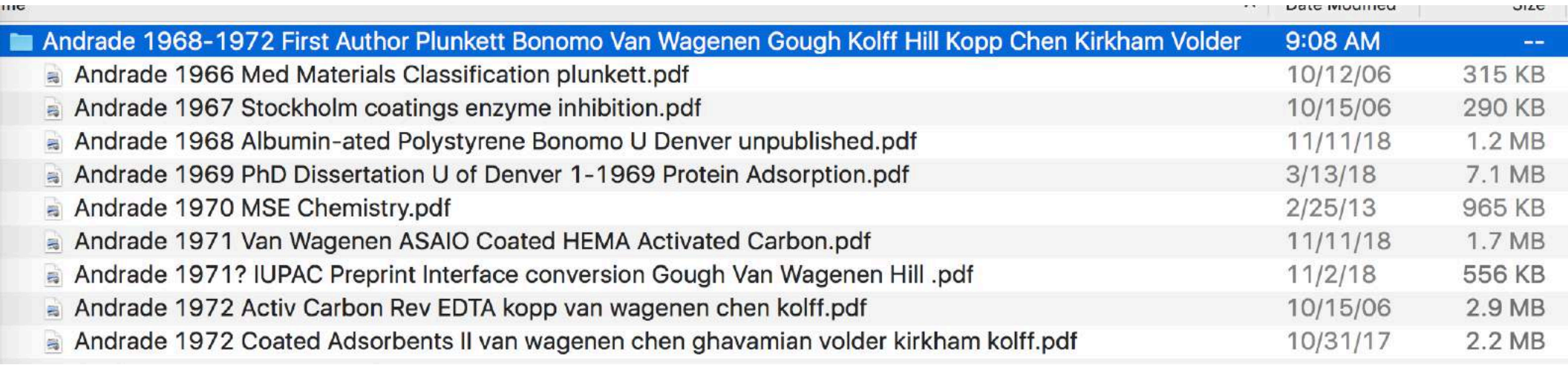
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16 November

A Medical Materials Literature Classification System

J.D. ANDRADE and J.D. PLUNKETT

University of Denver
Denver, Colorado

A materials research group at the University of Denver has been reviewing the medical, biological, and materials literature on surgical and experimental implant materials. In order to accomplish such a study systematically and efficiently, it was necessary to devise a literature classification system. The system will be briefly presented.

The publications dealing with implant materials are scattered throughout the medical and biological literature with little order or classification from a materials point of view. The interested investigator must generally weed these references out of the literature and is then faced with the problem of reading and trying to make some sense out of the jumble of papers he has obtained. Fortunately, Dr. Grau's bibliography (1) is available but it is limited to a listing of references by author and title, though there are plans to subdivide the bibliography by material types(1). One must still consult the original work and evaluate it. The silicone materials are excellently reviewed in Dow Corning's quarterly Bulletin (2). Unfortunately, no such publication exists for the other medical materials. Though there has been a recent flurry of interest in medical materials including several reviews (3) and conferences (4), the literature is still a librarian's nightmare.

We classify our references according to four major groups which are then subdivided into second and third-order groupings (5): I. Author-first author only; II. Materials-metals & alloys, ceramics, synthetic organics, silicones, natural products, and composites; III Applications-artificial organs, vascular substitutes, orthopedics, electronics, dental, "plastic" surgery, coatings and membranes; IV. Properties and Effects-coagulation, toxicity, foreign body reactions, corrosion and ionization, mechanical, thermal, electrical, chemical, and physical properties, standards and specifications, et al. Each reference is entered on a direct-retrieval card which is then coded and properly punched (5). The cards and accessories are commercially available (6)

This is a direct-retrieval system which is quite versatile. It can undergo extensive expansion and detailed sub-grouping. It is particularly useful to the individual investigator who wants to keep abreast of the literature without drowning in it.

We are currently using the method as a reference source for planned research and as an experimental literature classification for a computerized retrieval system which is under preparation.

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15–12 Coagulation-Resistant Coatings by Enzyme Inhibition. I. Initial Study

J. D. Andrade
Department of Metallurgy
University of Denver
Denver, Colorado, U.S.A.

Introduction: The blood-contact applications of prosthetic devices are severely hindered by the problem of coagulation on the foreign surface. This problem is combated by the use of low free energy surfaces,[1] such as the silicones, or by the use of heparin[2] or heparin-like[3] surfaces. The low energy surfaces tend to prolong whole-blood clotting time but do not prevent clotting. The heparin surfaces often suffer from heparin elution and require periodic heparinization, which causes additional problems.

Coagulation: Blood coagulation occurs on foreign surfaces by the activation of one of the blood coagulation enzymes, probably by an electrostatic attraction mechanism which results in denaturation and activation of the enzyme. In the "cascade" theory of coagulation, Factor XII (Hageman Factor) is activated and then reacts with Factor XI (PTA) to form a complex which in turn reacts in a cascade mechanism. The Seegers theory accounts for the contact activation phenomenon by the versatility of the prothrombin molecule.[4] Regardless of which theory one subscribes to, inhibitors of the coagulation enzymes involved in contact activation are known. Hageman Factor,[5] PTA,[6] prothrombin,[7] and thrombin[8] have all been inhibited by various enzyme inhibitors.

Rationale: If coagulation on foreign surfaces is initiated by a contact mechanism, and if certain chemicals are known whivh can inhibit the factors involved, then it is reasonable to attempt the development of an anti-thrombogenic surface by the bonding of enzyme inhibitors to a suitable substrate. Another approach is to include the inhibitor within the material, as has been done with heparin,[9] or by producing a special co-polymer of the inhibitor. This latter method is also under study in our laboratory.

Method: Many of the inhibitors of the coagulation enzymes are aromatic compounds, e.g., phenylhydrazine, DNFB, and phenylisocyanate. It is relatively well established that aromatic compounds are strongly adsorbed on a graphite surface.[10] Thus a colloidal graphite surface, such as that used by Gott,[11] would be expected to adsorb aromatic compounds. Assuming that the aromatic portion is adsorbed flat on the surface,[10] conformations of many of these compounds exist which allow the active groups to "stick-out" from the surface. Such arrangements would tend to increase the inhibitory effectiveness of the inhibitor, but its decreased mobility might hinder its effectiveness. The degree of inhibition would be a balance of these factors, along with the normal inhibition kinetics of the system.

Polystyrene and polycarbonate test tubes have been prepared with colloidal graphite coatings. The solutions used are similair to those described by Gott,[11] but the technique is much different because of the uneven air flow within a test tube. This problem is overcome by using a stream of compressed air or nitrogen introduced at the bottom of the test tube. The result is a uniform air flow, even drying, and a uniform coating. The coated and baked test tubes are then dipped in the inhibitor solution and dried. They are then used for the determination of in vitro whole blood clotting time.[12]

Results: Though preliminary results are very promising, many more tests must be performed before conclusions can be reached. The detailed results will be presented in a later paper.

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ALBUMINATED POLYSTYRENE: A THROMBO-RESISTANT SURFACE

J. D. Andrade* and F. S. Bonomo
Metallurgy and Chemistry Divisions
Denver Research Institute
University of Denver
Denver, Colorado 80210

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unpublished

* Present address: Division of Materials Science and Engineering and Division of Artificial Organs, University of Utah, Salt Lake City, Utah 84112.

SUMMARY

Albuminated polystyrene, prepared by an aqueous Friedel-Crafts reaction between bovine albumin and chloromethylated polystyrene, has been shown to be nonthrombogenic. This proteinated surface appears to be as thrombo-resistant as a heparinized surface. Preparation of the surface is described in detail; its properties are discussed.

INTRODUCTION

Most polymers rapidly adsorb a layer of proteins from blood and aqueous solutions (1-4). The structure of this coating ranges from a compact, uniform monolayer when a single species is adsorbed (4) to a nonuniform complex, structure when adsorption is competitive (1,5). In 1959 Copley (6) showed that blood coagulation times in fibrin-coated test tubes were much longer than in uncoated glass tubes. Certain heparinized surfaces are known to adsorb proteins (7,8), and it is known that heparin interacts strongly with plasma albumin (9,10). Lyman has shown (11) that surfaces precoated with a layer of intact protein do not adsorb platelets. These results lead one to consider surface proteination as a means of rendering materials thrombo-resistant, particularly the bonding of an individual protein to a synthetic polymer surface. Studies were begun to bind albumin to polystyrene.

Albumin may present a relatively passive surface to blood. It appears to function largely in the maintenance of osmotic balance, while the other proteins in blood, e.g., the gamma globulins and fibrinogen, have other specific tasks as well (12). Thus albumin may be especially unreactive and innocuous.

EXPERIMENTAL

Difficulties in using the classical diazo coupling reaction (see 13) to bind albumin to polystyrene led us to explore Friedel-Crafts reactions (14) in aqueous solutions. A modification of the technique described by Jenny (15), using zinc chloride as the catalyst in a saturated aqueous solution of HCl, was utilized. Polystyrene film (10 mils,

Westlake Plastics, Inc.) was cut into strips 18 x 50 mm for multiple attenuated total reflectance (ATR) infrared analysis; polystyrene tubing (1/4 inch ID, Westlake Plastics, Inc.) was machined into Gott vena cava rings (16) for in vivo blood compatibility studies. The polystyrene samples were washed in 95% ethanol, then chloromethylated (17) by immersing them in a solution of 5.0 g $AlCl_3$ and 3.6 ml chloromethylmethyl ether in 100 ml nitrobenzene at room temperature for 15 seconds. The salmon-pink samples were immediately plunged into 100% ethanol until a uniform white surface appeared, then thoroughly soaked and rinsed in 95% ethanol until no further trace of nitrobenzene was evident. ATR spectra of the tabs indicated extensive para substitution of the aromatic groups. The albumination solution was prepared by dissolving bovine albumin (Fraction V, Mann Research Labs) in a 0.7% solution of zinc chloride in distilled water, adjusted to pH 4 with HCl, to give a 1% solution of albumin; the control solution contained an equivalent amount of NaCl in place of the $ZnCl_2$. The chloromethyl polystyrene film and rings were immersed in the albumination solution at 40°C for at least 8 hours, then thoroughly washed for about 3 days in isotonic saline until there was no gross evidence (frothing) of protein remaining. While ATR spectra of the control surfaces shown little evidence of protein on the surface (there must be at least an adsorbed monolayer, however (2,4)), the spectra of the treated samples contained peptide bond absorption bands, indicating a significant amount of protein on the surface; a decrease in intensity of the C-Cl bands was also apparent, indicating Cl- displacement and probably protein binding (18).

RESULTS AND DISCUSSION

Control and treated vena cava rings (16, 19) were implanted in dogs by Dr. Vincent Gott. The acute (2-hour) control rings were thrombosed; the treated samples were essentially clot-free after both 2-hour and 2-week tests. The nonthrombogenic behavior of this albuminated polystyrene thus appears to be about as effective as that of heparinized surfaces for the conditions described here.

While the actual nature of the surface described here is not known, there appears to be a large amount of bound albumin. Whether the albumin is in its native form is not yet known; it is probably complexed with zinc ions (9). The fact that albumin undergoes a reversible conformation change at about pH 4 (9) must also be considered. The biological life of such a surface is also not yet known.

Lyman has shown (11) that surfaces precoated with a layer of undenatured protein do not adsorb platelets, while those containing a denatured layer do adsorb platelets. If our albuminated polystyrene surface consists of intact albumin, then Lyman's results are in agreement and offer an explanation for the thrombo-resistant behavior of our material.

Another possible explanation may lie in the nature of a proteinated surface. One must expect that such a surface is highly hydrated in solution and probably exhibits a gel-like surface to blood. Since the vascular walls are hydrophilic in nature (5,21), it is reasonable to suspect that the more ideal materials to be used in contact with blood will be hydrophilic (21). The blood compatibility of the nonionic hydrogels that have been studied is quite good (22-24) and would tend to support this hypothesis.

A mechanism for the interaction between water-containing surfaces and blood is not yet available. Andrade has examined the role of the

water-loaded surface in adsorption from aqueous solution (18). His model considered the intermolecular forces responsible for adsorption in certain limited systems and the role of the water content of the surface. The conclusion was that a nonpolar polymeric surface containing a high water content would not tend to adsorb apolar material from aqueous solutions. A molecule in the vicinity of such an interface would "feel" as if it were in bulk water and not under the influence of an interface. A molecule in the vicinity of an air/water or air/polymer interface experiences a force asymmetry which results in adsorption. Actual adsorption data to prove or disprove this hypothesis are not yet available; work is in progress. If the model and interpretation are correct, they may offer an explantion for the behavior of hydrophilic materials.

