Equilibrium Penetration of Monolayers IV: Dipalmitoyllecithin–Cetrimonium Bromide System

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Abstract □ Equilibrium surface pressure–area isotherms of dipalmitoyllecithin monolayers were measured on substrates containing various concentrations of the surfactant, cetrimonium (hexadecyltrimethylammonium) bromide. From these isotherms, the saturation adsorptions of surfactant for various surface lecithin concentrations were calculated. Plotting of these adsorptions against the inverse of the area per lecithin molecule, as required for the "accessible area" theory, revealed two linear segments, corresponding to penetration at high and at low monolayer areas. At both high and low areas, the adsorption into the accessible areas of the surface was similar to adsorption at a monolayer-free surface. The effective cross-sectional area of the monolayer molecule in the low area region was equal to the collapse area; in the high area region, it was equal to an area corresponding to the co-area, as calculated from the Amagat equation. The change in cross-sectional area corresponded to the transition in the monolayer from a liquid condensed state to a liquid expanded state.

Keyphrases □ Cetrimonium bromide—equilibrium penetration of dipalmitoyllecithin monolayers, surface pressure–area isotherms measured □ Dipalmitoyllecithin monolayers—equilibrium penetration by cetrimonium bromide, surface pressure–area isotherms measured □ Adsorption—cetrimonium bromide on dipalmitoyllecithin monolayers, calculated from surface pressure–area isotherms □ Surfactants—cetrimonium bromide, equilibrium penetration of dipalmitoyllecithin monolayers, surface pressure–area isotherms measured □ Monolayers—dipalmitoyllecithin, equilibrium penetration by cetrimonium bromide, surface pressure–area isotherms measured

The penetration of insoluble monolayers by surfactants injected into the aqueous subphase has been of interest since the work of Schulman and Hughes (1). Much interest has stemmed from the biological implications of penetration experiments involving biologically significant material (2–7), excited particularly by analogies between the monolayer and membrane structures (8). However, understanding of the penetration process has been slow to develop. To a large extent, this lack is a consequence of the early emphasis on kinetic studies and the neglect of equilibrium measurements that could provide essential adsorption data (9).

BACKGROUND

Using the little equilibrium penetration data available (10), Fowkes (11) and McGregor and Barnes (12) proposed theoretical treatments based on the osmotic approach to monolayer properties and on the concept of accessible areas, respectively. Equilibrium penetration can be regarded as the equilibrium adsorption of surfactant molecules at a monolayer-covered surface. In the accessible area theory, this adsorption is considered to occur in the spaces between monolayer molecules. Therefore, the adsorption, Γ_f, is a function of the area per monolayer molecule, $\hat{A}_M$:

$$\Gamma_f = \Gamma_w - a_M \Gamma_w (1/\hat{A}_M) \qquad \text{(Eq. 1)}$$

where Γ_w is the surface concentration of surfactant in the accessible area and a_M is the apparent cross-sectional area of the monolayer molecule. According to the theory, a plot of Γ_f against $1/\hat{A}_M$ should give a straight line and physically reasonable values for Γ_w and a_M.

Such linear plots have been reported for several systems (12–14). Usually, these plots consist of two or more straight lines, yielding different values for Γ_w and/or a_M and showing that the characteristics of penetration may differ for different conditions.

In this paper, data on the equilibrium penetration of dipalmitoyllecithin monolayers by cetrimonium (hexadecyltrimethylammonium) bromide are presented and analyzed in terms of the accessible area theory. Both compounds are available in a highly purified condition and, thus, fulfill one main experimental criterion for this type of study. Both also have some biological importance as representatives of their class: dipalmitoyllecithin as a model lecithin representing lecithins of biological origin and uncertain composition (15) and cetrimonium bromide as typical of the quaternary ammonium compounds used as antibacterial agents and thought to act by modifying the cell membrane (16).

EXPERIMENTAL

The monolayer-forming substance was β,γ-dipalmitoyl-L-α-lecithin[1]. The spreading solvent was *n*-hexane[2]–absolute ethanol[3] (9:1). A lecithin solution was made, sealed in 1-ml ampuls, and stored in a freezer. The surface pressure–area (π–$\hat{A}_M$) isotherm of the lecithin is shown in Fig. 1. This isotherm is in reasonable agreement with the isotherms given by Cadenhead and Kellner (17) for various spreading solvent mixtures.

