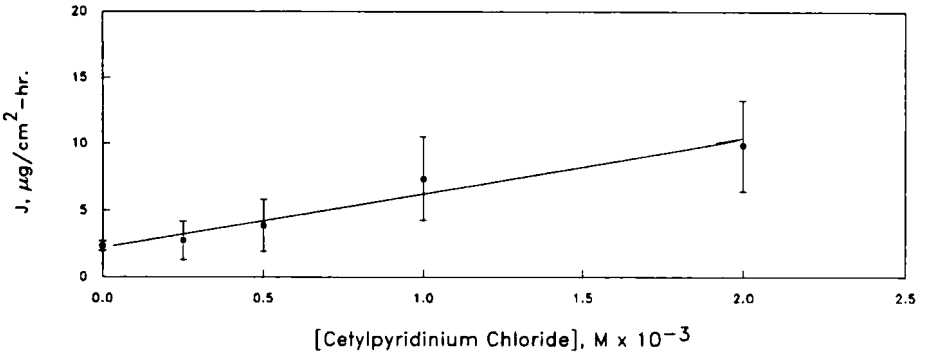
change in skin permeability of indomethacin is due to the effect of *Arquad 16*. In contrast, the addition of *Cetylpyridinium chloride* (Fig. 5) resulted in a linear increase in aqueous solubility of indomethacin as well as in its permeation and partition coefficients, but not diffusivity. The mechanism of increase in skin permeation rate is apparently the combined result of an increased solubility and improved partitioning.

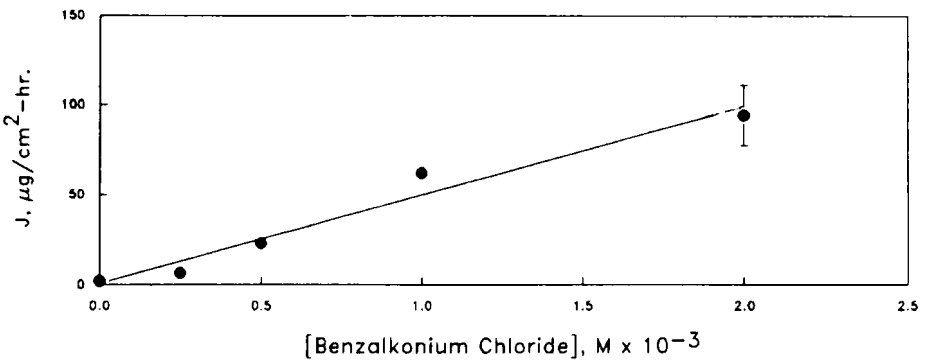
Benzalkonium chloride affects indomethacin in a different manner from the other surfactants (Fig. 6). Aqueous solubility was observed to decrease with an increasing *Benzalkonium chloride* concentration while partition coefficients increased substantially. A linear relationship is exhibited between the partition coefficient and the surfactant concentration. These results suggest that the increasing permeation rates is the outcome of increased partitioning of ionized indomethacin.

Diffusivity, which makes up part of the permeability term, reflects the inherent property of the penetrant molecule in a medium like skin. A difference in the diffusion coefficient may indicate a change in the medium's physical or chemical composition. Increasing partition coefficients, however, may indicate an increase in skin sorption due to ion-pair formation so long as drug solubility in the vehicle, C_d, does not increase.









Partition coefficients have been used as a criteria in determining the lipophilicity of a given molecule. Ion-pairing modifies the hydrophobic character of compounds and optimizes their transport across lipophilic membranes. An ion-pair would therefore have a higher partition coefficient than an ionic solute alone.

A very good correlation was found between the enhancement in the permeability coefficient of indomethacin and the increase in its partition coefficient in the presence of *Benzalkonium chloride* (Fig. 7). This strongly suggests that the enhanced skin permeation of indomethacin is due to the formation of ion-pairs. At its lowest concentration (0.00025M), *Benzalkonium chloride* enhanced the drug's skin permeability nine times. At the highest strength (0.002M), the permeability was increased nearly 150 times.