We thank Dr. Leo Vroman for suggesting the use of albumin as a potentially passive surface. His discussions and comments have been most helpful. We also thank Dr. Vincent Gott, Johns Hopkins University School of Medicine, Baltimore, Maryland, for performing the vena cava ring implantations and evaluations.

This work was supported by the Artificial Heart Program, National Heart Institute, NIH Contract No. PH-43-67-1407. One of us (J.D.A.) acknowledges the support of an N.S.F. training grant during a portion of the time this work was carried out.

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COAGULATION-RESISTANT SURFACES AND A MECHANISTIC MODEL OF ADSORPTION ON POLYMER SURFACES

A Dissertation

Presented to

The Faculty of the College of Engineering

University of Denver

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

by

Joseph D. Andrade, Jr.

January 31, 1969

THE UNIVERSITY OF DENVER

COLLEGE OF ENGINEERING

GRADUATE DIVISION

Upon the recommendation of the professor in charge of the thesis and of the chairman of the DEPARTMENT OF METALLURGY this thesis is hereby accepted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

_____

Professor in charge of thesis

_____

Associate Dean for the Graduate Division College of Engineering

_____

Date

Acknowledgements

Interdisciplinary studies are always dependent on a great deal of aid, interest, and advice from many people. I am especially indebted to Dr. Bruce Paton and Mike Heron of the Halstead Laboratory at the University of Colorado Medical Center for their interest and collaboration in the initiation of much of this work. The papers of two men are primarily responsible for my interest in the blood materials interface area: Doctors Leo Vroman and Donald Lyman. During the course of the work I had the good fortune to meet and get to know both of them; they have provided suggestions and encouragement. I wish to thank Dr. Leo Vroman for originally suggesting that albumin might be a particularly good protein to bind to solid substrates. The encouragement, advice, and support of a stimulating thesis advisor, Dr. Paul Predecki, was particularly important. I am also appreciative that the faculty and staff of the Dept. of Metallurgy and the Denver Research Institute allowed me to tackle a problem in the biomaterials area. Fran Bonomo was especially helpful and encouraging; he ran all of the MATR spectra, and we discussed many of the chemical problems which came up.

The assistance of a National Science Foundation Training Grant and the partial support of a National Heart Institute Contract (PH 43-67-1407) is gratefully acknowledged.

And to Barb and Tonio, for they made it all meaningful, happy, exciting, and worthwhile.

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CHAPTER I

INTRODUCTION

The use of artificial materials in surgery has grown considerably in the past decade. Many successful surgical techniques are now dependent on devices constructed from "foreign" materials. Every one of these applications faces a fundamental problem: the material/biological interface. There is no way to isolate. There is always an interface, and little is known about the processes and reactions which may be occurring at such interfaces. Perhaps the most critical and also the most fundamental problem in the use of materials in biomedical applications is that we do not understand the interfacial processes which occur, and, perhaps more importantly, we are most likely largely unaware of the existence of many processes which are occurring.

It is well established that blood coagulation can be initiated by contact with a "foreign surface." The exact reason or purpose for such a mechanism is not known, though Vroman[1,2] believes it may be an evolutionary remnant, a more specialized form of the general coagulation of cytoplasm. There is growing evidence that adsorption at the foreign surface, particularly protein adsorption, plays a fundamental role in the initiation of blood coagulation. Protein adsorption processes may also be important in other tissues. Synovial fluid, where protein adsorption on artificial joints may play a role in friction and wear processes, is but one example. There is now a growing acceptance

of the importance of fundamental surface studies in elucidating the mechanism of "surface-induced" blood coagulation, particularly work on protein, lipid, and platelet adsorption.

There is relatively little work available on protein adsorption at the solid-liquid interface. The work that is available deals primarily with adsorption on high-energy surfaces. The statistical theories that have been developed for the adsorption of linear flexible polymers cannot be expected to hold for a rigidly structured protein molecule. Most adsorption theories are thermodymanic in nature.[3] Levine's[4] recent thermodynamic theory is an attempt to treat protein adsorption on a fundamental level.

Proteins adsorb. It can almost be said that all proteins adsorb on everything, though that may not be quite true. Concepts such as surface charge or surface energy are not generally applicable. Perhaps the most perturbing fact about protein-polymer interaction is that there <u>is</u> an interaction. One can understand the adsorption of proteins on glass or metal or other high energy surfaces as a decrease in the overall surface energy. This is manifested in the decreased wettability of the proteinated surface. However, when proteins adsorb on low energy polymer surfaces, the surface energy is increased. This can be demonstrated by a change in the surfaces' wetting properties.[5] Thermodynamically, this makes little sense, as nature does not tend to produce situations of higher energy, particularly when the entropy must decrease as well.

Protein adsorption is not well understood. This is due not only to the complex nature of proteins, but also to the lack of a general mechanistic model of adsorption. Before one can hope to understand protein adsorption, one must understand the adsorption of simple compounds on a molecular level.

The original objective of this work was to develop and characterize a blood coagulation-resistant surface for use in medical implant applications. This proved to be an enormous and unfinished, though not necessarily unsuccessful, task.

The author's attempts to develop coagulation-resistant surfaces, particularly that of proteinated polystyrene, are briefly discussed in the next chapter; following these is a discussion of the use of fluorescence microscopy to detect protein adsorption on a microscopic level. For those readers unfamiliar with these general areas, it would be helpful to read the sections on proteins and adsorption in Chapter III before reading Chapter II.

The objective of the major part of this work, however, is to develop a molecular model of adsorption on hydrophobic surfaces, with applications to proteins, in the hope of gaining a rudimentary understanding of the mechanism of adsorption. Intermolecular and interfacial forces must be taken into consideration, but before such forces can be applied, the nature and structure of the medium through which they act, water, must

be considered. One must also consider the nature of the solute molecule and the properties of the polymer surface. These considerations will permit the interpretation of solid-solute, solid-solvent, and solute-solvent interactions, in addition to solvent-solvent interactions. With this background a model of adsorption on polymer surfaces will be developed. The model will first treat very simple species in the vicinity of a surface, then it will treat albumin, gamma globulin and fibrinogen. The results of the model and the computer calculations derived from it will then be compared to data on the adsorption of simple compounds and proteins. The development will be both qualitative and quantitative for the adsorption of simple molecules. The adsorption of proteins will be discussed more qualitatively.

The model will attempt to show that adsorption on the molecular level is a natural consequence of the asymmetric force field in the vicinity of an interface. It will also show that a large part of the asymmetry of that force field is due to solute-solvent effects. It will further attempt to show that one need not postulate active sites or binding sites to explain adsorption, though they may aid it. It will then show that one may minimize adsorption of a particular species by proper choice of certain surface parameters.

The development of the model will require knowledge of the structure of water, the nature of the polymer surface, the nature of adsorption, and the nature of intermolecular forces. These subjects will

be discussed before the model is presented.

All calculations and values are given in c-g-s units, where charge is expressed in electrostatic units (esu). All energies are in ergs, and all forces in dynes. All distances are given in angstroms, though centimeter units must be used in all of the calculations.

CHAPTER II

PRELIMINARY EXPERIMENTAL WORK

A. Coagulation-Resistant Surfaces

1. Polyorganofluorophosphates:

The intrinsic blood coagulation mechanism is believed to be dependent on the modification of a plasma protein by surface denaturation and its subsequent reactions. In the classical Ratnoff and MacFarlane cascade theory[6,7] Factor XII (Hageman Factor) is surface-activated to XII*, an enzyme, which can then catalyze the reaction XI → XI*, and the cascade mechanism is initiated.

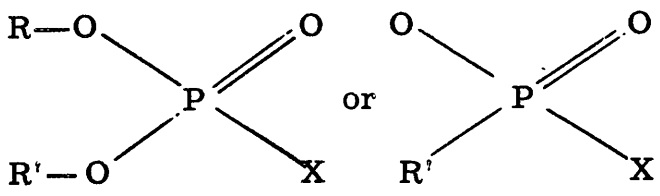
Though Factor XII has been isolated and purified, it is still a relatively uncharacterized protein, and controversy as to its role, and possibly even its existence, continues. It is generally accepted, however, that the intrinsic clotting mechanism is dependent on a modification of some plasma protein at a solid surface, be it Factor XII, Factor XI, or prothrombin.[8] Some very recent work casts doubt on the protein modification theory.[9]

If clotting is indeed due to the surface modification of some protein, the logical question is how to prevent that modification from occurring, and thus prevent the necessary activation. If one assumes that the denaturation process exposes certain reactive groups which are then capable of reacting with the next protein in the clotting sequence or

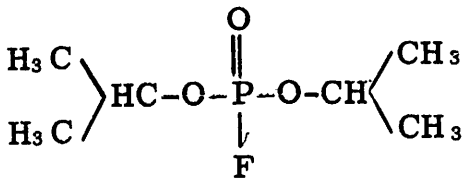
perhaps with other parts of the same molecule, the blocking of those reactive groups should prevent coagulation from occurring. This may be possible with enzyme inhibitors. The problem is that the active groups necessary for clotting have not been isolated, if indeed active groups are responsible at all. Most inhibitors tend to be effective in decreasing clotting activity in solution; the action of an inhibitor bound to a solid substrate is not known.

There are some clues as to the active site of prothrombin, but such information on Hageman Factor is virtually non-existent. Hageman Factor may be inhibited by diisopropylfluorophosphate[10] (DFP), an esterase inhibitor, but such a treatment may inhibit its esterase activity without inhibiting its clotting activity.[11] The work of Ray and Roy[12] and of Caldwell and Seegers[13] showed that disulphide and free amino groups are essential for prothrombin activity. Inhibition of these groups led to a loss in activity. The amino may be inhibited by dinitrofluorobenzene and phenylisocyanate; the sulfide can be inhibited by reducing agents.

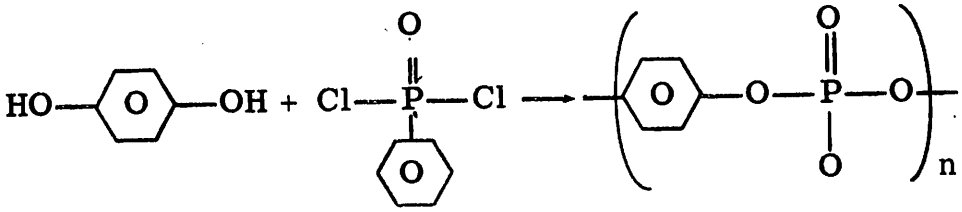
DFP is an extremely toxic and reactive member of a class of organophosphate compounds which irreversibly inhibit esterase enzymes. The general formula for these compounds is



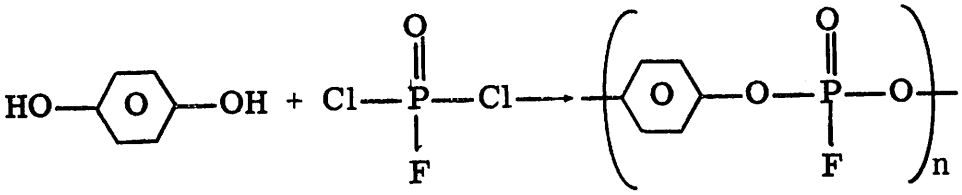
where X can be -F, -CN, or -O-  -NO_2. The formula for DFP is



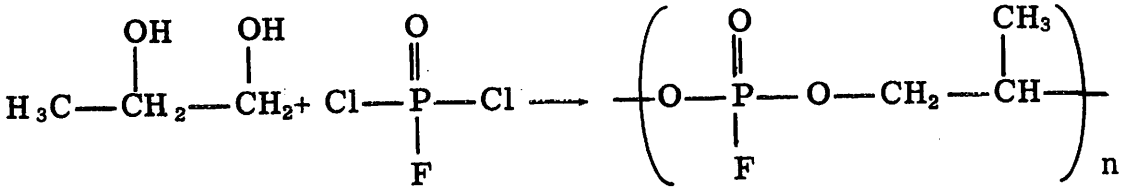
Sorenson's book on polymer chemistry[14] contains the reaction:



If this reaction is performed with phosphorousoxydichlorofluoride instead of with phenylphosphonyldichloride, one gets:



Such a polymer contains the reactive heart of the organofluorophosphate inhibitors. If now the polymerization is produced using propylene glycol instead of hydroquinone:



Such a polymer has a frightening resemblance to DFP. Molecular models of this polymer indicate that conformations exist wherein the P-F bond is directed up and out from the surface. Such a surface should have a very high reactivity for enzymes. By proper choice of the glycol, one can vary the R-groups on the organofluorophosphate, which should vary

its enzyme reactivity.