The surfactant, cetrimonium bromide[4], was tested by mass spectrometry and GLC; no impurities or homologs were detected, indicating a purity of >99.9%. The critical micelle concentration (CMC) found by surface tension measurements was 0.96 m*M*, which is within the literature range of 0.90–1.0 m*M* (13). Triple-distilled water was used for all subphases and surfactant solutions.

Penetration experiments were performed on a film balance, consisting of a trough and barriers of polytef; a Wilhelmy plate and strain gauge were used to detect surface tension variations (13). The apparatus for injecting the surfactant into the subphase was described previously (13, 18). Briefly, two perforated glass tubes were immersed in the trough and attached by polytef tubing to a pumping system (all polytef or glass parts) and a glass injection chamber. With this arrangement, it was possible to place the surfactant solution in the injection chamber and then to circulate and mix the subphase without appreciably disturbing a monolayer spread on the surface.

Penetration experiments were carried out as follows. The surface tension of the clean water surface was recorded, and then the monolayer was spread at a high area. Because of solvent loss when opening the ampuls, the concentration of the monolayer solution was not known accurately and the amount of monolayer delivered to the surface had to be determined by using the surface pressure–area isotherm for the lecithin (Fig. 1). Therefore, the monolayer was compressed to approximately 5 mN/m surface pressure, and the area was noted and compared with the isotherm.

After the monolayer had been expanded back to maximum area, a known amount of a concentrated surfactant solution was placed in the injection chamber and the pump was switched on. In about 10 min, the subphase concentration was homogeneous, as indicated by a constant value for the surface pressure. The monolayer was then compressed in a stepwise manner, with increases in surface pressure of about 5 mN/m for each step; it was allowed to stand after each compression until equilibrium had been reached, as shown by a steady value on the recorder output. This equilibration time varied from about 5 min to 2 hr as the pressure was raised. The stepwise compression was continued until the monolayer collapsed or overflow occurred. The π–$\hat{A}_M$ isotherm was calculated from the equilibrium surface pressure data.

With lower concentrations of surfactant, an alternative technique was used in which the monolayer was spread on a subphase containing the surfactant. At low surfactant concentrations, the results agreed well with

[1] A grade, Calbiochem.
[2] Spectroscopic grade, BDH Chemicals Ltd.
[3] Spectroscopic grade, E. Merck.
[4] Pro analysis grade, E. Merck.

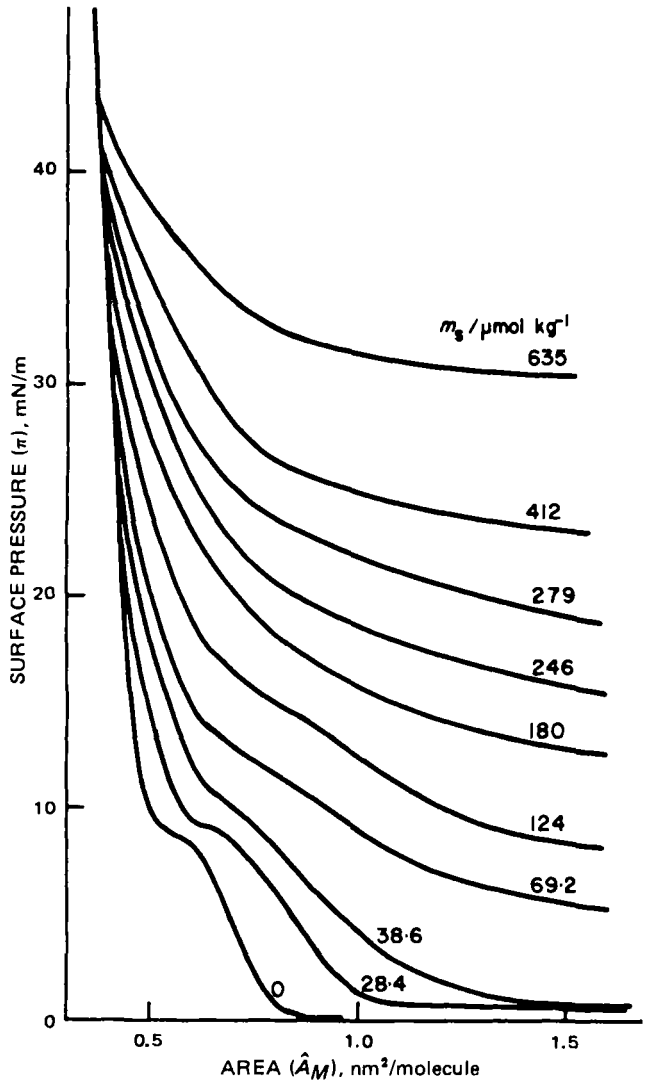

Figure 1—*Surface pressure-area isotherms of dipalmitoyllecithin monolayers on cetrimonium bromide solutions of the various concentrations shown (T = 298° K).*

those obtained by injection; but at high surfactant concentrations, the monolayer could not be spread satisfactorily.