Fig. 8 shows the relationship of K and P to be fairly good for the other surfactants except *Arquad 12*. With both *Arquad 16* and *Cetylpyridinium chloride*, we have seen indications that ion-pairing contributes to skin permeation enhancement although it may not be a dominant effect.

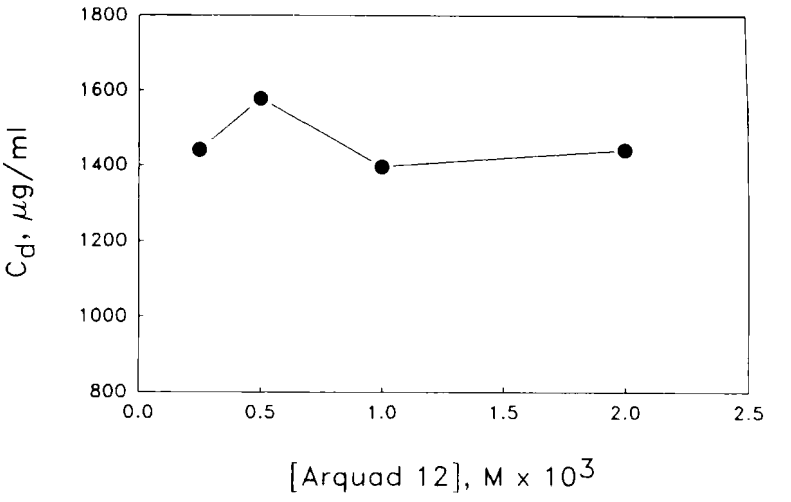
The effect of a surfactant is dependent upon its concentration range [1,7]. Ion-pairs may form between an anion and a cation only at concentrations below the critical micelle concentration, cmc. Table 1 lists the cmc values of the various cationic surfactants [8]. Included in the table are the partition coefficient values and permeation rates of indomethacin in response to the increase in surfactant concentration.

The working concentration range in this study falls below and above the cmc's of both *Arquad 16* and *Cetylpyridinium chloride*. In these two cases, there does not seem to be a distinctive shift in K values at concentrations past the cmc even though the partition coefficient has been considered the best indicator, among the permeation parameters, of ion-pair formation. Note, however, that skin flux notably increases at 2×10^{-3} M *Arquad 16* and 1×10^{-3} M *Cetylpyridinium chloride*. Both concentrations are just past the cmc for each respective surfactant.

Different effects on ion absorption arise when the counter ion is at a concentration where only ion-pairs are formed than when it is at a concentration where the physicochemical equilibrium enables both ion-pairs and complex coacervates to exist [9].

FIGURE 2

Effect of Variation in Surfactant Concentration on the Skin Permeation Rate of Indomethacin



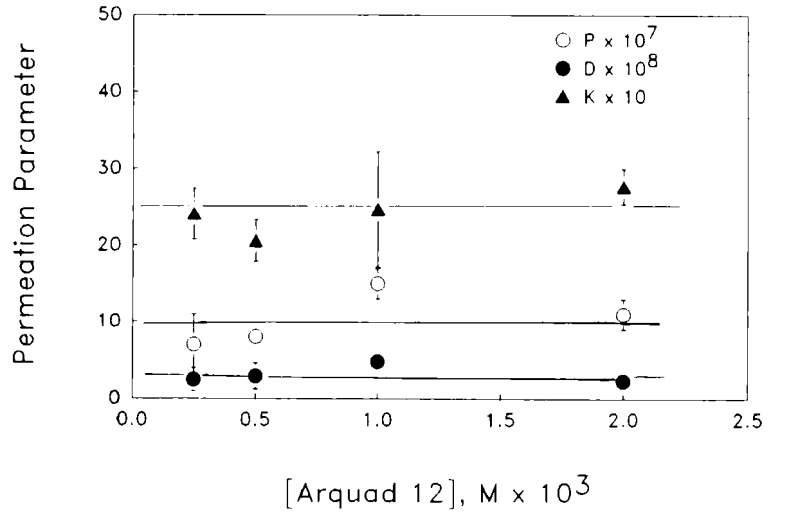
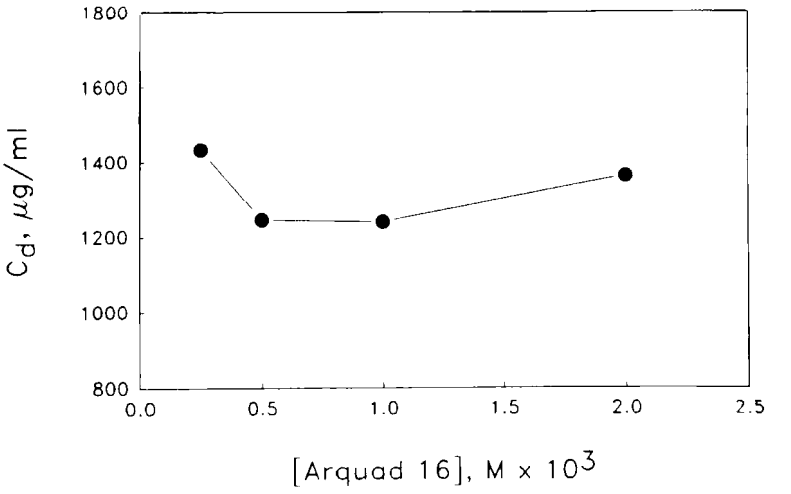


