moregulation of the column might also be desirable. The slight variations in retention times do not appreciably affect peak heights for quantitation.

Acidification of the mobile phase was shown to affect chromatography favorably. It shortened the retention times of plasma residues and lengthened retention times of phenytoin and I, thereby minimizing plasma interference with the assay.

The column gave good resolution of the compounds for at least 6 months of operation with systems of this sort, being used about 3 days/week.

In conclusion, this method should be useful for clinical monitoring of plasma phenytoin concentrations. Its metabolite, I, can be detected in moderately high concentrations, as shown by a preliminary *in vivo* rabbit investigation. The method is extremely rapid, economical of plasma and reagents, and simple.

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Comparison of Adsorbed Films of a Polyvinylpyrrolidone Copolymer with Spread Monolayers

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Received November 28, 1977, from the *College of Pharmacy, Rutgers—the State University, Piscataway, NJ 08854.* Accepted for publication February 23, 1978.

Abstract □ The adsorption of a polyvinylpyrrolidone–polyvinyl acetate graft copolymer from solution was studied by surface pressure measurement. Adsorption from the dilute solutions was slow, limited, in part, by diffusion of polymer molecules to the surface. When adsorbed monolayers were compressed on a surface balance, the resulting surface pressure values paralleled those of a spread monolayer, strongly suggesting that the structures of adsorbed and spread monolayers are the same.

Keyphrases □ Polyvinylpyrrolidone copolymer—adsorption from solution studied by surface pressure measurement, films compared to spread monolayers □ Adsorption—polyvinylpyrrolidone copolymer from solution, studied by surface pressure measurement, films compared to spread monolayers □ Surface pressure—measurement used to study polyvinylpyrrolidone copolymer adsorption from solution, films compared to spread monolayers □ Polymers—polyvinylpyrrolidone–vinyl acetate copolymer, adsorption from solution studied by surface pressure measurement, films compared to spread monolayers

The surface properties of polymers are studied conveniently by spreading them as monolayers on a water surface. In such studies, polymer molecules are spread onto an aqueous substrate in small quantity so as barely to disturb the surface tension. Once the spreading process is complete, the surface area is reduced to smaller values (thereby concentrating the two-dimensional polymer systems) and the surface properties are measured as a function of the area available to each unit of polymer.

However, in systems of practical interest such as pharmaceutical dispersions, a polymer in the formulation is in solution and polymer molecules migrate to the interface and are adsorbed. The first few polymer molecules adsorbed encounter an uncrowded interface and have space in which to adopt the energetically most favorable orientation. But the interfacial region gradually becomes crowded as adsorption proceeds, and polymer molecules arriving later may be unable to spread completely. The conformation of molecules in concentrated adsorbed monolayers may thus be different from that in spread monolayers, so the properties of the two types of systems may differ.

BACKGROUND

Most studies comparing the structures of spread and adsorbed monolayers have been carried out on proteins. Yamashita and Bull (1) found that adsorbed films of lysozyme were thicker than spread monolayers. They suggested that the adsorbed protein molecules largely retain their native configuration at the surface while spread lysozyme molecules unfold more completely. Surface films of trypsin formed by applying the enzyme to a clean surface showed a complete loss of enzymatic activity (2–4). However, when the films were formed by adding more trypsin to an interface that already had some trypsin present, some enzymatic activity was retained. In this situation, complete spreading did not occur and the film properties depended on the method of film formation.

Musselwhite and Palmer (5) prepared monolayers of bovine serum albumin using two different techniques. In one experiment, the monolayers were spread in the usual way, and the film was concentrated by compression to a smaller surface area. The second approach involved concentration of the monolayer by maintaining the same surface area and adding more protein to the surface. The newly added protein molecules encountered a surface already partially occupied. This mode of increasing the surface concentration of the protein is similar to the process that occurs during adsorption. The force–area diagrams for the two techniques were quite different. These reports indicated that adsorbed and spread films of proteins are not equivalent.

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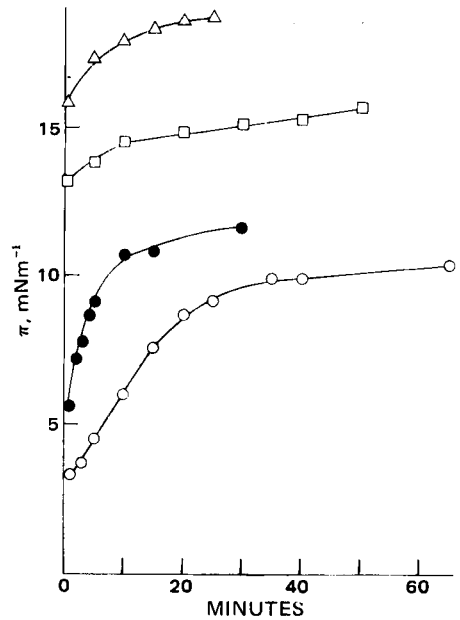

Figure 1—*Surface pressure of solutions of copolymer as a function of time. Key: △, 8.05 × 10⁻³%, unstirred; □, 2.75 × 10⁻³%, unstirred; ○, 0.20 × 10⁻³%, unstirred; and ●, 0.20 × 10⁻³%, stirred.*

Previously (6, 7), the properties of monolayers of some polyvinylpyrrolidone–polyvinyl acetate graft copolymers were presented. The copolymer with the smallest vinyl acetate content is sufficiently water soluble to permit study of its adsorption from aqueous solution. This report describes the adsorption of the copolymer at the air–water interface and compares adsorbed films with the monolayers studied earlier.