After an experimental run, the monolayer was removed by sweeping; a surface tension reading was then taken for the clean surface. This surface tension was used to determine the concentration of the subphase from a calibration curve (13).

RESULTS

The equilibrium surface pressure–area isotherms for dipalmitoyllecithin monolayers on subphases containing various surfactant concentrations are given in Fig. 1. From these curves, plots of π against log m_S (molality of surfactant) for selected values of $\hat{A}_M$ were prepared (Fig. 2). These plots show considerably more scatter at low areas where the isotherms are steeper than at high areas. All curves are approximately linear at surface pressures greater than 15 mN/m, indicating saturation adsorption. The slopes of these linear sections are shown in Fig. 3.

Figure 3 has two significant features. First, at high areas, the slopes tend toward the slope for a monolayer-free surface, as expected. Second, an inflection point at approximately 0.6 nm²/molecule corresponds to the transition between the liquid-expanded and liquid-condensed states of the lecithin monolayers on water shown in the isotherm in Fig. 1.

The data of Fig. 3 were used to calculate the saturation adsorptions of surfactant in the monolayer-covered surface. For these calculations, the Gibbs equation must be modified to allow for the presence of the monolayer. The equation takes the form derived by Pethica (10):

$$\Gamma = \frac{1}{\Phi\Psi RT}\frac{d\pi}{d\ln m_S} \qquad \text{(Eq. 2)}$$

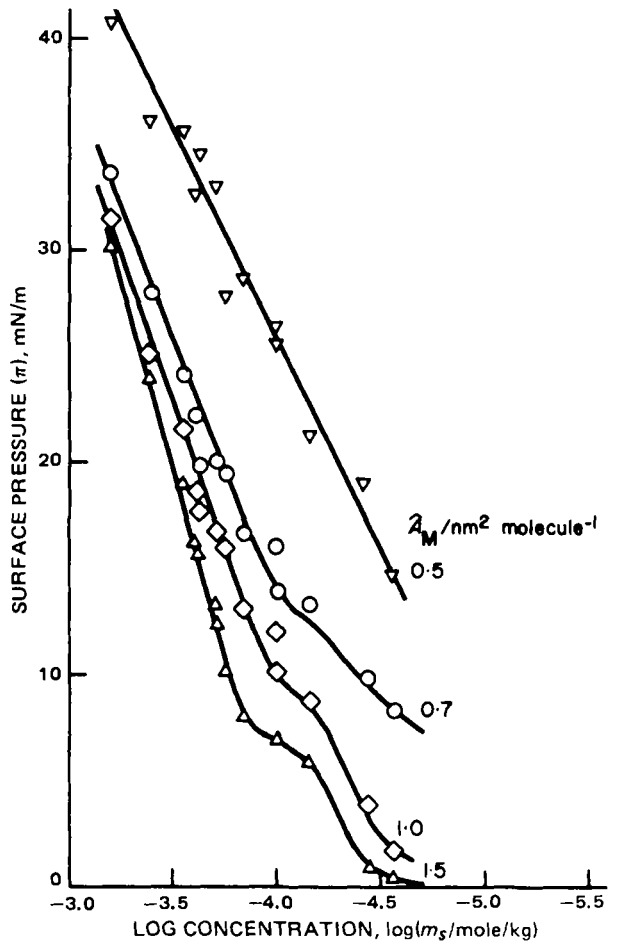

Figure 2—*Surface pressures of cetrimonium bromide solutions under dipalmitoyllecithin monolayers as a function of surfactant concentration. Curves were calculated from the data of Fig. 1 for the areas per dipalmitoyllecithin molecule,* $\hat{A}_M$.

