Sorption of Benzalkonium Chloride by an Insoluble Polyamide

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Abstract □ Sorption of an ingredient from solution by an insoluble polymeric material will reduce the concentration of the product and may lead to alterations in the properties of the material. Since cationic surface-active agents are used for a number of medical and paramedical applications, it was felt desirable to study their sorption characteristics by a specific insoluble polymeric material (a polyamide, Nylon 6,6). Results of the equilibrium sorption experiments revealed that maximum sorption occurred in the region of the critical micelle concentration of the surface-active agent. It was theorized that the hydrophobic moiety of the cation interacted with the nonspecific sites in the hydrocarbon units of the polyamide. As the agent was bound, a chloride ion followed giving electrical neutrality. With solutions having high concentrations of benzalkonium chloride, the associated molecules and micelles are also interacted to the nonspecific sites along the hydrocarbon section of the polyamide. Permeation experiments revealed the importance of the original concentration on the rates of diffusion and permeation.

Keyphrases □ Benzalkonium chloride—sorption by insoluble polyamide □ Diffusion coefficient—benzalkonium Cl-polyamide system □ Permeability constant—benzalkonium Cl-polyamide system □ CMC effect—benzalkonium Cl sorption □ UV spectrophotometry—analysis

When a compound is sorbed from a solution by an insoluble polymeric material, two major consequences can occur: (*a*) loss of potency of a product in contact with the polymeric material and (*b*) alteration in the physical-chemical-mechanical properties of the container or device having contact with the solution. The increasing use of insoluble polymeric materials (plastics) as containers, administration assemblies, collection devices, and a host of prosthetic devices helps to focus attention on the sorptive potential of these new man-made materials and the possible problems they may present in medical and paramedical applications.

Sorption studies of compounds by selective polymeric materials, besides having immense practical value, also permit theoretical consideration as to the behavior of the compound (or compounds) in the matrix of the polymeric materials. This paper reports on the study of the sorption of a cationic surface-active agent (benzalkonium chloride) by a specific polyamide (Nylon 6,6).

EXPERIMENTAL

Equilibrium Sorption Studies—Benzalkonium chloride[1] was diluted with freshly prepared distilled water to prepare solutions of the following concentrations: 0.05, 0.08, 0.1, 0.2, 0.5, 0.8, and 1.0%. Exactly 100.0 ml. of each solution was pipeted directly into glass sorption tubes. Each of the tubes was then sealed with suitable ground-glass stoppers. The tubes were placed in a water bath of the desired temperature and when the solution temperature had reached the temperature of the water bath, the stoppers were removed and polyamide samples[2] [2.54 × 10.2 cm. (1 × 4 in.)] weighing a total of approximately 10 g. were added to the tubes and the tubes were resealed. These experiments were conducted at 40, 50, 60, and 70 ± 0.02°. Control solutions (no polyamide) were run side-by-side with the test solutions.

Previous sorption experiments demonstrated that an equilibrium state or, more correctly, a pseudo-equilibrium state was attained within a 120-hr. period. For this reason, all of the experiments were continued to 120 hr., at which time the solutions were assayed[3] for remaining benzalkonium chloride content. The difference between the original solutions and the quantity remaining in the solution at the conclusion of the experiment was considered as having been sorbed by the polyamide.

Results of equilibrium sorption experiments are illustrated in Fig. 1. Each curve, except the 60° curve, shows a region of maximum sorption.

Permeation and Diffusion Studies—Permeation studies were conducted using a permeation cell as described by Gonzales *et al.* which contains two glass chambers separated by the test plastic under study (1). Exactly 500 ml. of benzalkonium chloride of a specific original concentration was preheated to the temperature of the water bath and then added to the left chamber of the cell. To the right chamber, freshly prepared distilled water was added. In these studies, stirring in both chambers was accomplished by the use of immersible magnetic stirrers[4] which had certain advantages over the previous method by which stirring rods attached to an external source of power were used. At times, the older method would lead to the locking of the stirring rod as it passed into the chamber and generally disrupted the experiment.