EXPERIMENTAL

The copolymer[1] [61.2% (w/w) vinylpyrrolidone and 38.8% vinyl acetate] was purified as described previously (3). Water was deionized and distilled from an all-glass still. Glassware was cleaned with chromic acid cleaning solution and then rinsed repeatedly with distilled water. Aspiration was used to remove impurities from the surfaces of the polymer solutions. Surface tension was measured by the Wilhelmy plate method. All experiments were performed at room temperature, 24.5 ± 1°.

RESULTS AND DISCUSSION

Surface tension of the polymer solutions was measured as a function of time. Results are expressed in terms of surface pressure, π, defined as the difference between the surface tension of pure water and that of the polymer solution. The solution surfaces were cleaned by aspiration, and surface pressure was monitored for 10 days. The values of surface pressure rose quickly at first and then leveled off. Attainment of a limiting value of surface pressure indicated that adsorption underwent no further change. The more concentrated solutions approached within 1 mNm^{-1} of the limiting value of surface pressure within 1 hr. Very dilute solutions required about 3 days to reach the same point.

Some data obtained during the first 60 min after aspiration of the solution surface are presented in Fig. 1. Previous work (8) showed that bulk diffusion is important in limiting the adsorption rate in very dilute solutions. The dependence of the rate of copolymer adsorption on bulk concentration suggests that diffusion is an important step in the adsorption process, at least during the early stages. Further evidence for this conclusion is provided by the fact that gentle stirring speeded adsorption from the very dilute solutions (Fig. 1).

At higher values of bulk concentration, the approach to apparent equilibrium is limited by the presence of previously adsorbed molecules. Molecules approaching the surface must find an opening to which to anchor and then bring other polymer segments into the interface. The attainment of a quasiequilibrium conformation at the interface requires expansion of the molecule against the force exerted by neighboring molecules. This condition has been referred to as a surface pressure barrier to adsorption (9).

[1] PVP/VA 735, GAF Corp., New York, N.Y.

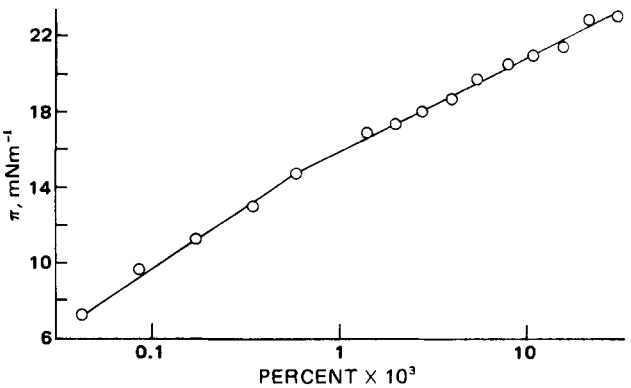

Figure 2—*Limiting values of surface pressure of solutions of the copolymer as a function of concentration.*

In Fig. 2, the limiting surface pressures are plotted against the logarithm of solution concentration. The experimental points may be resolved into two linear segments. The slope of the plot is steeper at low concentrations than at higher concentrations. This type of behavior is opposite to that found with compounds of low molecular weight but was observed in studies of other polymers (10, 11).

Unfortunately, the Gibbs isotherm cannot be employed to calculate values of surface excess. The Gibbs equation assumes a state of equilibrium, and there is no assurance that the reported surface pressures represent equilibrium values since surface films of polyvinylpyrrolidone copolymers are not readily desorbed (6). Furthermore, the copolymer molecules cannot be considered to be a single species since they differ in molecular weight and in relative composition of the monomeric building blocks. Therefore, the polymer solutions represent multicomponent systems, to which the Gibbs equation is inapplicable (12).

In several independent experiments, copolymer solutions were placed in a surface balance and permitted to stand for about 15 min after the surface was swept. The adsorbed monolayers were then compressed, and surface pressure readings were taken as a function of available surface area (Fig. 3). To determine whether compression of these adsorbed films yielded the same pattern as the spread monolayer, the specific surface area, in square meters per milligram, at full trough area was determined for each experiment from the π–A curve for spread monolayers of the copolymer (6). The surface area corresponding to the other surface pressures measured on the compressed adsorbed films then was calculated from the percent of the trough area. With these calculated areas and the experimentally measured surface pressures, it was possible to construct π–A curves representing compression of the adsorbed films.