where Γ is the relative adsorption of surfactant with reference to water. The factor Φ arises from the presence of the monolayer and, following Pethica (10), is calculated from:

$$\Phi = \hat{A}_M/(\hat{A}_M - \bar{\bar{A}}_M) \qquad \text{(Eq. 3)}$$

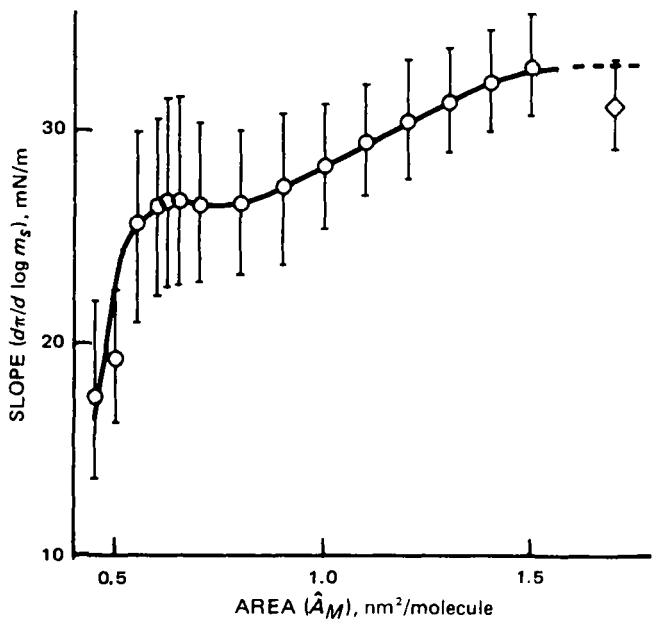

Figure 3—*Slopes of the π–log* m_S *curves in the region of saturation adsorption for cetrimonium bromide solutions under dipalmitoyllecithin monolayers (○) and with no monolayer present (◇).*

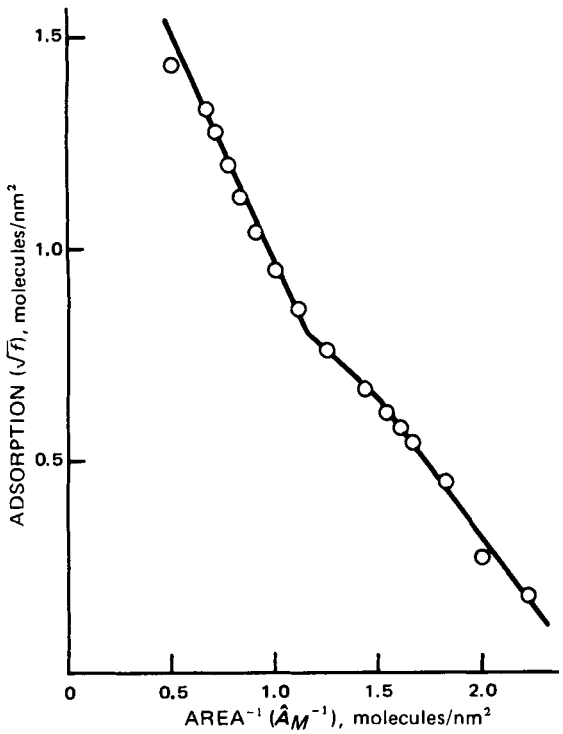

Figure 4—*Adsorptions of cetrimonium bromide into dipalmitoyllecithin monolayers plotted according to Eq. 1.*

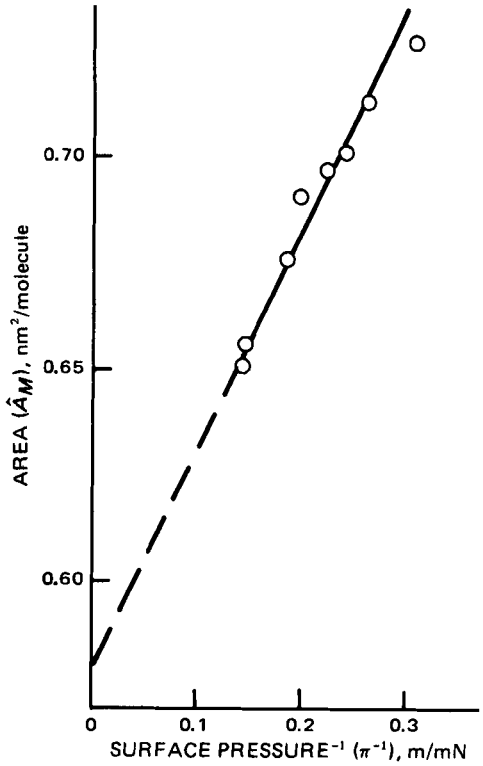

Figure 5—*Monolayer area, $\hat{A}_M$, plotted against the inverse of surface pressure in the high area region of the dipalmitoyllecithin isotherm.*