Experiments were conducted using benzalkonium chloride solutions prepared at concentrations of 0.1, 0.2, 0.5, and 1.0% at four different temperatures (40, 50, 60, and 70°). At various time periods, aliquots of solution from the "no-drug" side were removed and assayed for benzalkonium chloride. Plots of the data as concentration of benzalkonium chloride appearing on the no-drug side *versus* time permitted the evaluation of both the diffusion coefficient and the permeability constant.

The diffusion coefficient (D) was calculated by the use of Barrer's expression (2):

$$D = L^2/6\tau \qquad \text{(Eq. 1)}$$

where L is thickness of the polyamide film in centimeters and τ is the time-lag obtained from the previously mentioned plots. Actual time-lag values were calculated by the method of least squares.

From the slopes of each of the lines (evaluated by the method of least squares), it was possible to calculate the permeability constant (P) using the following expression:

$$P = c/t \cdot LV/CA \qquad \text{(Eq. 2)}$$

where c/t is the slope of the linear portion of the permeation plot, L is the thickness of the film, V the total volume of solution, C the original concentration of the solution, and A the surface area of the film exposed to the solution. Table I includes both the D and P values for each of the experiments.

By knowing D and P, it is possible to calculate the solubility coefficient (S) since $P = SD$, but in the case of the benzalkonium chloride-polyamide system, it was deemed inappropriate since the S values would have little meaning because of the very large influence which concentration of the agent has upon the diffusion process.

[1] Benzalkonium chloride, aqueous, 10% (Roccal 10%), Winthrop Laboratories, New York, N. Y.

[2] Nylon-6,6 or poly(hexamethylene adipamide) (Polypenco 101), film of 5-mil thickness, Polymer Corp. of Pennsylvania, Reading, Pa.

[3] Quantitative determination of benzalkonium chloride was conducted by a spectrophotometric method at 263 mμ, using base-line technique. Distilled water was used as the reference solution. Recording spectrophotometer, model DK, Beckman Instruments, Inc., Fullerton, Calif.

[4] Model MS-7, Tri-R Instruments, Inc., Jamaica, N. Y.

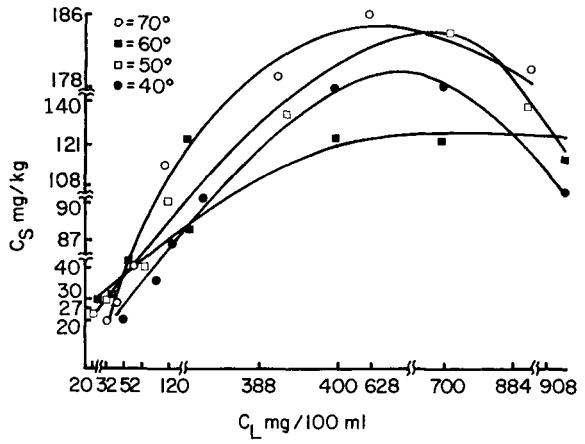

Figure 1—*Equilibrium sorption isotherms of benzalkonium chloride in polyamide. Key:* C_L, *concentration of solute in liquid phase (120 hr.);* C_S, *concentration of solute in solid phase (120 hr.).*

Since the permeation experiments were conducted at several temperatures, the activation energies of diffusion (ΔE_D) and permeation (ΔE_P) were evaluated by the use of the Arrhenius relationship in a manner similar to that reported in a previous paper. These values are listed in Table II for each of the original concentrations.

DISCUSSION

Equilibrium Sorption Studies—When the concentration of benzalkonium chloride in the polyamide was plotted, as shown in Fig. 1, a pronounced curvature of each of the isotherms resulted, conveying that within the concentration range studied maxima sorption occurred. In 1944 Aicken first reported this type of unusual sorption pattern with surface-active agents and solid substrates (3). In this case, the author was studying the sorption of sodium alkyl sulfates by cotton and wool. Since then, other investigators have reported similar observations with surface-active agents and solid substrates (4–13). In general, the sorption pattern was as follows. Below the critical micelle concentration (CMC) there was a proportional increase in sorption with an increase in concentration of the agent. In the vicinity of the CMC, an inflection occurred in the curve, denoting a relatively large increase in sorption. Above the CMC, a maximum in sorption occurred and with further increase in the concentration of the surface-active agent a decrease in sorption took place, generally leveling off as the concentration of the agent was further increased.