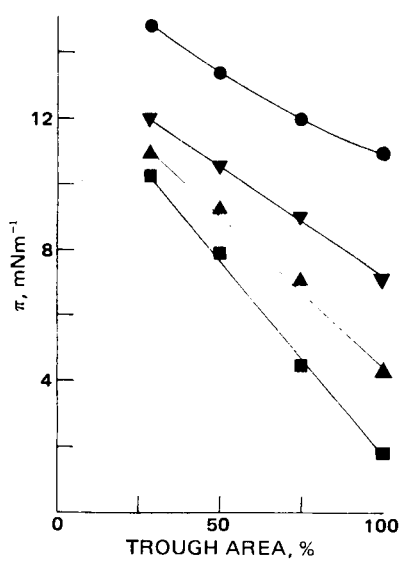

Figure 3—*Surface pressure of adsorbed monolayers compressed on a surface balance. Each symbol represents a different adsorbed monolayer.*

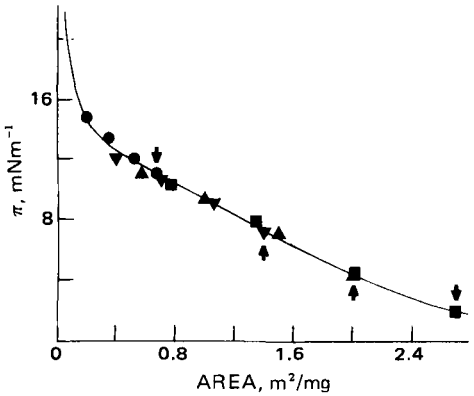

Figure 4—*Comparison of π–A results obtained from adsorbed films (see text for details) with those from a spread monolayer. This solid line is the π–A curve for the compressed monolayer (6). Symbols correspond to those in Fig. 3. Arrows indicate the data points that were fit to the π–A curve of the spread monolayer and from which surface area values of the other points were calculated.*

In Fig. 4, all of the data are combined and compared with the π–*A* curve for a spread monolayer. The correspondence obtained suggests that the structure of the adsorbed monolayers is identical or very similar to that of a spread monolayer, in contrast to what was observed with other polymers.

Concentration of a monolayer by addition of more of the copolymer (in solution) to the surface leads to surface pressure values equivalent to those obtained by compression of a monolayer (6). Therefore, the added copolymer molecules are incorporated to yield a system identical to that obtained by compression of an extremely dilute surface film. Spreading in the presence of a monolayer is similar to the process that occurs during adsorption in that the molecules arriving at the surface in both situations must take their place against the resistance of an existing surface pressure (5). Thus, the ability of the copolymer to spread in the presence of a monolayer to reach a quasiequilibrium state is consistent with the evidence obtained in the present study for the structural equivalence of spread and adsorbed monolayers of the copolymer.

Attainment of the final shape and orientation of an adsorbing polymer molecule is a two-step process. In the first step, the polymer molecule touches the interface and "sticks." At this point, the conformation of the molecule is the same, or nearly the same, as in the bulk phase. In the second step, if it occurs, the molecule rearranges itself to minimize contact of hydrophobic groups with the aqueous liquid while permitting immersion of polar groups. This rearrangement usually involves a change from a coiled conformation to one that is more extended.

Adsorption of the open conformation typical of completely spread molecules requires breaking intramolecular bonds, which represents an energy barrier to molecular rearrangement. In addition, the surface pressure of neighboring molecules in the interface must be overcome since the change to the extended conformation requires additional area at the interface. Another possible hindrance to spreading is intermolecular interaction between adsorbing molecules or between an adsorbing molecule and one that has achieved the open conformation. This interaction would tend to fix the adsorbing molecule in place, making it difficult to reorient in the interface. If these barriers are operative, molecular reorientation can be extremely slow, and the majority of adsorbed molecules will then retain a conformation similar to that in bulk.

Polyvinylpyrrolidone copolymer molecules are highly flexible, and the monolayers are fluid (6). These findings argue against significant intra- or intermolecular association and help to explain why adsorbed films are fully spread. Proteins, however, show significant interactions (13). This fact may account for the differences between spread and adsorbed monolayers of proteins.

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NOTES

Polarographic Analysis of Cephalexin

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Received November 14, 1977, from the *Department of Physical Chemistry and Chemical Pharmacology, Faculty of Chemical Sciences, University of Chile, Santiago 1, Chile.* Accepted for publication February 14, 1978.

Abstract □ Cephalexin was found to be polarographically reducible after hydrolysis in an acidic medium, producing two polarographic waves. Both waves were diffusion controlled. The concentration–diffusion plot method was used for the analysis of cephalexin in capsules.

Keyphrases □ Cephalexin—polarographic analysis in dosage forms □ Polarography—analysis of cephalexin in dosage forms □ Antibacterials—cephalexin, polarographic analysis in dosage forms

Cephalexin[1] is a semisynthetic analog of cephalosporin C in which the α-aminoadipic acid of cephalosporin C is replaced by phenylglycine and the ester-linked acetic acid is condensed to a simple methyl group.

Electrochemical analysis of cephalosporins, specifically, cephalosporin C and derivatives (1, 2) and cefamandole

[1] Recalcine Inc.

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