FIGURE 3

Effect of Variation in *Arquad 12* Concentration on the Solubility and Permeation Parameters (P, D, and K) of Indomethacin



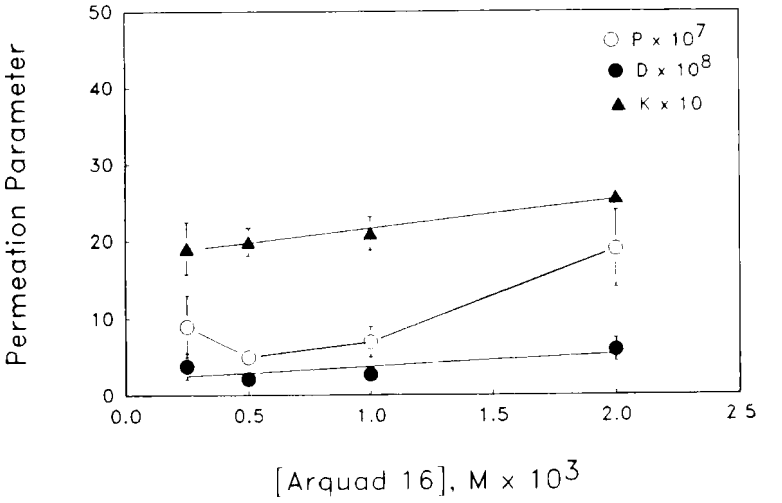


FIGURE 4

Effect of Variation in *Arquad 16* Concentration on the Solubility and Permeation Parameters (P,D, and K) of Indomethacin