The results of the present study with benzalkonium chloride (Fig. 1) show a rapid uptake in the early phases reaching maxima in the region of 400 mg./100 ml. This concentration range is above the CMC of benzalkonium chloride which has been estimated to be approximately 80 mg./100 ml., following the general pattern of maxima as noted by previous workers.[5]

The results in Fig. 1 suggest as a very first approximation that below the CMC benzalkonium chloride is sorbed as single ions. With an increase in concentration toward the CMC, dimers and aggregates are forming and these in turn are being sorbed. Above the CMC, micelles are being sorbed, leading to the maxima. Further increase in concentration of the benzalkonium chloride solution releases single molecules, aggregates, and micelles to the solution. It also seems probable that as penetrating molecules enter the matrix of the polyamide they may alter in some manner the steric and spatial arrangements of the polymer chains, increasing or decreasing the sites for interaction.

The sorption isotherms (Fig. 1) also demonstrated the effect of temperature upon the uptake of benzalkonium chloride. Generally there was an increase in sorption with an increase in temperature. The 60° sorption experiment did not follow this trend, however, and even after the same type of experiment was repeated several times there was no indication of the reason this curve did not fall between the 70 and 50° curves.

[5] Information supplied through Winthrop Laboratories, New York, N. Y.

Table I—Diffusion Coefficients and Permeability and Solubility Constants for Benzalkonium Chloride at Four Temperatures and Four Concentrations in Nylon 6,6 (5 mil) Film

Original Concn., mg./cm.^{3}	Temp., °C.	Diffusion Coefficient ($D \times 10^{-9}$ cm.^{2}/sec.)	Permeability Constant ($P \times 10^{-8}$)[a]	Solubility Coefficient
10.14	70	2.06	0.715	3.47
	60	0.673	0.475	7.06
	50	0.578	0.202	3.49
	40	0.569	0.068	1.19
5.145	70	4.59	1.14	2.49
	60	0.831	0.570	6.86
	50	0.609	0.313	5.14
	40	0.503	0.153	3.04
2.035	70	0.678	1.490	22.0
	60	0.444	0.850	19.1
	50	0.279	0.517	18.5
	40	0.143	0.159	11.3
0.9900	70	0.903	2.600	28.8
	60	0.725	1.080	14.9
	50	0.207	0.577	27.9
	40	0.101	0.474	46.9

[a] [(mg.)(cm.)]/[(sec.)(cm.^{2})(mg./cm.^{3})].

White *et al.* did note a slight decrease in equilibrium sorption with a cationic agent and a cellulosic material as the temperature was increased (10). However, others have demonstrated that, depending upon the specific solute and specific substrate, there may be an increase in sorption with an increase in temperature (5). The increase of sorption with temperature raises the question as to the possibility that true equilibrium was not reached or that degradation or alteration of the polyamide might have occurred.

Diffusion Coefficient—Table I includes the apparent diffusion coefficients for each of the original concentrations of benzalkonium chloride at each of the temperatures studied. As would be expected, the D values increased with temperature for each of the original concentrations. The effect of concentration on D becomes very evident, demonstrating quite vividly how D can be influenced by the original concentration.

A comparison of patterns of diffusion as a function of original concentration at each of the temperatures shows that for the 40 and 50° conditions diffusion increases with an increase in concentration, reaching a maxima at the 0.5% and then dropping slightly at the 1.0% concentration. The D values for the 50 and 60° conditions reveal a higher D at the 0.1% concentration, then a drop in the D value at the 0.2%, followed by an increase in D at the 0.5% and finally, another drop in D at the highest original concentration. The unusual patterns illustrated in Fig. 2 can best be explained by the properties of the surface-active agent, benzalkonium chloride, and upon its propensity to form micelles.