where $\bar{\bar{A}}_M$ is the partial molecular area of the monolayer substance. The factor Ψ, arising from the presence of the counterion, is to allow for the ionization of the surfactant and was given a value of 2 for the present experimental situation: an ionic surfactant, with no other salt added to the subphase solution (13). The adsorption values are shown in Fig. 4.

DISCUSSION

There are no adsorption data in the literature for direct comparison with the results in Fig. 4. Hendrikx and Ter-Minassian-Saraga (19, 20) measured the penetration of egg lecithin monolayers by radiolabeled cetrimonium bromide. At comparable areas per molecule, their adsorption values are lower than the present values, but the differences possibly could be attributed to the different lecithins used and the presence of buffer ions in their system. It is not possible to extract any adsorption values from the published results of Vilallonga *et al.* (21).

When the saturation adsorption values are plotted against the inverse of the area per monolayer molecule, as required by the accessible area theory (Eq. 1), two distinct linear sections can be seen (Fig. 4). A third middle section or transition region corresponds to the transition in the monolayer state from liquid condensed to liquid expanded (Fig. 1).

By using the accessible area approach, values for the two parameters Γ_w and a_M were determined for the two linear sections (Table I). There is no significant difference between the values of Γ_w for the low and high area regions, and both agree with the value for adsorption on a monolayer-free surface of 1.71 ± 0.20 molecules/nm^2.

There is a substantial difference, however, between the values for a_M in the low and high area regions. At low areas, the value obtained for a_M, 0.41 ± 0.06 nm^2/molecule, agrees well with the collapse area of the lecithin, 0.37 ± 0.01 nm^2/molecule. At high areas, a rearrangement of the equation used by Schofield and Rideal (22), the Amagat equation:

$$\hat{A}_M = \hat{A}_0 + qkt/\pi \qquad \text{(Eq. 4)}$$

Table I—Analysis of the Equilibrium Penetration of Dipalmitoyllecithin Monolayers by Cetrimonium Bromide in Terms of Eq. 1

	Γ_w, molecule/nm^2 (π = 30 mN/m)	a_M, nm^2/molecule (π = 30 mN/m)
Low $\hat{A}_M$	1.70 ± 0.19	0.41 ± 0.06
High $\hat{A}_M$	1.97 ± 0.19	0.51 ± 0.07
No monolayer	1.71 ± 0.20	—

where q is a measure of the lateral adhesion of the molecules in the film, was used to find an estimate, $\hat{A}_0$, for the effective cross-sectional area of a lecithin molecule in the liquid-expanded state. The fit of the experimental data to Eq. 4 is good, as shown by the linear plot of $\hat{A}_M$ against $1/\pi$ in Fig. 5. From the intercept of the linear plot, a value of 0.58 ± 0.07 nm^2/molecule is obtained from $\hat{A}_0$. This value agrees within the experimental error with the value for a_M, 0.51 ± 0.07 nm^2/molecule, in the high area region.

Hence, the transition in the lecithin monolayer from the liquid-condensed state to the liquid-expanded state accounts for the two linear regions in the adsorption curve (Fig. 4). In the low area region, the monolayer is in a condensed state with the polar head groups anchored on the surface and the long chain tails upright. In this condensed state, the collapse area of the lecithin is a good estimate of the effective cross-sectional area of a dipalmitoyllecithin molecule and, therefore, is the appropriate area to be used for the accessible area theory.

However, in the high area region, the monolayer is in an expanded state with its polar heads anchored on the surface and the long chain tails slightly bent and disordered and probably divergent because of a higher proportion of *gauche*-conformers (15). Thus, a lecithin molecule in the expanded state would occupy a larger area on the surface. This area was estimated by calculating the co-area, $\hat{A}_0$, (using Eq. 4), which represents a correction to the ideal two-dimensional gas equation for the effective area of the molecules. Therefore, the co-area is the same as the area required. In both regions, the surfactant molecules are adsorbing into available spaces between the clusters of monolayer molecules. Hence, the value obtained for adsorption is similar to that on a monolayer-free surface.