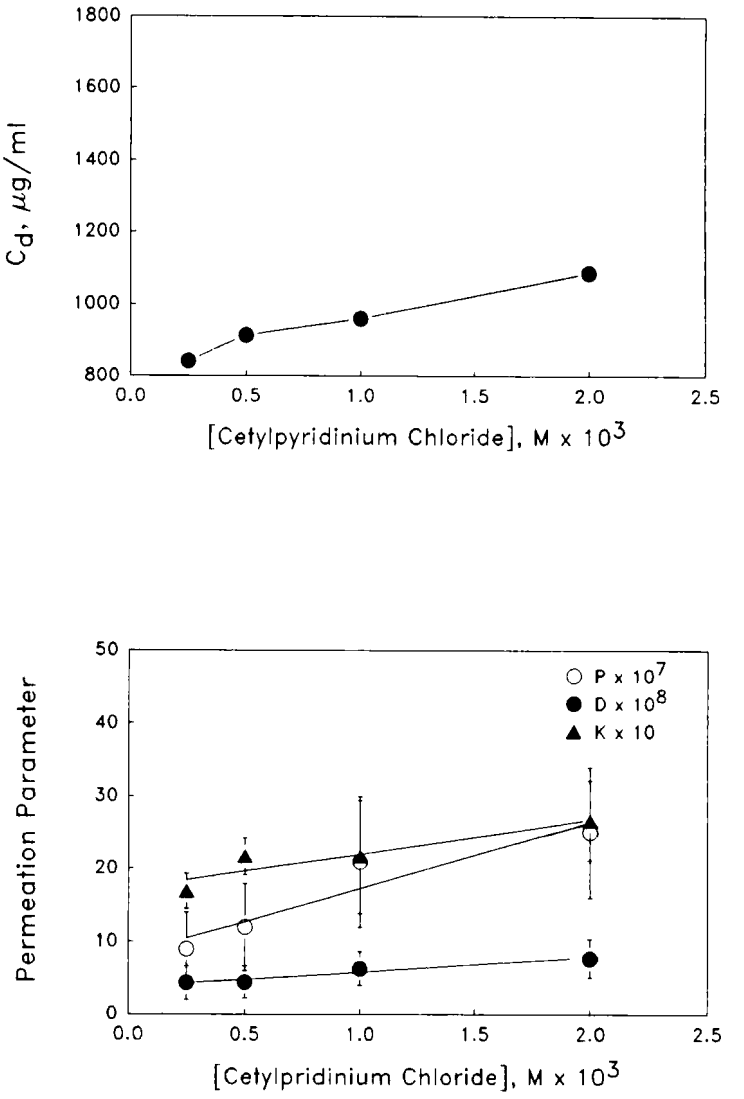


FIGURE 5

Effect of Variation in *Cetylpyridinium Chloride* Concentration on the Solubility and Permeation Parameters (P,D, and K) of Indomethacin