At the 0.1% concentration, the benzalkonium chloride in the solution will exist primarily as single molecules in equilibrium with dimers. These particles will enter and diffuse in the polyamide matrix as monomers and dimers and their rate of transport in the substrate will be a direct function of temperature. As the original concentration of the solution is increased to 0.2%, the benzalkonium chloride at the two lower temperatures is primarily in the monomer and dimer configurations and enters the substrate and diffuses in the same manner as in the lower concentration situations. A slight increase in D might be expected since a higher concentration gradient is probably operating in the 0.2% solution than in the 0.1% solution. At the 60 and 70° situation, a greater degree of association of molecules appears to take place, preventing or

Table II—Activation Energies of Diffusion and Permeation for Benzalkonium Chloride in Nylon 6,6 Film

Original Concn., %	ΔE_D (kcal./mole)	ΔE_P (kcal./mole)
1.0	2.75	14.0
0.5	5.80	13.8
0.2	13.0	12.2
0.1	16.1	12.1

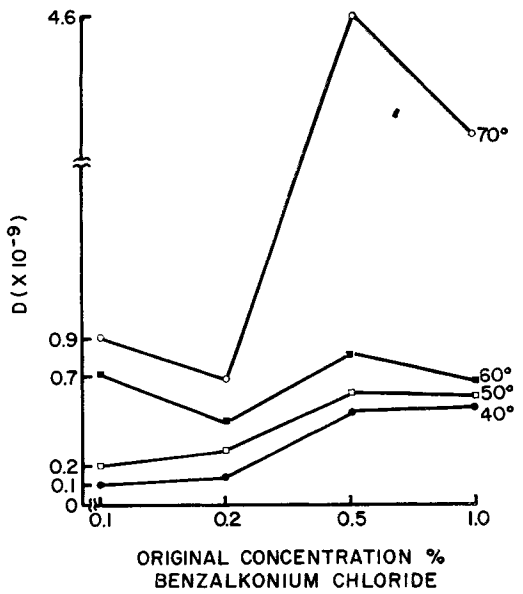

Figure 2—*The effect of original concentration on the diffusion coefficient of benzalkonium chloride in polyamide.*

hindering the benzalkonium chloride from entering areas of the substrate for binding which was available at the 0.1% concentration. This is speculative, of course, but appears to be reasonable in light of a cluster formation of particles in plastics. Further increase in concentration to 0.5% produces a solution with an increased number of associated molecules and micelles. The slight increase in D values at the 40, 50, and 60° conditions and the extremely large increase in D at 70° may not be consistent with what has been said previously, since larger particle sizes could be expected to travel through a matrix at a slower rate. Therefore, the increase in D for all of the temperatures at the 0.5% concentration must be explained by the plasticizing effect the benzalkonium chloride has on the polyamide. This effect becomes much more operative for the 70° diffusion. As the associated molecules and micelles travel through the polymer matrix, they push the polymer chain apart revealing new sites for binding. At the highest concentration studied (1.0%) there are even more micelles present, and even though plasticizing action may still be taking place, this has less of an effect on diffusion. The larger number of aggregates attracts individual benzalkonium chloride molecules and/or prevents other molecules from being bound to the polyamide.