In the octadecanol–cetrimonium bromide system, there are also two distinct linear sections joined by a transition region (13). However, in this system, the value for Γ_w changes from the low area region to the high area region and the value for a_M is constant over the entire range. In the high area region, the surfactant adsorbs into large areas of free surface between the clusters of close-packed monolayer molecules; the value obtained for Γ_w, therefore, is for a monolayer-free surface. In the low area region, the value for Γ_w is significantly higher than the adsorption into a monolayer-free surface. The surfactant apparently adsorbs into small holes in the monolayer, with only one surfactant molecule per hole. Since the surface concentration of surfactant is small, the surfactant ions are too widely separated for electrostatic effects to be apparent, and it is the size of the surfactant ion that is important. In this region, the inverse of the

value obtained for Γ_w, 0.37 nm^2/molecule, is in good agreement with the estimated cross-sectional area of the surfactant ion (13).

There is no similar region in the present system. The explanation of this difference appears to concern the ionic nature of the choline group in comparison with the nonionic nature of the alcohol. With octadecanol monolayers, the surfactant ion is being adsorbed into holes in an uncharged matrix of octadecanol molecules; with the lecithin monolayers, the matrix consists of the zwitterionic phosphatidyl choline. The positively charged quaternary ammonium part of the lecithin molecule apparently dominates the penetration process so that, even when the adsorption of surfactant ions is small, the ionic environment is roughly similar to that found with the higher adsorption at a monolayer-free surface. Hence, in both situations, the adsorption is limited by electrostatic effects and not by the size of the surfactant ion.

The shape of the surface pressure–area isotherms of the penetrated lecithin monolayers is interesting (Fig. 1). At very low areas, all surfactant molecules are squeezed out of the surface, as indicated by the tendency of the isotherms of penetrated monolayers to join up with the surface pressure–area isotherm for the lecithin monolayer on water. Unfortunately, there was too much scatter in the data to allow adsorptions to be calculated at areas per molecule lower than 0.45 nm^2/molecule, but qualitatively the ejection of surfactant is clearly shown by the isotherms in Fig. 1.

CONCLUSION

Analysis of the equilibrium penetration of dipalmitoyllecithin monolayers by cetrimonium bromide according to Eq. 1, derived from the accessible area theory (12), shows that: (*a*) good straight lines are obtained, as required by the theory; and (*b*) the values of the parameters a_M and Γ_w obtained from these analyses are physically reasonable in that they correspond to the effective molecular areas of the lecithin in the various monolayer states and to the adsorption of the surfactant at a monolayer-free surface, respectively.

It is concluded that the accessible area theory provides a satisfactory description of equilibrium penetration in the present system. Alternatively, the theory could have been used to predict equilibrium penetrations from the surface pressure–area isotherm of dipalmitoyllecithin and the adsorption of cetrimonium bromide at a monolayer-free surface.

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Effects of Paper on Performance of Antibiotic-Impregnated Disks

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Received February 7, 1977, from the *Antibiotic Residue Branch, National Center for Antibiotics Analysis, Food and Drug Administration, Washington, DC 20204.* Accepted for publication June 23, 1977.

Abstract □ Grades of paper used in the manufacture and assay of antibiotic susceptibility disks have significant effects on the diffusion of antibiotics from the paper when compared to a control grade of paper. The papers also evoke different microbiological responses to changing concentrations of some antibiotics. Regulatory implications and the need for further standardization of assays among control laboratories are explored. Grades of paper generally used for assay and control of susceptibility disks, on the other hand, appear to be comparable to each other in both respects.

Keyphrases □ Antibiotic susceptibility disks—effect of various grades of paper on performance □ Paper, various grades—effect on performance of antibiotic susceptibility disks □ Disks, paper—effect of various grades on performance in antibiotic susceptibility tests

That different grades of paper affect diameters of zones of inhibition produced by known antibiotic concentrations has been recognized for some time (1, 2). One study (1) compared two grades of paper[1] to a control grade. One grade produced inhibition zones somewhat larger than the standard disk for each of four antibiotics; the other grade produced either significantly smaller zones or none at all.

Marth *et al.* (2) determined that use of 6.35- and 12.7-mm disks of the same grade of paper permitted detection of the same low levels of penicillin in milk but that 6.35-mm disks of another grade were generally able to detect only higher concentrations of antibiotic under the same conditions.

[1] Whatman.