The synthesis and characterization of these polyorganofluorophosphates was performed by Mr. Herbert Yen and are described in his thesis.[15]

Shortly after beginning this work it became evident that a DFP-like polymer would probably bind other proteins long before it ever contacted Hageman Factor. Thus a polyorganofluorophosphate surface would tend to become rapidly and irreversibly proteinated as soon as it contacted blood. A proteinated surface could surely be produced by more direct means than synthesizing special, new, and possibly toxic polymers.

2. Albuminated Polystyrene:

Ten years ago Copley[16] showed that blood coagulation times in fibrin-coated test tubes were relatively long. Recent work on the blood compatability properties of collagen[17] "...suggest that the collagen surface is remarkably free of thrombogenic properties." It has also been clearly demonstrated that the first thing that happens to most materials when contacted with blood is the rapid formation of a film of adsorbed protein[18-20] (see also Chapter III). Such a film is not necessarily stable, however, as competitive adsorption is known to occur with proteins;[1,18] just as with synthetic polymers[21] (see also Chapter III); indeed, this is the basis for the adsorption chromatography of proteins. It has been well documented that heparinized surfaces adsorb proteins.[22,23] Adsorption on a heparinized surface is quite strong,[23] thus competitive adsorption may not occur. It seems, therefore that thrombo-

resistant surfaces may contain a layer of bound or complexed and relatively immobilized protein.

The above discussion leads naturally to the concept of surface proteination as a potential method of rendering surfaces non-thrombogenic.

Protein bonding to synthetic polymers has been extensively utilized in biochemistry; the methods and techniques were thoroughly reviewed recently.[24] Polystyrene was selected as a substrate for this experimental work because polystyrene and its derivatives have been widely used as insoluble supports for the binding of enzymes and other proteins.[24] Another reason for using polystyrene is that it is inexpensive and readily available in sheet and tube form.

The diazo coupling reaction can be used to couple proteins to supports containing amino groups. It is fairly well established[25] that the coupling of diazonium salts of $H_2N-Ph-X$ with proteins introduces $-N{=}{=}N-Ph-X$ groups to the imidazole and phenol residues, though epsilon-amino, guanidino, and imino groups may also be involved.[26] Polyaminostyrene can be prepared by nitration and then reducing to the amine; Falb's method[27] was used for this work. The polyaminostyrene surface was then diazotized and coupled to protein following the technique of Gyenes and Sehon.[28] The surface modifications and protein binding were detected by multiple attenuated total reflection (MATR) infra red spectroscopy; the MATR spectra were made by Mr. Fran Bonomo,

Chemistry Division, Denver Research Institute. The nitration, amination, and proteination steps were evident in some of the spectra. Generally, however, the reactions were not reproducible and were thus somewhat unreliable. A simpler and more reproducible reaction was sought.

Friedel-Crafts reactions in aqueous solutions are discussed briefly in Olah's treatise.[29] Jenny[30] prepared diphenylmethane by reacting benzene with benzyl chloride. The catalyst was zinc chloride in a saturated aqueous solution of hydrochloric acid. The reaction was carried out at the interface between the aqueous and organic solutions. The reaction conditions used (3 hours at 50 C) prompted the use of a similar technique for the proteination of polystyrene, where the reaction would take place at the interface between the polystyrene and the protein solution.

Polystyrene sheet (10 mil) and tubing ($\frac{1}{4}$ inch ID) were obtained locally.[31] The sheet was cut into 18 × 50 mm tabs for ease in MATR analysis; the tubing was machined into Gott vena cava rings[32,33] for the in vivo blood compatibility studies.

The polystyrene samples were washed in ethanol and then chloromethylated according to Falb's method.[38] The dry samples were immersed for 15 seconds at room temperature in a solution containing 5 grams of $AlCl_3$ and 3.6 ml of chloromethylmethyl ether (Aldrich Chemical Co.) in 100 ml of nitrobenzene. The salmon-pink samples were then plunged immediately into 100 per cent ethanol until a uniform white surface appeared; they were then thoroughly soaked and rinsed in 95 per cent

ethanol until no further trace of nitrobenzene was evident. MATR spectra of the tabs indicated extensive para substitution of the aromatic groups (Fig. 1). The differences between the MATR spectrum of polystyrene and chloromethylated polystyrene are evident from Figure 1.

The use of albumin for the chemical proteination of surfaces was first suggested by Dr. Leo Vroman.[35] Albumin is probably the best choice as it is a relatively innocuous protein whose primary biochemical function appears to be the maintenance of osmotic pressure. The other proteins in blood, e.g., fibrinogen and the gamma globulins, all have rather specialized functions in addition to their role in the osmotic balance.[36] Thus an albuminated surface may be an especially passive or inert surface for application in contact with blood.

About 0.7 gram of $ZnCl_2$ was added to 100 ml distilled water; the solution was slowly adjusted to pH 4 with 0.1 N HCl. One gram of albumin[37] was then added with stirring. The control solution was identical except that 0.9 grams of NaCl were substituted for the $ZnCl_2$. The chloromethylated samples were immersed in the albumination and control solutions at 40 C for at least 8 hours. The samples were then thoroughly washed and rinsed in 0.9 per cent saline until there was no evidence of protein remaining; this required many changes of the saline over a period of 2-3 days. MATR spectra of the control and albuminated surfaces are given in Figures 2a and 2b. Note that the control surface shows little evidence of protein (peptide bands) but that the peptide band is strong in

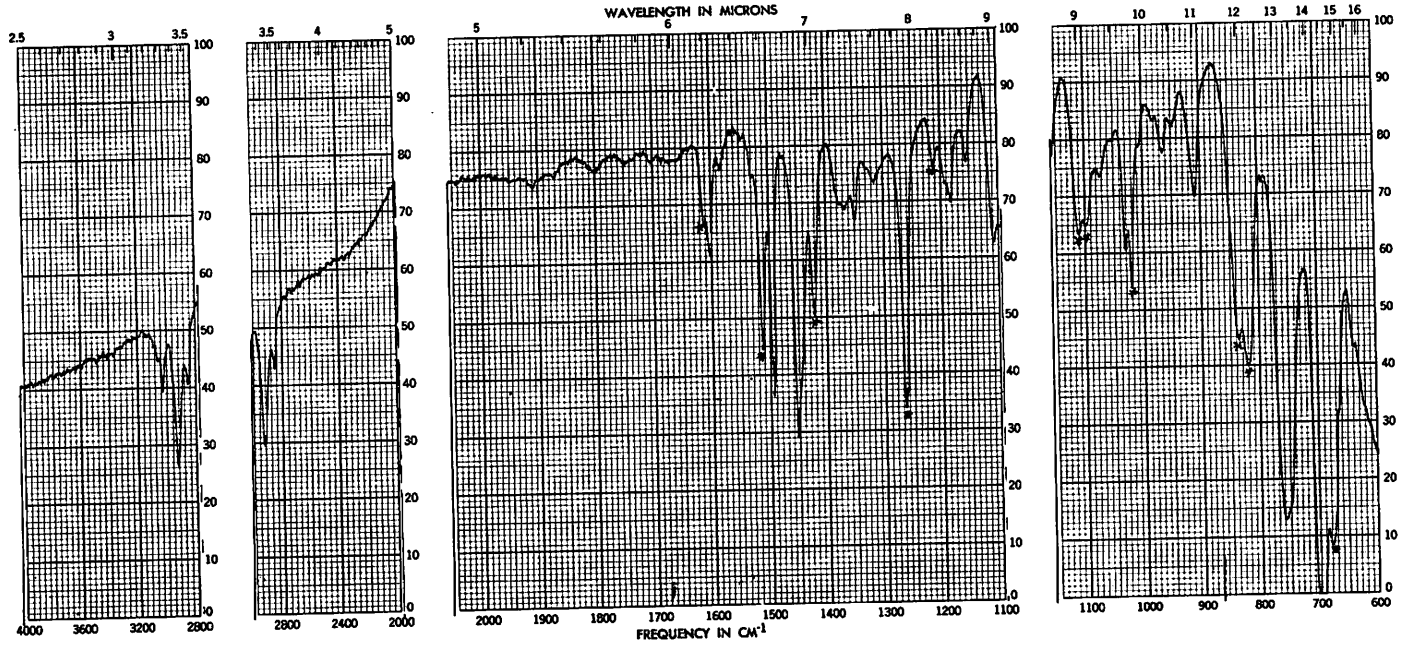


Figure 1. MATR Infra-Red Spectrum of Chloromethylated Polystyrene. Characteristic Chloromethyl Bands are Present. The Decrease in Intensity of Some of the Polystyrene Bands Indicates Para Substitution of the Aromatic Ring.

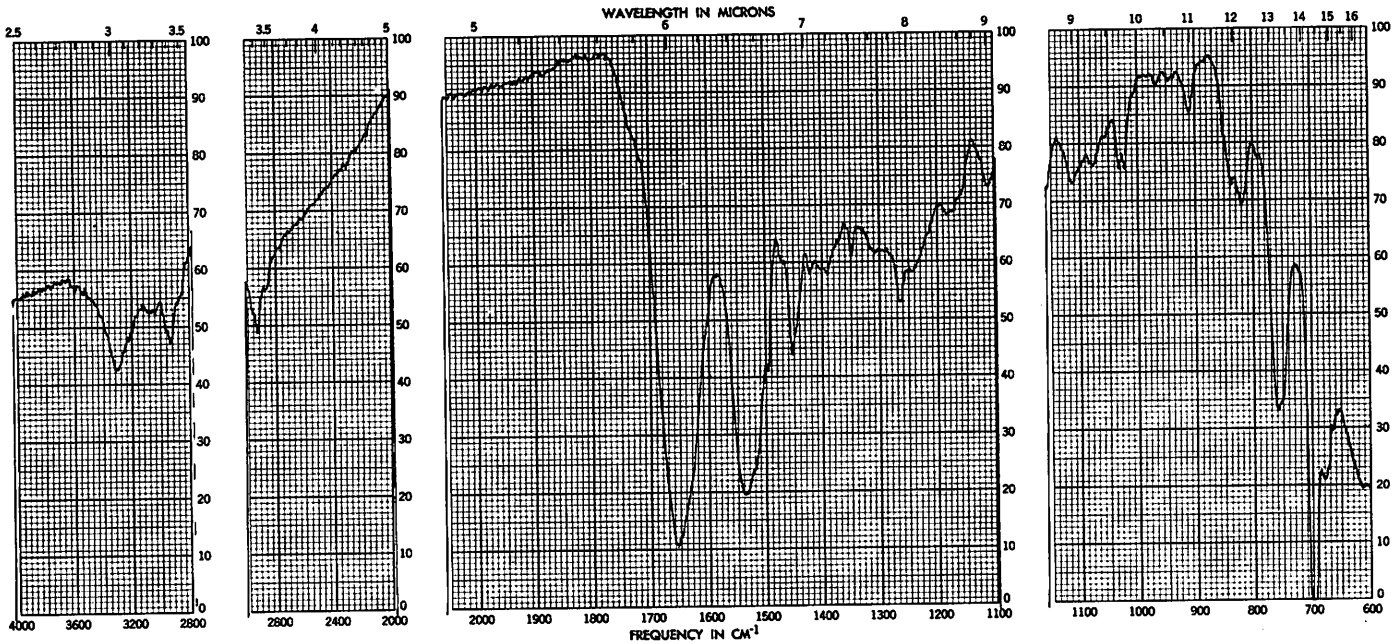


Figure 2a. MATR Infra-Red Spectrum of Albuninated Polystyrene. The Spectrum is Essentially That of Chloromethylated Polystyrene With Some Protein Bands Superimposed on it. The Peptide Absorption Band at About 1650 cm^{-1} is Particularly Evident.