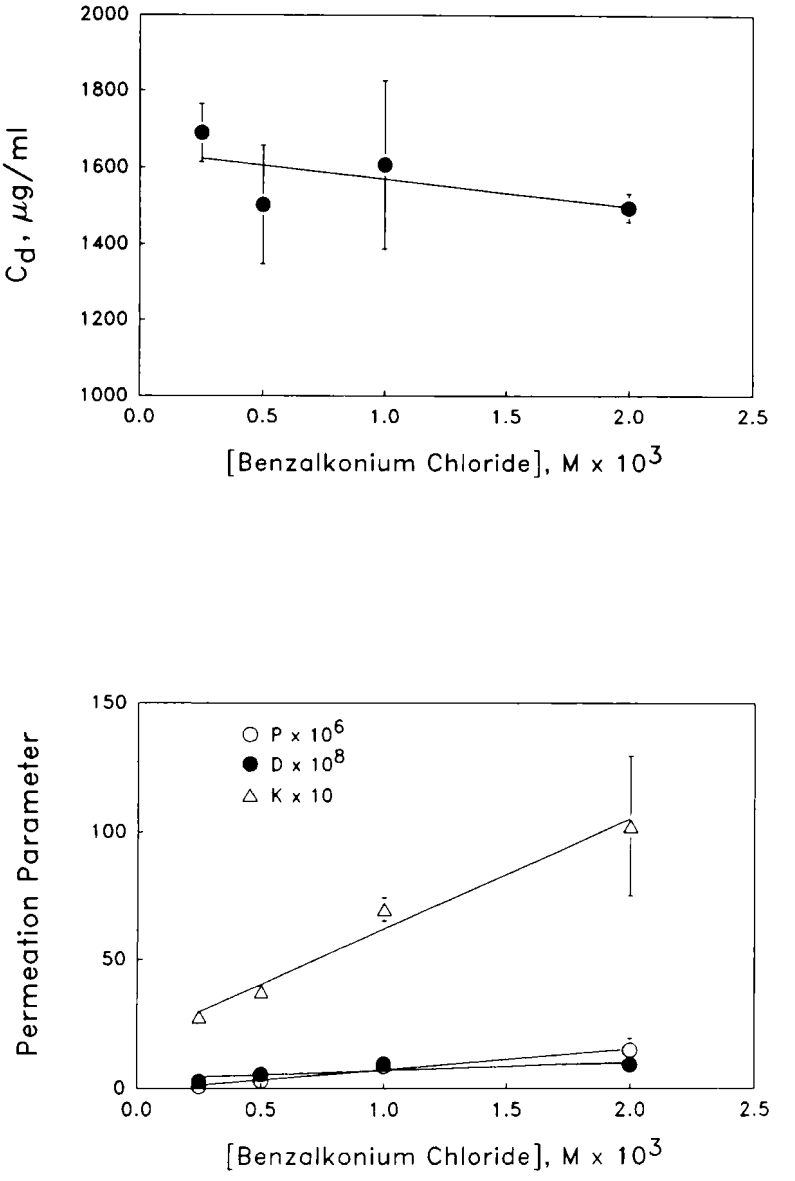


FIGURE 6

Effect of Variation in *Benzalkonium Chloride* Concentration on the Solubility and Permeation Parameters (P,D, and K) of Indomethacin

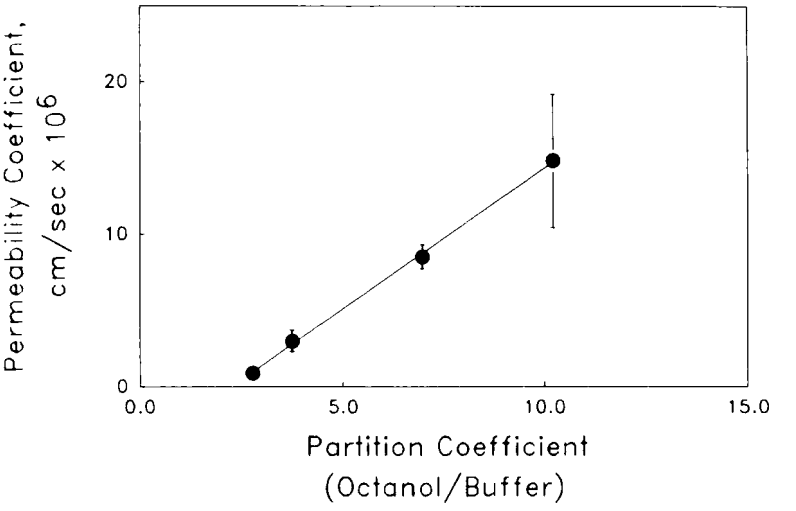


FIGURE 7

Relationship Between the Permeability Coefficient of Indomethacin and Its Partition Coefficient in the Presence of *Benzalkonium Chloride*

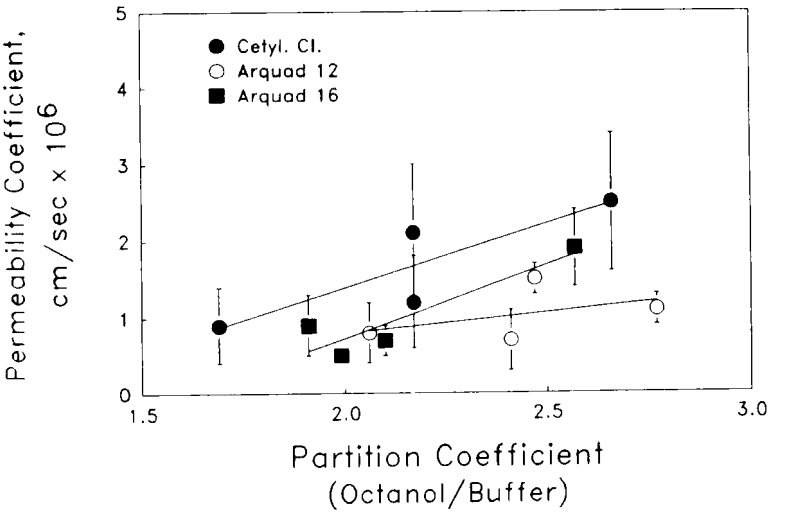


FIGURE 8

Relationship Between the Permeability Coefficient of Indomethacin and Its Partition Coefficient in the Presence of Various Quaternary Ammonium Compounds

TABLE 1

Critical Micelle Concentrations (**cmc**) and Effect of Surfactant Concentration on the Permeation rate (**J**) and Partition Coefficient (**K**) of Indomethacin