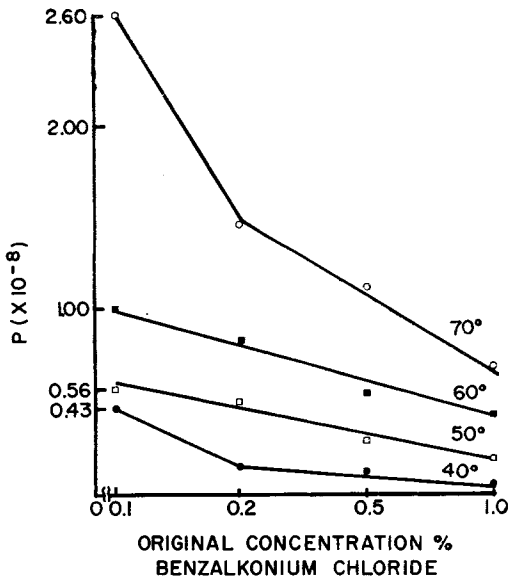

Figure 3—*The effect of original concentration on the permeability constant of benzalkonium chloride in polyamide.*

Permeability Constant—As would be anticipated, P values increased with an increase in temperature. The influence of concentration may also be noted by referring to Fig. 3. At all of the temperatures studied the permeability constant decreased with an increase in concentration. Since permeability is composed of two factors (diffusion and solubility) either factor or both factors could account for the results. In general, it is believed that the solubility factor was the more predominant in influencing the values obtained for the permeability constants.

Activation Energies of Diffusion and Permeation—The activation energies of diffusion in polyamide are shown in Table II and reveal that the energy requirement increases from 2.75 kcal./mole for the 1.0% solution, reaching a value of 16.1 kcal./mole for the 0.1% solution. These values would indicate that more energy is needed for "pushing" the diffusing molecule (or micelles) through the polymer matrix at the lowest concentration than for the higher concentrations. Other investigators have also noted the influence of the original solution concentration on the activation energy of diffusion.

In a study of the dyeing of wool, Alexander and Hudson found that a low dye concentration gave an activation energy of approximately 5.0 kcal./mole which increased to 13.0 kcal/mole when the dye concentration was high (14). These authors concluded that at the very low dye concentration a film of stagnant solution was present and that diffusion through this liquid film would be very slow and even be the rate-determining step in dyeing. As the concentration of the solution increases, however, the film becomes thinner and, in essence, will have a negligible effect on the overall dyeing. In the diffusion experiments reported in this paper, sufficient stirring took place to minimize the stagnant layer adjacent to the plastic.

Rodell *et al.* in their studies of the sorption of sorbic acid by polyamide, noted that when the original concentration of the sorbic acid was increased the activation energy of diffusion decreased from 26.2 kcal./mole to a low of 3.07 kcal./mole for the highest original solution concentration (15). The benzalkonium chloride activation energies were similar to those of Rodell even though the magnitude was much smaller. It should be pointed out that the apparent diffusion coefficients for the sorbic acid decreased with an increase in the original concentration. This pattern of diffusion for the benzalkonium chloride was essentially the same.

The activation energy of permeation for each of the original concentrations of benzalkonium chloride are included in Table II and it is interesting to note that the ΔE_P values fell into a rather narrow range (12 to 14 kcal./mole). Theoretical implications of the activation energy of permeation are vague. It must be remembered that the permeation process actually involves both diffusion and solubility, thus the activation energy of permeation is the sum of the activation energy of diffusion (ΔE_D) and the heat of solution (ΔH).

Mechanism of Binding—The hydrogen-ion concentration of the benzalkonium chloride solutions used in this study was in the range of 6.0. Mathieson *et al.* have reported that the polyamide (Nylon 6,6) has an isoelectric point occurring at a pH of 6.20 (16). It would seem unlikely that the cationic moiety of benzalkonium chloride could interact with the polyamide through an ion-ion interaction since no negative ionic charge is present in the polyamide at pH 6.0. It is possible, of course, that even in the pH range used in these studies, some ionized groups of the carboxyl moiety of the polyamide exist, but if this is the case, the number would be extremely small and could not account for all of the sorption. Cation ion-exchange with hydrogen in the carboxyl groups in polyamide also appears to be ruled out for the most part since this could not account for the large amount of sorption. The most possible mechanism for the interaction must come through the attraction of the rather large hydrophobic moiety of the cation to nonspecific sites in the polyamide, these being the hydrocarbon sections in the polyamide. As the agent is bound, a chloride ion follows, giving electrical neutrality. With solutions having high concentrations of benzalkonium chloride, the associated molecules and micelles also interact to the nonspecific sites along the hydrocarbon section of the nylon polymer. Admittedly, the micelle interaction is most likely more complex than depicted here, but further rationalization cannot be given until detailed investigations lead to the exact mechanism of interaction.