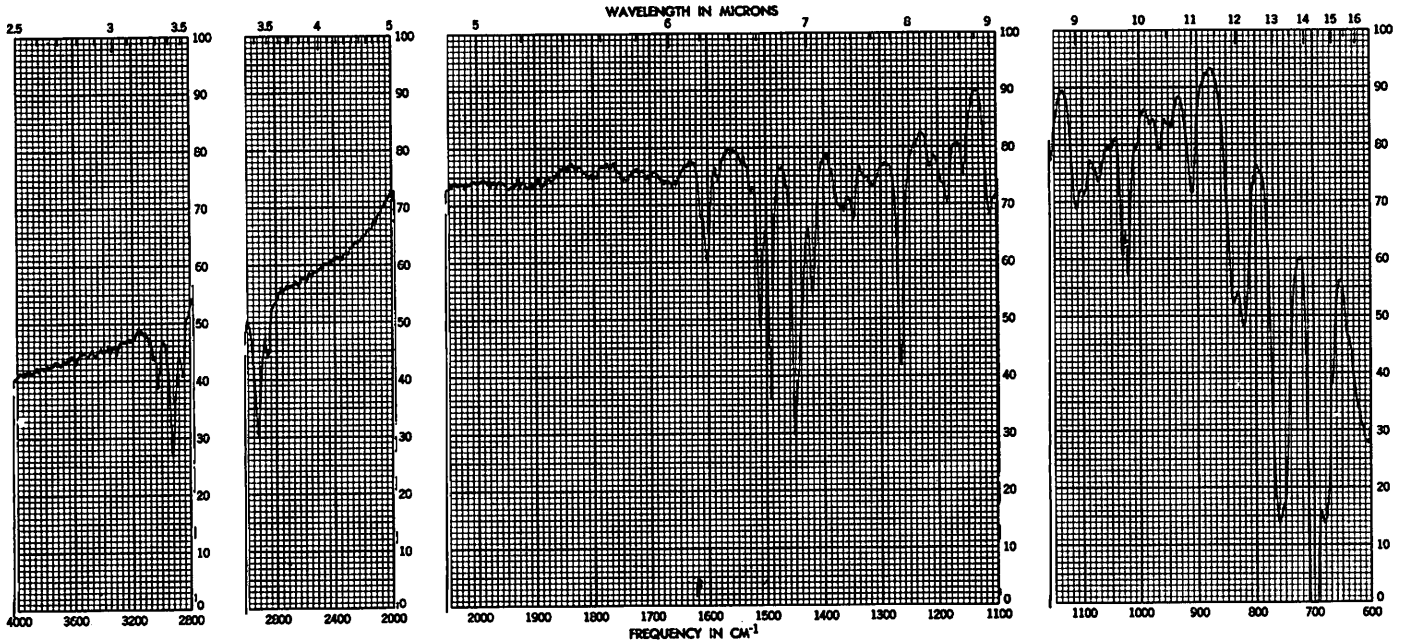


Figure 2b. MATR Infra-Red Spectrum of a Control Sample of Chloromethylated Polystyrene. This Sample was Treated in Exactly the Same Way as That in Figure 2a, Except that the $ZnCl_2$ Catalyst was Replaced by NaCl. There is no Evidence of Protein. The Spectrum is Essentially That of Chloromethylated Polystyrene (Fig. 1).

the treated samples. The treated samples also show a decrease in intensity of the C-Cl bands, indicating Cl^- displacement and protein bonding.

The reactions described above were performed on vena cava rings under sterile conditions and shipped to Dr. Vincent Gott in sterile saline. Control and treated rings were implanted in dogs[33] for acute (2 hour) and chronic (2 week) tests. The acute tests showed that the control rings were thrombosed; the treated rings were essentially free of thrombus. Of the four rings submitted for chronic testing, two were completely free of thrombus, and two "...had relatively minimal thrombus."[38] The non-thrombogenic behavior of this albuminated polystyrene thus appears to be about as effective as a heparinized surface.

In vitro tests were not performed. The problems and artifacts produced in conventional in vitro clotting tests, probably by the transfer of denatured protein monolayers to the test surfaces, make such tests unreliable, unless the air/blood interface is completely eliminated.[19]

The actual nature of the albuminated polystyrene surface described here is not known. It is a rough surface, as the nitrobenzene attacks the polystyrene surface during the chloromethylation reaction. The surface obviously contains a great deal of protein, as evidenced by the MATR spectra. Whether that protein is in its native form or not is not known; it may be complexed with zinc ions. The surface may even consist of protein fragments, though this is doubtful, as the reaction conditions are

not particularly severe. The biological half-life of such a surface is not known.

A mechanism for the non-thrombogenic behavior of the surface is not known. Perhaps it is due to the innocuous nature of albumin, discussed earlier. Lyman has shown[9] that surfaces precoated with a layer of undenatured protein do not adsorb platelets, while those containing a denatured layer do adsorb platelets. If the albuminated surface consists of undenatured albumin, then Lyman's results may explain the non-thrombogenic behavior of albuminated polystyrene.

Another possible explanation lies in the water-containing or gel-like nature of a proteinated surface. This mechanism is discussed in Chapter III, as it is dependent on the adsorption model developed in that chapter.

B. Protein Adsorption by Fluorescence Microscopy

The coagulation of blood has been discussed as a surface energy-dependent process.[39] If this is so, then microscopic changes in surface energy might have significant effects on the overall behavior of the materials. Both Lyman [39] and Merrill [23] have mentioned this possibility. Studies of polymer morphology have clearly shown striking differences in surface energies (see the discussion of the polymer surface in Chapter III). Therefore, it was of interest to look at protein adsorption on a microscopic level to see if there was any correlation between adsorp-

tion and surface morphology. Only two techniques appeared to be suitable to detect adsorption at the microscopic level with the sensitivity necessary to detect a monolayer: fluorescence microscopy [40, 41] and microautoradiography. [42] The former was chosen, largely because of convenience. It will be shown that the fluorescence microscopy method left much to be desired.

The study of protein adsorption on a microscopic level places severe limitations on the techniques which can be used. The microscopic requirements requires that the technique be compatible with microscopic observation. The low concentrations involved* require that extremely sensitive techniques be used. The concentrations* are at the limit of detectability of both fluorescence microscopy and microautoradiography. The microautoradiography method would require long exposure times, even with proteins of high specific activity. Also, radiolabeled proteins were not commercially available. Thus the fluorescence microscopy technique was selected. It was also felt that if the method proved successful, it might be used with specific fluorescent antibodies; thus studies of competitive adsorption of proteins could be undertaken.

* The projected areas of albumin for side-on and end-on orientations are about 4,600 A^2 and 1,700 A^2, respectively.[19] If one considers a monolayer of adsorbed albumin, a one micron square area of surface would contain about 6,000 molecules in the end-on orientation, which is 10^{-19} moles or 7×10^{-15} grams; in the side-on orientations, only 23,000 molecules could be accomodated, giving 4×10^{-20} moles or 3×10^{-14} grams.

The properties of a protein are relatively unchanged after conjugation with the highly fluorescent molecule, fluorescein isothiocyanate (FITC). Changes in solubility, net charge, stability, and molecular size and shape are slight, though there can be a 1 to 2 percent increase in molecular weight. FITC is not a small molecule, and there may be up to 10 FITC molecules conjugated to each molecule of protein,[40, 41] thus one might expect significant changes in surface properties. The results with fluorescent antibodies do not tend to bear out this suspicion, however. Antibodies conjugated with FITC and other fluorochromes retain their antigenic specificity. The interaction between a protein and its antibody is very subtle and sensitive. If fluorescent conjugation does not significantly affect such interactions, it can probably be assumed that solid surface interactions will also not be significantly affected, though the possibility must be kept in mind.

FITC-conjugated bovine albumin, gamma globulin, and fibrinogen were obtained from Mann Research Labs.[37] The material was conjugated by the Rinderknecht technique [43] in phosphate-buffered saline.[40] The buffer system may have been a poor choice as it is known to have a desorption effect on denatured albumin.[44] It is also quite probable that the rapid Rinderknecht method may yield a lower degree of conjugation than slower methods.

A drop of protein solution was placed on a freshly prepared polymer film in an inverted Petri dish. After adsorption times of $\frac{1}{2}$ to 2 hours the

drop was washed off with distilled water, quickly dried, and stored in a clean container until examined.

The filter system chosen was that recommended by Goldman.[45] The exciter filter was a UV-blue transmitting Schott BG-25 filter[46] coupled with a heat absorbing filter. The blue excitation light then excited fluorescence in the specimen. The barrier filter was a single piece of Kodak Wratten No. 12 gelatin, followed by a clear Wratten 2B to absorb the UV component which is transmitted by the No. 12 filter. The arrangement is given in Figure 3.

The optimum excitation system was found to be transmitted light dark-field illumination, which prevents most of the excitation light from entering the objective; the barrier filters need not be as absorbent, therefore, as for bright field illumination. The use of a reflected light excitation system was inferior to the above, largely because of absorption in the half-silvered mirror. At first a 150 watt xenon arc was used unsuccessfully; a 200 watt high pressure mercury arc proved somewhat better.

After proper alignment and adjustments, the sample was placed on the inverted microscope stage and an interesting morphological field was selected. The initial observation was performed with a Wratten No. 15 filter in the light path to prevent excitation of the sample. The No. 15 filter was then replaced with 3 mm of BG-25 and the fluorescence observed in a dark room under a photographer's cloth.

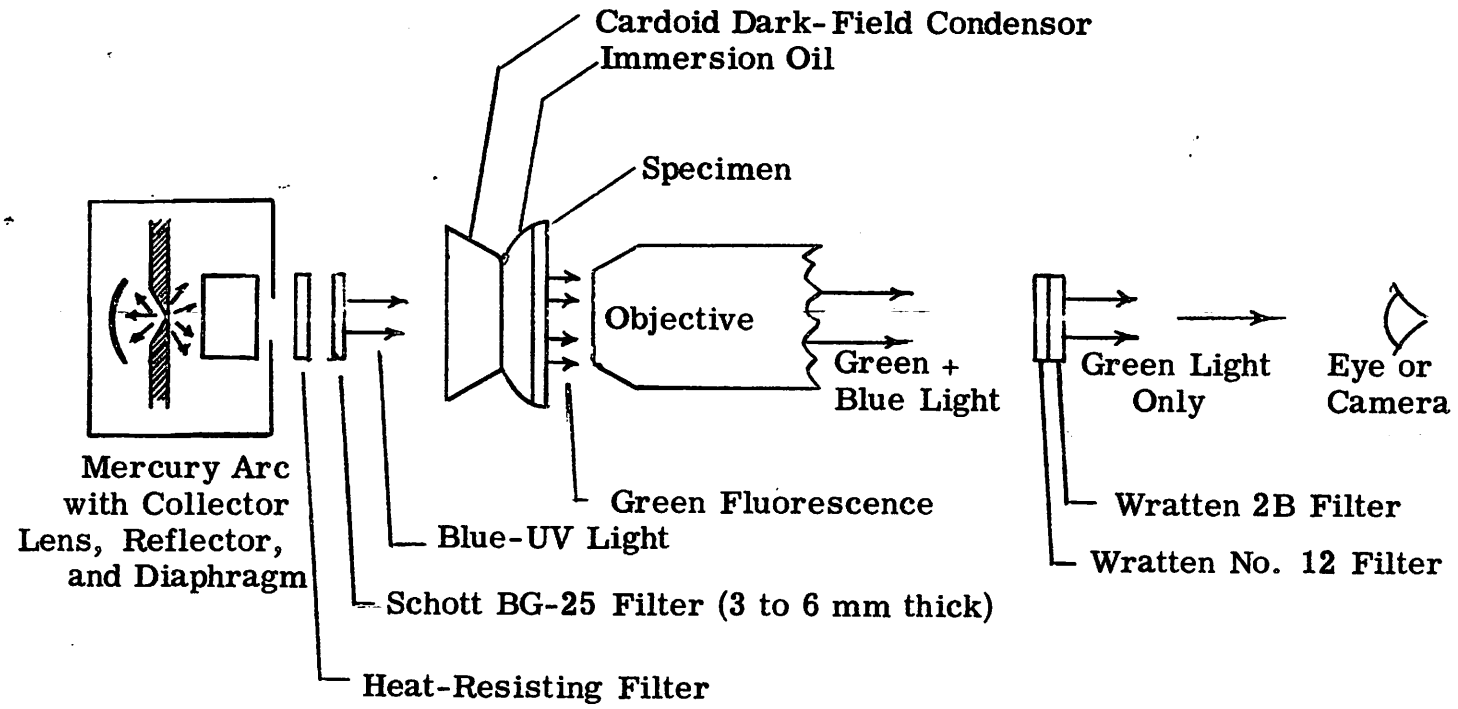


Figure 3. The Optical Arrangement for Excitation and Observation of Fluorescently Labeled Proteins.