	Arquad 12	*Arquad 16*	*Cetylpyridinium Chloride*	*Benzalkonium Chloride*
A. **cmc**, M	2×10^{-2}	1.3×10^{-3}	9×10^{-4}	2.3×10^{-3}
B. **K**				
Surfactant Concentration				
0.00025M	2.41 (0.33)	1.91 (0.34)	1.69 (0.24)	2.77 (0.25)
0.0005M	2.06 (0.27)	1.99 (0.18)	2.10 (0.22)	2.57 (0.50)
0.001M	2.47 (0.75)	2.10 (0.22)	2.17 (0.78)	6.97 (0.46)
0.002M	2.77 (0.23)	2.57 (0.50)	2.66 (0.55)	10.22 (2.77)
C. **J**, $\mu g/cm^2$-hr.				
Surfactant Concentration				
0.00025M	3.79 (2.21)	4.57 (2.06)	2.74 (1.46)	6.53 (1.50)
0.0005M	4.21 (2.45)	2.37 (0.47)	3.88 (1.97)	22.95 (5.52)
0.001M	7.34 (0.84)	3.21 (0.95)	7.39 (3.12)	62.34 (5.81)
0.002M	5.92 (0.81)	9.19 (2.44)	9.86 (3.42)	94.34 (29.10)

NOTE: ( ) indicates standard deviation from triplicate samples.

This is probably why it has also been suggested that the effect of a surfactant above the cmc represents the net result of drug absorption enhancement and retardation through a membrane [1].

From the results that have been presented, it appears that *Benzalkonium chloride* is the only probable ion-pairing agent in the group of surfactants that was studied. Assume

$$Q_{T_{max}} = Q_o + Q_{ion\text{-}pair}$$

where

$Q_{T_{max}}$ = theoretical maximum amount of drug that can permeate through the skin

Q_o = actual amount of drug that can permeate through the skin without the addition of *Benzalkonium chloride*

TABLE 2

Comparison of Experimental and Calculated Amounts of Indomethacin Permeating Through Skin

Benzalkonium Chloride, M	Amount of Indomethacin Permeated (μg)*	
	Experimental	Theoretical
0.0	16.52 (3.73)	-----
0.00025	12.36 (4.81)	329.60
0.0005	64.81 (10.50)	642.67
0.001	245.22 (69.49)	1268.82
0.002	264.54 (95.84)	2521.12

NOTE: ( ) indicates standard deviation from triplicate samples
*At 22 hours

$Q_{ion\text{-}pair}$ = possible amount of drug that can permeate through the skin with 1:1 pairing between ionized indomethacin and *Benzalkonium chloride*

The data in Table 2 suggest the experimental amounts of indomethacin that go through rat skin following a 22 hour permeation run correspond, at best, to only 19% of the calculated amounts. The difference between the two sets of data suggests several possibilities. The rate of dissociation of the ion-pair complex may be high in solvents of a greater dielectric constant such as the aqueous buffer used in this investigation. If this was the case, then the efficiency of ionized drug transport is lessened even if ion-pairing does occur.

Another reason for the disparity in Q values may be the presence of a physical phenomenon such as drug binding onto the skin. Assuming that the formed ion-pairs successfully increase drug availability for transfer across the lipophilic membrane, skin binding may prevent more drug from being detected in the receptor solution.

CONCLUSIONS

This study shows that the skin permeation rate of ionized indomethacin can be altered by the presence of surfactants of opposite charge. Though of the same basic

quaternary ammonium structure, the counter-ions that were evaluated affect the permeation properties of the solute to varying degrees. In some cases, the increase in flux cannot be attributed solely on changes in the partition coefficient which is assumed indicative of ion-pair formation as long as drug solubility in the aqueous vehicle does not change. It is also important to work only in concentration ranges below the cmc, otherwise experimental results may not reflect true ion-pair behavior.

An attempt was made to predict the theoretical amount of drug that can permeate through the skin given a known concentration of surfactant. The calculated numbers do not correlate with the experimental values obtained. Two reasons for the difference were presented. It is not however, within the scope of this paper to make any conclusions about the given hypotheses.

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