SUMMARY

Sorption experiments were conducted using benzalkonium chloride as the solute and a polyamide (Nylon 6,6) as the insoluble

polymeric material. Results of the experiments revealed that maximum sorption values occurred above the region of the critical micelle concentration for three of the four temperatures studied. Permeation experiments led to the evaluation of diffusion coefficients and permeability constants at several original concentrations of the cationic agent at four different temperatures. It was theorized that the binding of the benzalkonium chloride to the polyamide most likely occurred between the hydrophobic portions of the cation and the hydrocarbon regions of the nylon. The unusual sorption characteristics and the effect of original concentrations on the diffusion and permeation were attributed to the propensity of the benzalkonium chloride to form aggregates and micelles in aqueous mediums.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 14, 1968, from the *Materials Science Toxicology Laboratories, University of Tennessee Medical Units, Memphis, TN 38103* and *The Drug-Plastic Research and Toxicology Laboratories, University of Texas, Austin, TX*

Accepted for publication February 24, 1969.

This investigation was supported in part through an extramural grant from the *Division of Biologics Standards*, National Institutes of Health, Bethesda, Md.

Presented to the Industrial Pharmaceutical Technology Section, APhA Academy of Pharmaceutical Sciences, Miami Beach meeting, May 1968.

Comparative Pharmacokinetics of Coumarin Anticoagulants V: Kinetics of Warfarin Elimination in the Rat, Dog, and Rhesus Monkey Compared to Man

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Abstract □ Small (1–4 mg./kg.) and large (10–12.5 mg./kg.) doses of sodium warfarin were administered intravenously to rats (Sprague-Dawley, male), dogs (mongrel, male), and monkeys (rhesus, male). Warfarin concentrations in the plasma declined exponentially with time in each species. The plasma half-life of warfarin was independent of dose in the dog, appears to decline slightly with increasing dose in the rat, and increases markedly with increasing dose in the monkey. Apparent volumes of distribution (dose/C_p^0) increased slightly with increasing dose in most of the animals. The half-life of warfarin, as observed in this study, increases in the order: rat < monkey < dog < man. It is shown that the dose-dependent elimination kinetics of bishydroxycoumarin, known to occur in man and monkeys, can be observed also with the other widely used coumarin anticoagulant, warfarin, when sufficiently high doses of the latter are administered. This effect is not seen clinically perhaps because the therapeutic dose range of warfarin is much lower than that of bishydroxycoumarin. Concomitant administration of a large dose (≥10 mg./kg.) of bishydroxycoumarin with sodium warfarin (2 mg./kg.) to monkeys tended to increase the half-life of warfarin. These observations are consistent with other indications suggesting that the two coumarin anticoagulants are subject to the same major biotransformation pathway(s).

Keyphrases □ Coumarin anticoagulants, elimination—pharmacokinetics □ Warfarin elimination—pharmacokinetics □ Pharmacokinetics comparison—warfarin elimination, rat, dog, monkey, man □ UV spectrophotometry—analysis □ Fluorometry—analysis

The pharmacokinetics of the coumarin anticoagulants have been the subject of considerable interest and study (reviewed in *Reference 1*). The kinetics of bishydroxycoumarin (BHC) elimination have been investigated in the rat, guinea pig, dog, and rhesus monkey (2), and a detailed multicompartmental analysis of BHC elimination has been attempted in man (3). More recent studies have resulted in the elucidation of the kinetics of the prothrombinopenic effect of the coumarin anticoagulants in man (4, 5). The present report is concerned with the pharmacokinetics of warfarin (3-α-phenyl-β-acetylethyl-4-hydroxycoumarin) elimination in the rat, dog, and rhesus monkey, as compared to man.