Extensive evaluation tests were conducted with fast negative films and developer combinations—none was as satisfactory as Polaroid's "Polascope" film (ASA rated at about 10,000), not even Kodak 2475 and 2485 high-speed recording films, even with extensively forced development. The fast Kodak films are panchromatics and apparently could not respond as well to the green fluorescence (about 5250 A) as the Polaroid film could.

Unfortunately, even the Polaroid film could not successfully record the very faint fluorescent images which resulted. The images were barely observable by eye but not with sufficient clarity or resolution to relate the results to the morphology of the sample. An image intensification system might have made it possible to record the results, but the resources for such an expensive system were not available. A more important problem was that in many cases the polymer itself was fluorescent, probably due to commercial additives; in a few cases (a commercial polypropylene) the polymer fluorescence was so intense that it was literally blinding. Extraction of the polymer (in hexane, for example) succeeded in removing much of the fluorescence, but never all of it. In nearly all cases the fluorescence remaining was greater than the contribution from adsorbed protein. In addition to contamination with additives which may be fluorescent, polymers are known to adsorb organic compounds from the atmosphere; the behavior can result in highly fluorescent species on the surface.[47] In practically all cases these fluorescent

artifacts completely overwhelmed the contribution due to adsorbed protein.

It was thus clear that the fluorescence microscopy technique was an unsatisfactory choice, and the effort was abandoned.

CHAPTER III

A MECHANISTIC MODEL OF ADSORPTION ONTO APOLAR POLYMER SURFACES

A. Background

1. The Polymer Surface:

The clean polymer surface is generally considered to be a relatively homogeneous structure with quite reproducible surface properties. Detailed surface studies of the wetting properties of high polymers have justified this assumption;[48] but there is now a growing body of evidence to indicate that polymer surfaces may not be as homogeneous as previously suspected.[49]

The crystallinity of high polymers is now firmly established, and several monographs on the subject are available.[50, 51] The crystallinity of polymers can vary from 0 percent, as for atactic polystyrene, to in excess of 90 per cent, as for polytetrafluoroethylene. The degree of surface crystallinity of polymers has recently been shown to strikingly affect their surface properties.[52, 53]

The characteristic mode of growth of crystalline polymers from the melt and viscous solutions is spherulitic. The spherulite is considered to grow from some nucleus, possibly a tiny single crystal;[51] nucleated heterogeneously on particles or on a substrate. Because of viscosity effects and temperature gradients, the single crystal cannot continue to grow, and the growth degenerates into lamellar fibrils.

The lamellae tend to grow radially from the nucleus, producing a structure of spherical symmetry whose growth rate tends to be a linear function of time. The spherical symmetry results from non-crystallographic branching of fibrils (groups of adjacent lamellae) at an unstable growth interface.[54, 55]

Commercial crystalline polymers (polyethylene, polytetrafluoroethylene, polypropylene, cellulose, nylons, etc.) are normally composed of very small crystallites, generally unresolvable in the optical microscope. Nevertheless, though the surface of a crystalline polymer may appear homogeneous to a macroscopic contact angle drop, it certainly must appear heterogeneous to a microscopic protein or a simple compound. Amorphous polymers and elastomers may be relatively homogeneous, e.g., polystyrene, polymethylmethacrylate, etc.

Zisman[48] has related the surface energy of polymers to their chemical constitution by detailed studies of their wetting properties. He obtains a quantity called the "critical surface tension," γ_c, which is probably closely related to the surface free energy. The range for common polymer surfaces is from about 18 or 19 dynes/cm for polytetrafluoroethylene to 46 dynes/cm for 6/6 nylon.[48]

It is reasonable to expect that the different faces of a polymer single crystal or lamella will have different surface energies. Hoffmann[56] estimates that the lateral surface energy is about 10 ergs/cm^2

and the energy of a fold surface is 57 ± 5 ergs/cm^2 for polyethylene; Keller[57] believes that a significantly higher value is more accurate. The energy of a "typical" polyethylene surface is probably some average of the two, possibly close to Zisman's[48] γ_c value of 31 ergs/cm^2. Hoffman[56] also gives data for polychlorotrifluoroethylene, where the lateral energy is 4 ergs/cm^2 and the fold surface energy is 40 ergs/cm^2; Zisman's γ_c for this polymer is also 31. Thus the γ_c value does not necessarily shed light on the energies of the crystallites.

Polymer single crystals are microscopic and have not been grown in large enough sizes to allow one to use contact angle techniques for surface energy determination. Because of their lamellar nature, however, single crystals can be allowed to deposit from dilute solution to form an aggregate with the C-axis fairly well oriented perpendicular to the aggregate surface. Schornhorn and Ryan[58] have studied the wettability properties of polyethylene single crystal aggregates by contact angle measurements. The aggregates were highly crystalline. Their value for the surface of polyethylene single crystal aggregates is 53.6 dynes/cm^2. As the surface of an aggregate must be almost completely composed of fold surfaces, their results are in excellent agreement with Hoffman's[56] value of 57 ± 5. It is thus clear that the surface energy of a crystalline polymer is not only a function of its chemical nature but is also a function of how the molecules are oriented.

Polymers are usually cast or molded against low-energy surfaces

which tend to reduce sticking. As the polymers crystallize, the low molecular weight or impurity species are rejected from the growing crystal.[54,55] A polymer crystal thus tends to be surrounded by uncrystallized material, which is probably why γ_c and other surface properties are not particularly sensitive to crystallinity or bulk density.[58,59] If the polymer is cast against a high energy substrate, which can furnish many heterogeneous nucleation sites, its surface properties are different from those of conventionally formed polymers. This has been demonstrated by Schornhorn in several papers.[52,53] He studied the surface properties of both crystalline and non-crystallizable polymer surfaces prepared by melting on both high energy (gold) and low energy (nitrogen gas) substrates. A portion of his data is given in Table I.

TABLE I

SURFACE PROPERTIES OF POLYMERS

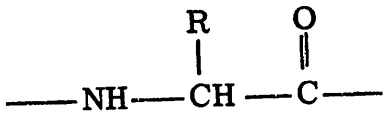
NUCLEATED AGAINST GOLD SUBSTRATES[53]

Polymer	Bulk Density	$(\gamma_c)_{N_2}$	$(\gamma_c)_{AU}$
1. Polyethylene	0.95	35	69.6
2. 6/6 Nylon	1.14	46	74.4
3. Polychlorotrifluoroethylene	2.12	31	58.9
4. Polypropylene (Isotactic)	0.90	29	39.5
5. Polypropylene (Atactic)	0.86	29	28.0

The surfaces of crystalline polymers are not necessarily homogeneous. The surfaces of a crystallite can have energies significantly different than the "amorphous" surface. An even greater energy difference exists between the lateral and fold surfaces of crystallites. The assumption will be made that a polymer surface consists of randomly arranged molecules, though the effect of different surface orientations will be qualitatively discussed.

2. Proteins:

A protein is a complex, highly structured, nylon-like copolymer to which many of the principles of polymer chain statistics are not applicable. A protein can contain up to about twenty different monomer units, called amino acids, each linked together by a peptide bond with its very prominent hydrogen-bond forming properties. The mer unit can be represented as



where R can be any of twenty different groups, some acidic, some basic, others hydrophobic. These are discussed in all biochemistry texts. The amino acid sequence of a protein is termed its primary structure. The alpha-helix conformation along many portions of the chain, resulting primarily from hydrogen bonding between the peptide linkages, is called the secondary structure. If one folds and fastens the protein back on

itself at several points, by disulfide linkages, hydrogen bonding, and hydrophobic interactions, a three-dimensional configuration of the chain is obtained. This is the tertiary structure. Finally, to further increase the complexity, most proteins are composed of two or more chains bound together into a complex quaternary structure.

The great variability of properties among different proteins and the phenomenal specificity exhibited by many of them is due largely to their fragile and complex tertiary and quaternary structures. The fragile structure can be stabilized or disrupted, depending on the ionic environment of the protein solution. The ionization tendencies of the various pendant groups depend on the pH of the medium. The pH of plasma is approximately 7.4. Buffer systems are employed in the body and in biochemical preparations to keep the pH constant. A very important property of a protein is its isoelectric point (IEP).

> The total charge on a protein molecule depends on the pH of the solution and the relative number of each kind of amino acid in the molecule. When the net charge density of the molecule is zero, that is, when total negative and positive charges effectively neutralize each other, the protein will not migrate in an electrical field, and that pH is its isoelectric point (IEP). At a pH alkaline to its IEP, the protein will carry a negative charge; at a pH acid to its IEF, the protein will carry a positive charge.
>
> Ref. 60, p. 41.

The fact that water tends to avoid apolar groups (discussed in the next section) while it is drawn to charged or polar groups leads to some important results. When a flexible molecule containing both hydrophobic

and hydrophilic groups is dissolved in water, the molecule assumes a stable configuration where its hydrophobic groups tend towards the center of the molecule (away from the water), while the hydrophilic groups are on the surface, nearest to the water. The hydrophobic groups can only interact with water by dispersion forces. These forces cannot compete with hydrogen bonding, and therefore water tends to "stay together" rather than interact with hydrocarbons. The situation is different at an interface, as now the force field is asymmetric and the London interactions are very significant.

The various side chains of amino acids have different ionization tendencies. Several of the groups have their isoelectric point around pH 7 and may be either charged or uncharged; acidic groups are negatively charged, basic ones positively charged. At pH 7 there are two amino acid residues which are negatively charged (acidic) and two that are positively charged (basic), in addition to the acid terminal of the peptide chain. The amino terminals may be either positively or negatively charged. The net charge and charge distribution of a protein molecule and its consequent dipole moment at pH 7 will be primarily dependent on the number and distribution of four different amino acids.[61] Amino acids capable of hydrophobic bond interactions are indicated by an AP notation in Table II. The other amino acids are assumed to be hydrophilic (P), except for glycine. For those amino acids which are charged at natural pH, the charge is given in parenthesis in Table II.

TABLE II

PROPERTIES OF SOME SELECTED PROTEINS[36, 68]

Amino Acid Composition (Moles/mole protein)	P or AP*	Albumin	Gamma Globulin	Fibrinogen	Ribonuclease
Lysine (+)	P	56	87	214	10
Arginine (+)	P	23	41	153	4
Aspartic Acid (-)	P	52	107	336	5
Glutamic Acid (-)	P	81	140	336	5
Amide NH_3	P	38	146	30	17
Glycine (*)	*	12	94	236	3
Alanine	AP	62	72	142	12
Valine	AP	41	131	119	9
Methionine	AP	5	10	59	4
Isoleucine	AP	8	30	125	3
Leucine	AP	60	102	184	2
Phenylalanine	AP	30	47	95	3
Histidine	P	15	27	57	4
Threonine	P	28	114	175	10
Serine	P	24	178	230	15
Proline	AP	25	100	169	4
½ Cystine	P	33	34	77	8
Tyrosine	P	16	62	104	6
Tryptophan	?	1	33	55	0
Total Carbonate		0.08%	2.9%	2.5%	---
N Terminals		Asp. Ala	Asp. Gly	Ala	Tyr Lys
C Terminals		Leu. Ala Val. Gly	Ser. Gly	-----	Val
Molecular Weight		69,000	160,000	340,000	14,000
Isoelectric Point (pI)		4.9	5.8 to 7.3**	5.8	9.4
P/AP Ratio*		1.58 (1.52)	1.58 (1.31)	1.92 (1.52)	2.27 (2.10)
Approximate Net Charge @ pH 7		-54	-119	-300	+4

* Glycine is often considered to be apolar, though steric effects would greatly inhibit its hydrophobic interactions. The P/AP value is calculated ignoring glycine; the value in parentheses considers glycine as apolar.

**The pI of the gamma globulins varies with the protein fraction tested. Many ionizable groups are no doubt immersed in the interior of the molecule for some configurations and thus cannot contact the solvent and ionize.

Table II presents data for ribonuclease and for the common plasma proteins albumin, gamma globulin, and fibrinogen. The structure of the plasma proteins is not known, nor are the exact amino acid sequences. The polar (P)/apolar (AP) ratios are also given in Table II. These ratios have been used by Ghosh[62] and Vroman[18] and may indicate the protein's tendency to adsorb by hydrophobic interactions. The P/AP ratio is determined by adding all the amino acids in the chain capable of polar interactions and dividing by the total number capable of only apolar (dispersion) interactions. The probable net charge at pH 7 is also given in the table.

The various properties of the plasma proteins have been succinctly reviewed by Putnam.[63]

Albumin is an ellipsoidal globular protein, usually considered to have an axial ratio of about 6:1. It is composed of a single polypeptide chain containing 16 to 18 disulfide bridges.[63] These bridges must all be intramolecular links tying and knotting the chain together; this accounts for albumin's stability. Albumin has a high concentration of acidic and basic amino acids and must therefore be quite polar, though its P/AP ratio indicates a significant number of hydrophobic residues. There is some evidence that albumin may have a somewhat hollow cylindrical shape.[64] It also has a strong tendency to dimerize,[63] which could indicate some type of mirror image charge distribution or structure.

The gamma globulins are a group of proteins which all have relatively similar solubility and electrophoretic properties. Their structure is commonly considered to be ellipsoidal and globular with a slightly greater axial ratio than albumin. "They have been considered to be a family of proteins, varying continuously, subtly, and ineluctably in their properties." (Ref. 63, p. 229). The gamma globulins have a relatively low alphahelix content, thus their structure is probably quite disorganized with respect to proteins of higher alphahelix content. Their folding into a compact configuration may be due to hydrophobic rather than hydrogen bonding.[63] The low alpha-helix content is in part due to the high proline concentration; proline cannot fit into an alpha helix. Gamma globulins also have an unusually large proportion of -OH containing residues (serine and threonine). Though they have over double the molecular weight of albumin, they have about the same number of disulfide bridges. Thus, the gamma globulins must have a structure which is capable of subtle changes in response to subtle influences. The structure is not encumbered by alpha-helices or by an excessive number of disulfide bridges, yet is is capable of extensive polar and dipolar bonding. The number of peptide chains in a typical gamma globulin is not well established,[63] but it is believed to consist of two small and two large polypeptide chains.[36]

Fibrinogen is a very large nodular or rod-like protein. The entire coagulation cascade appears to serve only to catalyze or modify fibrinogen so that it can polymerize with itself to form fibrin polymer, the network of a blood clot. It has been widely studied, mainly because of its importance in blood coagulation; an entire book is devoted to this one protein.[65] Fibrinogen appears to be composed of three pairs of polypeptide chains, each of molecular weights of about 50,000 to 65,000. Dipole moment measurements indicate "...a very high degree of charge symmetry with respect to the long axis of the molecule. The most likely arrangement, therefore, is mirror-image-like halves on both sides of the center of the molecule!" (Ref. 66, p. 69). Fibrinogen may have a very high water content, thus estimations of its shape vary greatly depending on the technique used and the assumptions made. The length of the molecule in solution is probably around 600 A;[66, 67] though electron microscope work gives a value of about 475 A for the dehydrated protein.[66] Electron microscope observations show a long needle-like structure with a nodule on each end and in the center; this structure is illustrated later in Figure 25. The nodules appear to be able to rotate, both perpendicular and parallel to the long axis. The structure can be summarized as "... Three nodular formations connected by loose, sponge-like segments" (Ref. 66, p. 84). About one-third of the chains are in the alpha-helix configuration. Fibrinogen is also known to dimerize.

3. Adsorption:

a. Introduction

A surface is a discontinuity. A surface is defined wherever a phase terminates. The phase may terminate in a vacuum or at the surface of another phase. The surface formed where two phases meet and terminate is an interface. The concept of a surface is, in most instances, really that of an interface. Perhaps the most complete and up-to-date treatment of surface science is given by Adamson.[69] Fowkes' comprehensive and recent review [70] and Davies' and Rideal's Interfacial Phenena[71] are also very useful. Treatments of interfacial energies and forces are more difficult to find: the American Chemical Society's Chemistry and Physics of Interfaces[72] is one of the most readable expositions, particularly the paper by Fowkes.[73] Good briefly deals with the subject in his review[74] and treats it in detail in his papers;[75-8] Fowkes' other papers also treat it.[79-82]

The attraction of the molecules in the surface layer of a liquid by the bulk phase results in a decrease in the number of molecules in the surface region, thus increasing the intermolecular distance on the surface; the result is a surface tension. Usually the surface tension or energy resides in the outermost layer, but in some systems there are contributions from the second and third layers. The forces responsible for the surface tension are the intermolecular forces which will be discussed. In the case of water, there are London dispersion and hydrogen-

bonding (dipole-dipole) attractions. Following the principle of additivity of intermolecular forces,[83] the interfacial tension, γ can be written as:[73]

$$\gamma_{water} = \gamma^{London}_{water} + \gamma^{hydrogen\text{-}bonding}_{water} \quad .$$

Consider the interaction between water and a saturated hydrocarbon. The only intermolecular force available for the interaction of hydrocarbons with themselves or other uncharged species is the dispersion force. The interface between water and a hydrocarbon can be considered to be composed of two adjacent interfacial regions; the sum of the surface tensions of the two regions gives the overall interfacial tension. The hydrocarbon molecules at the interface are not only attracted by their bulk phase, but they are also attracted by the dispersion interactions of the other phase. Therefore molecules at an interface between two different phases are in a different environment than those at the surface of a single phase. The molecules at the interface are not only involved in intermolecular interactions with their own kind, but they are also interacting with the molecules in the adjacent phase. The result is that the interfacial tension must be lower than the surface tension of water itself.

Adsorption is an interfacial process. It is generally defined as a surface excess of some component in or near an interface. The interfacial region can be treated as a separate phase with its own thermodynamic properties. This approach was pioneered by Gibbs (see Ref. 69)

and has been exhaustively developed.[3]

Interfaces can be divided into two general types: condensed-phase/vapor and condensed-phase/condensed-phase. Adsorption can also be divided into two classes: physical adsorption and chemisorption. Physical adsorption refers to interactions other than direct chemical bonds, while chemisorption is due to actual chemical bonds. There is a wide overlap between the two types; an excellent discussion is given in Chemisorption.[84]

Adsorption data is usually treated in terms of an adsorption isotherm, which is a plot of amount of material adsorbed against the concentration in solution; the data are taken at constant temperature and after equilibrium has been established.

The adsorption of gases on solids or liquids has been treated by a consideration of intermolecular interactions,[85,86] but adsorption from solution has been deprived of such mechanistic treatments and has had to depend on thermodynamic analyses. This is understandable, as a treatment of adsorption from solution in terms of inter-molecular interactions is complicated by solvent-solid and solute-solvent interactions. A more difficult problem is posed by considering adsorption from solution at the solution/air interface: how can intermolecular interactions account for such adsorption? The air certainly cannot significantly interact with the solute molecules. Thus one must resort to a consideration of solute-solvent interactions to attempt to explain the phenomenon.

This is the basis of the mechanistic model of adsorption from solution which will be presented and developed in the next chapter.

b. Simple Compounds

The surface tension of water is 73 dynes/cm. Addition of a second component to pure water usually results in a decrease in the surface tension. The decrease is due to solute adsorption at the air/water interface. Thus a measure of surface tension as a function of solute concentration is an indication of adsorption. Such surface tension isotherms are common in the literature. An example is given in Figure 4 for both surface-active and surface-inactive solutes. If the decrease in surface tension is due to the fact that some solute molecules are statistically in or near the interface at all times, thus affecting its properties, then the plot in Figure 4 should be a linear function of concentration (dotted lines). The surface tension should linearly decrease for a low-energy (surface-active) solute and should linearly increase for a high-energy (surface-inactive) solute. It is clear from Figure 4 that this is not the case. The surface tension drops very rapidly below the linear line (dotted) for the surface-active solute, indicating that a concentration of solute builds up at the surface or adsorption of solute occurs. For the surface-inactive solute, the line is below that expected for a linear increase, indicating that a surface deficiency of solute exists or negative adsorption occurs.

It has been suggested that adsorption at the water/air interface can be divided into two processes,[21] the diffusion of solute to the vicinity of

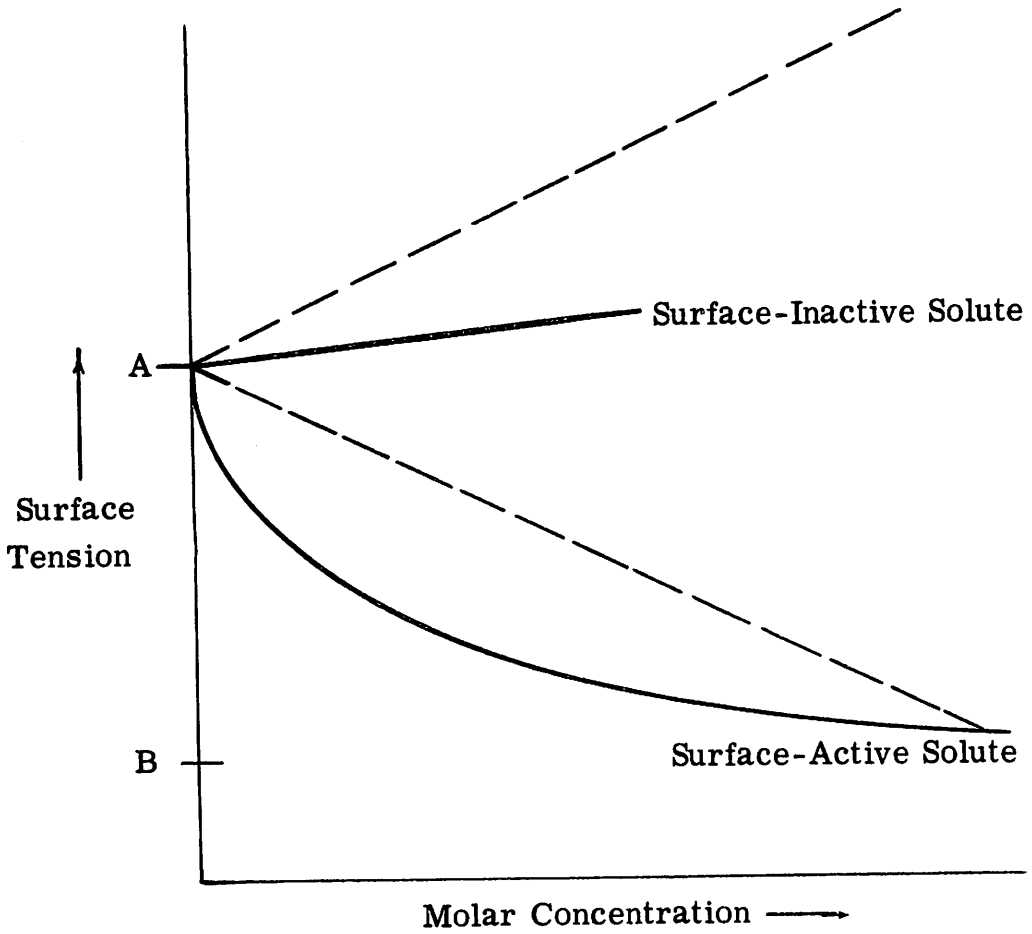


Figure 4. Hypothetical surface tension—concentration isotherms for surface-active (low energy) and surface-inactive (high energy) solutes (after Ref. 60, p. 211). A represents the surface tension of the solvent (73 dynes/cm. for water): B represents the surface tension of pure solute.

the interface, and the actual adsorption and orientation of the solute at the interface. The adsorption process tends to be very rapid for relatively small molecules, but becomes quite slow for larger molecules, probably due to the slower diffusion rates. Protein adsorption at the water/air interface often takes several hours or longer to equilibrate.

Though most of the adsorption occurs in a very short time, there is usually a small increase in adsorption for a long time before equilibrium is attained. One suggestion[21] is that the first molecules arrive at an empty surface and are free to orient and adsorb while later arrivals have more difficulty finding a place. Thus, the process should slow down rapidly when the surface is nearly completely covered, as observed. It is probable that adsorption at the liquid-air interface may continue to occur after an adsorbed layer has formed, particularly if the solute molecules can significantly interact with each other. Multiple layers are not, however, detectable by the more common techniques of change in surface tension or surface potential. If enough material is present it can form a second phase, as with oil films on water.

If the solute molecules are capable of extensive intermolecular interaction among themselves and with the water, as for stearic acid, the adsorbed molecules may form a stable, insoluble monomolecular film. Monomolecular films are very intriguing and have been studied a great deal.[87]

Unfortunately, there has been very little work or discussion of the relationships between adsorption processes at different interfaces. Kipling has attempted some discussion;[21] but could draw very few conclusions. Adsorption at the liquid/air interface does appear to be preferential for that component which most reduces the surface tension, though the same generalization is not necessarily true for adsorption at the liquid/solid

interface. The amount of material adsorbed at a liquid/solid interface is often greater than at a liquid/air interface.[21] Also, adsorption at the solid/liquid interface can be highly specific. Polar adsorbents preferentially adsorb polar compounds; apolar adsorbents preferentially adsorb apolar compounds. Solvent competition effects are also very important.

A detailed discussion of the adsorption of simple compounds will be given in Section C. 2.

c. Polymer Adsorption

The principles of polymer adsorption have been well summarized by Ullman and coworkers;[88] a recent review[89] is also available, as is a chapter in Kipling's book.[21] The general principles noted above for simple compounds also apply to polymers. Their large molecular weights tend to make interpretation of data more difficult.

Molecules of synthetic polymers in solution tend to assume a random-coil shape unless there are strong polymer-solvent interactions or strong interactions between portions of the polymer chain.

Polymer adsorption on solid surfaces tends to follow a Langmuir isotherm, implying monolayer formation. Data on the amount of polymer adsorbed clearly show that much more than a flat monolayer adsorbs, thus the monolayer must be composed of relatively random coils. The amount of polymer adsorbed is a function of molecular weight, increasing

with increasing molecular weight. Polymers are not homogeneous because of their molecular weight distribution. Thus, polymer adsorption produces a fractionating effect, because the higher molecular weight material is preferentially adsorbed. Polymer systems give good evidence of competitive adsorption. Kipling[21] cites data where low molecular weight species are adsorbed first, and then displaced by the more strongly interacting high molecular weight component.

Though initial polymer adsorption is relatively rapid, equilibrium is often not attained for weeks or months. Polymer adsorption is somewhat irreversible in that it is difficult to desorb polymers from a polymer surface. This is interpreted as due to the statistical improbability of breaking all of the many polymer-surface interactions simultaneously.

There has been little work on polymer adsorption from aqueous solutions due to the inherent insolubility of polymers in water. The adsorption of synthetic polyelectrolytes has been studied. Lauria's study[90] which included adsorption on polystyrene beads, utilizes the conventional techniques of high surface area adsorbent and solution concentration changes. His data seem "...to fit a type of Langmuir isotherm with repulsion between the adsorbed units." (Ref. 90, p. ix)

Polymer films at the liquid/air interface have been well studied and reviewed[87 91-2] If the polymers contain both polar and apolar groups, stable monomolecular films can be formed. Most of the work has dealt

often purposefully prepared. Here the only concern is with adsorption from solution; films formed by gently depositing protein solutions onto a water surface will be ignored. Practically all the isotherms available for the air/water interface are film-pressure isotherms and are thus difficult to relate to other data.

When there is a relatively small amount of protein at the interface (less than 1 $mg./m^2$), the film is dilute[95] and essentially completely unfolded (denatured). In compressed films all of the molecules are not necessarily denatured, and some unfolded, intact proteins are usually present. The most reasonable model of protein films is the duplex model.[95] This states that the first molecules to arrive are probably denatured and a denatured monofilm is formed. Cumper and Alexander believe that "...surface denaturation of each molecule as it reaches a clean interface must be an almost instantaneous process," (Ref. 94 p. 134). As additional protein molecules arrive, they form undenatured multilayers. Other models are also available.[95] The tendency to form a monofilm is quite strong, as films can continue to form against an applied surface pressure.

Protein adsorption is always maximized when the solution pH is at the isoelectric point. At pH's away from the isoelectric point, adsorption is probably retarded by repulsive forces due to the charged protein molecules in the surface.[95] There is apparently a molecular weight effect as well, as the high molecular weight proteins form films less readily than smaller proteins.

There is evidence that only a small portion of a protein molecule need interact with a surface to enable denaturation to occur.[95] It is apparent that the formation of protein films is a slow process, as surface tension measurements do not approach a constant value until several hours after spreading. This is probably due to reorientation effects and more complete unfolding with time.

Denaturation at the oil/water interface is a function of the interfacial energy (about 73 ergs/cm^2 for the air/water interface). In systems where this energy is quite low, denaturation apparently does not occur. Adsorption does occur, however, but the molecules are probably in a more or less unmodified state. Adsorption at oil/water interfaces is more rapid than at air/water interfaces[95], but a satisfactory mechanism for this behavior has not been postulated.

There is a fundamental difference between solid/liquid and liquid/liquid interfaces. A liquid/liquid interface is relatively mobile, as both liquids are experiencing thermal motion, and their molecules are dynamic. Thus, it is relatively easy for an adsorbed molecule to orient at such an interface and even penetrate into one or both liquid phases. There is no such mobility at the solid/liquid interface. The methods of studying such an interface are also quite different, thus the results are not directly comparable.

Protein adsorption on solids tends to conform to the Langmuir isotherm, again implying monolayer adsorption of some type. Adsorption

from moderately concentrated solutions tends to produce films in which the proteins are not denatured and are probably in a relatively compact configuration. Adsorption from dilute solutions most likely produces a denatured film with an additional layer or two of weakly adhering intact molecules.[62,95]

Perhaps the surface most extensively studied is glass;[96] but the results are difficult to interpret. The variable nature of the surface of glass[97] is usually not considered in adsorption studies. Different surface treatments lead to strikingly different results.[95]

Much of the available work on protein adsorption stems from work on blood coagulation. It has long been known that blood coagulation occurs rapidly in glass containers but much more slowly on polymeric surfaces or paraffin-coated surfaces. This has led to the postulation of a surface-induced or surface-activated mechanism of blood coagulation, in addition to the intrinsic mechanism. These mechanisms are now fairly well established[6-8,98] though there is a great deal of controversy about the details within each mechanism. The two major theories are discussed by Vroman in a very readable little book;[1] in another work[99] they are synthesized by him in an adsorption model of coagulation as a sequence of protein-protein adsorption interactions. The activation role of the solid surface is believed to be due to the adsorption of a particularly surface-susceptible protein called Hageman Factor (after Mr. Hageman) and its resultant distortion.[100] This approach is not very satisfying, as Hageman Factor is present in

much lower concentrations in blood than many other readily adsorbable proteins. This puzzling situation has led to a great deal of effort to determine the true role of protein adsorption and surface-induced processes in blood coagulation. This work, and other more detailed adsorption studies, will be presented in Section C, where it will be compared against the model which will be developed in Section B of this chapter.

There are few studies where reliable and complete adsorption isotherms have been developed. The lack of a suitable model makes it difficult to put the available information in any sort of order or perspective.

4. Water:

The structure and properties of water play a fundamental and perhaps major role in protein-surface interactions? The most popular theory of water structure is the "flickering cluster" model first proposed by Frank and Wen in 1957 [101] and developed quantitatively by Nemethy and Scheraga.[102] The basis of the model is that hydrogen bond formation is a cooperative process, due to the acid-base nature of the bond.[101] When one bond forms, many tend to form; when one breaks, many break. Thus, small clusters are formed, constantly appearing and dissolving, whose lifetime is long enough to be physically meaningful. This model has been analyzed in depth, and its thermodynamic properties are

available.[102] The clusters are mixed with non-hydrogen bonded water molecules which are involved in dipole-dipole interactions. The Frank-Wen-Nemethy-Scheraga (FWNS) model accurately represents the known thermodynamic properties of water. It does not, however, account for the observed structural transitions in water.[103] One of these transitions occurs in the vicinity of body temperature and may eventually have a large significance in the understanding of body chemistry.

The other major theory of water structure is Pauling's model of water as a continuous clathrate or hydrate, though his model is not as popular as the FWNS theory. Pauling's model is visualized as a network of hydrogen bonded water cages, composed of 20 to 24 molecules and enclosing nearly spherical cavities in which a host molecule can be accomodated without disturbing the structure.[104] The host molecule does not collide with the cages and is in a symmetrical field, thus there is little hindrance to internal rotation. The host molecule may be a clathrate-forming atom, such as xenon, or it may be a water molecule.

Pauling has formulated a general theory of anesthesia[105] based on water-anesthetic interactions resulting in clathrate formation. The Pauling hydrate model has been thoroughly analyzed by Frank and Quist.[106] In their analysis the Pauling model is discussed in terms of flickering clusters of water cages.

The strong charge-dipole interactions between an ion and water dipoles produces a tightly bound and oriented hydration layer around an ion. The extent of this effect is dependent on the ion's polarizing power.[104] The hydration layer is rather rigid and at least one molecule thick. In addition to these relatively short-range effects, the electrostatic field of the ion can exert a torque on farther removed dipoles and thus interfere with structure-forming and structure-breaking. These effects are usually described in terms of the Frank and Wen[101, 107] multizone hydration hypothesis. In this model the ion is surrounded by two concentric zones: in the inner zone, the water molecules are oriented, immobilized, and compressed; in the outer zone, the water structure is disrupted and the molecules perhaps partially oriented. Outside these regions the water structure is relatively unaffected by the presence of an ion. Because of changes in the water structure, the dielectric constant near an ion is different then in the bulk. The value of the dielectric constant near the ion is not known, but it can be assumed that beyond eight angstroms it is essentially the bulk value.[104]

One might expect that the structure of water at an air/water interface would be well established. It is generally accepted that the surface of water is polar,[107-8] the oxygen portion pointing towards the surface and the hydrogens pointing toward the bulk. This arrangement is compatible with the high surface energy of water, but now there is evidence that the true arrangement might be just the opposite.[108]

It is well accepted that water tends to isolate apolar solutes, forming a more ordered structure around the foreign material, often called an "ice-berg." Such an arrangement minimizes the total energy of the system.[109] The tendency to reject or isolate apolar material has led to the concept of the hydrophobic bond.[102] Salem[110] has suggested that a more direct approach to an understanding of the hydrophobic bond may come from a consideration of intermolecular interactions. There is also evidence[107] that the water surface tends to exclude ions, as demonstrated earlier in Figure 4.

5. Intermolecular Forces:

The subject of intermolecular forces has been thoroughly treated in the book by Hirschfelder et al.[111] Shorter discussions have been given by Hildebrand and Scott;[83, 112] Margenau;[113] and Pitzer.[114] Several conference proceedings on intermolecular[115] and surface forces[116] are also available. Good's very recent review[74] neatly summarizes the subject and discusses some applications to solid polymers. Salem's papers[110, 116-119] review the nature of intermolecular forces and treat biological applications in a very lucid manner. Other discussions and reviews are also available[120,121]

Intermolecular forces can be artificially divided into short-range and long-range forces. The short-range forces are due to electron cloud overlap at very close separations, producing a repulsive force. Short-range forces are ignored here, as the separation distances which will be

considered will always be greater than several angstroms. The long-range forces can be divided into three major types: electrostatic, induction, and London dispersion forces. The electrostatic forces can be further divided into charge-dipole, dipole-dipole, and quadrupole interactions. Quadruple contributions will be ignored as they are usually negligible. [74] Intermolecular forces are responsible for deviations from ideal gas behavior and are, therefore, classed together under the general heading of Van der Waals forces, though often the term Van der Waals is used only in reference to London dispersion forces. These forces are not necessarily weak and may approach magnitudes of the order of chemical bonds.[74]

a. Electrostatic Interactions

The charge-dipole interaction energy, $U(Q - \mu)$, between two like particles is represented as:[111]

$$U(Q - \mu) = - (Q \cdot \frac{\mu}{r^2}) \cos \theta, \qquad (1)$$

where θ is the angle between r and the dipole axis, μ is the dipole moment, and Q is the charge. The expression for dipole-dipole interactions is more complex:[74]

$$U(\mu - \mu) = \frac{\mu_1 \mu_2}{r^2} [\, 2 \cos \theta_1 \cos \theta_2 - \sin \theta_1 \sin \theta_2 (\cos(\phi_1 - \phi_2))] \qquad (2)$$

where the angles are identified in Figure 5.

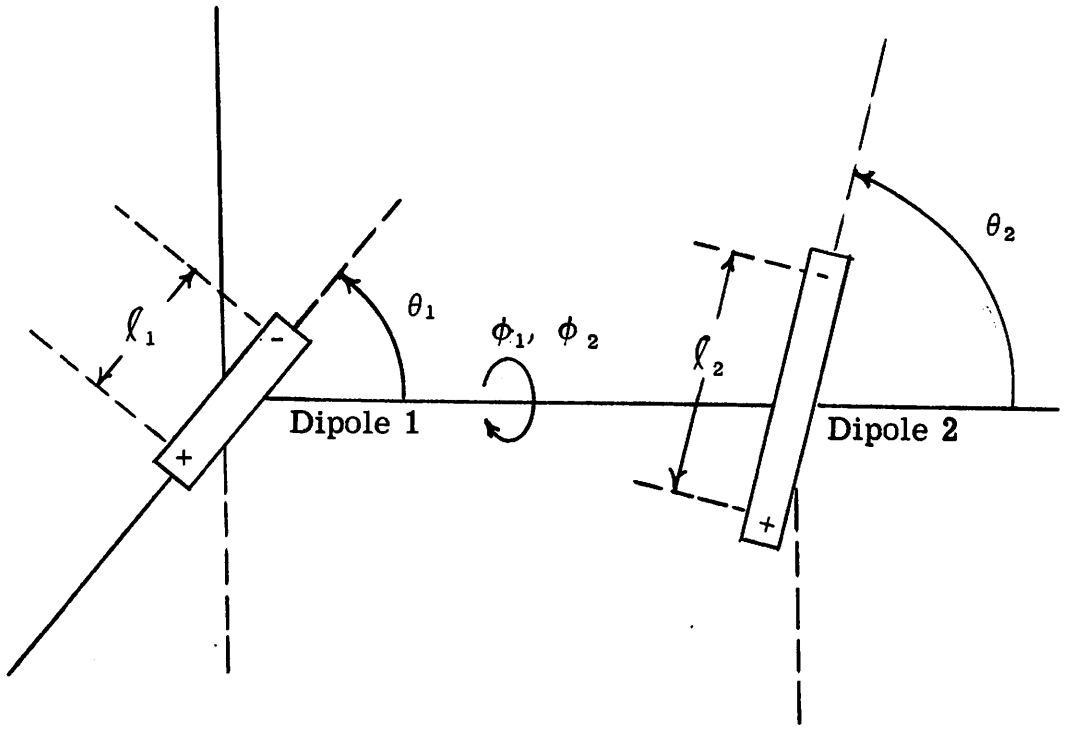


Figure 5. Orientation Relations Between Two Stationary Permanent Dipoles (After Ref. 74, p. 22).

If the two dipoles are free to rotate, they will assume the low energy head-to-tail configuration where all the angles go to zero, thus reducing equation (2) to:

$$U = -\frac{2\mu_1\mu_2}{r^3} \tag{3}$$

Equations (2) and (3) are obtained on the assumption that $\ell \ll r$. When this condition does not hold, the system cannot be treated as dipoles, and the interactions must be summed over the entire charge system. Hirschfelder[111] and Good[74] discuss the case where ℓ is not $\ll r$. When r/ℓ is less than 2.5, the expressions are in error by 25% or more.

The average distance of closest approach of two water molecules is estimated as 3.2 A.[102] The effective charge separation distance, ℓ, corresponds roughly to the C-H bond distance and can thus be estimated at about 1 A.[122] Thus r/ℓ is about 3.2 for water and r is sufficiently greater than ℓ to use the dipole expressions.

These expressions only apply to bi-molecular dilute gas phase interactions of molecules in fixed orientations. If the dipoles are free to rotate and the interaction energy is greater than the thermal energy of randomization, then there will exist a distribution of orientations with those of lowest energy predominating. The distributions are expressed by Boltzmann functions, and one obtains expressions for the average interaction energies; after expansion and simplification, the expressions reduce to[111,113]:

$$\overline{U}(Q - Q) = \frac{Q_1 Q_2}{r} \tag{4}$$

$$\overline{U}(Q - \mu) = \frac{1}{3kT} \frac{Q_1^2 \mu_2^2}{r^4} \tag{5}$$

$$\overline{U}(\mu - \mu) = \frac{-2}{3kT} \frac{\mu_1^2 \mu_2^2}{r^6} \tag{6}$$

where the $\overline{U}$ indicate time-average energies. Because of the requirements of the Boltzman expansion, equations (4-6) are only valid for relatively large separations, i.e., r must be greater than 3 A.

The hydrogen bond is often treated as a special type of inter-

molecular attraction,[74] but it is an unusually strong dipole-dipole interaction and can be treated in the same manner. For short hydrogen bonds, less than 2.5 A, there can be an appreciable covalent character to the bond (20 - 25%); this is usually negligible for bond distances greater than 2.8 A.[74] It will be treated as a strong dipole-dipole interaction.

b. Induction Interactions[111]

When a charge or permanent dipole interacts with a neutral molecule, a dipole moment is induced in the neutral molecule. The two species can then interact electrostatically:

$$U(Q - \text{ind. } \mu) = \frac{Q_1^2 \alpha_2}{2r^4} \quad , \tag{7}$$

where α_2 is the polarizability of molecule 2. For dipole-induced dipole interactions,

$$U(\mu - \text{ind. } \mu) = \frac{-\mu_1^2 \alpha_2}{2r^6} (3 \cos^2 \theta_1 + 1). \tag{8}$$

Averaging over the angles, Equation (8) becomes:

$$\overline{U}(\mu - \text{ind. } \mu) = \frac{-\mu_1^2 \alpha_2}{r^6} \tag{9}$$

Dipole-induced dipole forces are usually negligible[74] and will therefore be ignored, but charge-induced dipole contributions cannot be neglected.

c. London Interactions

London contributions to intermolecular and interfacial interactions may in many cases be greater than polar contributions, even in

The terms $\bar{C}_1$ and $\bar{C}_2$ are often set equal to the first ionization energy, I, though Pitzer[114] suggests that $\bar{C} \simeq 2I$ is a more reasonable approximation for some simple molecules. Fortunately, Salem has carried out a rigorous analysis of the problem,[117] making fewer assumptions, and has calculated the interaction energy between two $-CH_2-$ groups:[118]

$$U(d) = -9.18 \times 10^{-11} \text{ erg/(d in A)}^6 = \frac{-3}{4} \bar{C} \alpha^2/d^6. \qquad (12)$$

If one uses a static polarizability value for $-CH_2-$ of[118] 1.84×10^{-24} cm^3 (see Table IV), equation (12) indicates that, for a $-CH_2-$ group, $\bar{C} \cong 2.16 I_{-CH_2-}$, in reasonable agreement with Pitzer's[114] suggestion. Using Salem's numbers[110] for water-water dispersion interactions, the $\bar{C} \simeq 2.2$ I approximation yields α- values for water within 7% of the commonly accepted value (Table IV). If the reasonable approximation, $C_i \simeq 2.2 I_i$, is used in equation (11) along with the generally accepted static polarizability and ionization potential values, dispersion interactions can be calculated. Equations (10) and (11) then become:

$$U(d) = \frac{3}{2}(2.2) \frac{I_1 I_2}{(I_1 + I_2)} \alpha_1 \alpha_2 / r^6 . \qquad (13)$$

This expression will be used for calculating dispersion interactions. Values of I and α are tabulated in Table IV for the groups and molecules to be considered. The quantities I, α, and μ are discussed in more detail later.

d. Multi-Molecular Interactions

The superposition principle of electrostatics [123] allows one to determine the force on any charge as a vector sum of the forces from each of the other charges.

Unless the orientation of each ion or dipole is exactly known, equations (1) and (2) cannot be employed. In liquid or solid media the molecules are usually not free to rotate, thus equations (4-6) are not valid. In the case of a solid polymeric surface each unit will be handled as a separate entity, or "bead", as suggested by Good,[74] and the appropriate group and bond dipole moments and polarizabilities (Table III) will be used.

London interactions are different from electrostatic effects in that the bi-molecular interaction is to a first approximation independent of the interaction with other atoms.[124] This is proved in Margenau's review.[113] Thus, the direct additivity of London forces is generally accepted.[69] Often the total contribution can be found by an integration. London interactions at large separation distances become complicated by a retardation effect, due to a phase difference between the fluctuating and induced dipoles. This has been treated[125] and is only important at large separations (of the order of 1000 A or greater); therefore it will be ignored.

The additivity of London forces in condensed media has been challenged.[126] The expressions are nevertheless valid if the medium

is accounted for[127-9] in the equations. Direct measurements of London attractions between solids have been made by Deryagin and his colleagues.[130] They found that the values obtained using the additivity concept agree with their results if the retardation correction is included (as they were working at distances greater than 1000 A). General theories of London forces in which retardation and media effects are included have been reviewed.[126, 131] Equations to calculate London interactions between atom distributions of different geometric shapes have been derived[124, 127, 132] and tabulated.[69] The appropriate expressions for the geometries to be considered here will be developed and discussed in Section B.

e. Determining α, μ, and I

The polarizability can be calculated from the expression[122, 133]

$$\alpha = \frac{3}{4\pi N_0} \left(\frac{\epsilon - 1}{\epsilon + 2}\right) \frac{M}{\rho}, \qquad (14a)$$

where ϵ is the high frequency dielectric constant, N_0 is Avogadro's number, ρ is the bulk density, and M is the molecular weight. Also, as $\epsilon = n^2$ (if ϵ and n are measured at the same frequency*) one can use

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* The dielectric constant is a function of frequency, as is the refractive index. At sufficiently low frequencies, the dielectric "constant" becomes a true constant, independent of frequency (see Ref. 134 for a lucid discussion). The value used for electrostatic problems is the constant value (78.3 for water at 25 C) but for dispersion interactions the high frequency value (1.76 for water) must be used, as the frequency of charge fluctuations responsible for dispersion forces is about 10^{15} sec.$^{-1}$.

the expression

$$\alpha = \frac{3}{4\pi N_0}\left(\frac{n^2 - 1}{n^2 + 2}\right)\frac{M}{\rho} \simeq 4 \times 10^{-25}\left(\frac{n^2 - 1}{n^2 + 2}\right)\frac{N}{\rho}, \qquad (14b)$$

when n is the refractive index, usually for the sodium D line.

These expressions yield a sub-microscopic polarizabilty from macroscopic data. The results are probably valid for isotropic substances or for anisotropic materials where ϵ or n was determined as a function of drawing or crystallographic direction. The polarizability would, of course, then also be direction-dependent. Values of ϵ or n in the literature[135] are not usually listed as function of direction, though such information is available for polyethylene.[136]

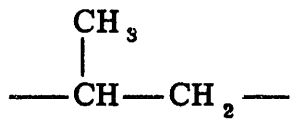
If directional polarizabilities are required, one may have to resort to bond polarizabilities. Values of $\alpha_{\parallel}$ and $\alpha_{\perp}$, the polarizabilities parallel and perpendicular to the bond direction, respectively, are available[111,137] (Table III). If the polarizing field is at some arbitrary angle, θ, to the bond direction, then:[111,137]

$$\alpha_\theta = \alpha_{\parallel} \cos^2 \theta + \alpha_{\perp} \sin^2 \theta. \qquad (15a)$$

If this expression is averaged over all angles, then

$$\alpha = \frac{1}{3}(\alpha_{\parallel} + 2\alpha_{\perp}). \qquad (15b)$$

Saturated polymer chains can be treated as "strings of beads".[74] For example, the $-CH_2-$ group can be selected as the representative segment in polyethylene, the



in polypropylene, the $-CH_2CHCl-$ in polyvinyl chloride, etc. The polarizability of the bead can then be calculated by equations (14a) to (15b). Unfortunately, equations (14a) and (14b) yield results different from equation (15b) in some cases. For benzene, the results are identical, but for polyethylene they are different. The $-CH_2-$ in polyethylene can be considered to have 2 C-H and 2 ($\frac{1}{2}$) C-C bonds. Equation (15b) then yields (see Table III for values) $\alpha = 1.94 \times 10^{-24}$ cm.^{3} Salem;[118] determining bond polarizabilities from molar refraction* data,[138] obtained an α-value of 1.84×10^{-24} cm.^{3} An average value of n^2 for high density ($\rho = 0.96$) polyethylene is 2.37 (Table IV). Putting these values in equation (14a), $\alpha = 1.82 \times 10^{-24}$ cm.^{3} Good[74] gives a value of 1.76×10^{24} for high-density polyethylene. The difference between the four results are quite significant, particularly when it is recalled that the London interaction energy (or force) is directly proportional to α.

When orientation is not a problem, α-values calculated from equations (14a-b) should be used, as they are dependent on macroscopic

*The molar refraction, R, is defined as

$$R = \left(\frac{n^2 - 1}{n^2 + 2}\right) \frac{M}{\rho} = \left(\frac{4}{3}\right) \pi N_0 \bar{\alpha},$$

where M = molecular weight and ρ = bulk density. See equation (14b) and Refs. 133 and 138.

properties. It is believed that polyethylene contains extraneous dipoles, possibly carbonyls.[139] They could produce a local field which would decrease the polarizability, in qualitative agreement with the results just calculated. Because of these impurities the polarizability of commercial polymers cannot be accounted for by only considering bond polarizabilities. However, if orientation effects are important, one must determine α from bond polarizability data (Table III).

The London expression also required ionization potential values. Tables of ionization potentials are available.[140-2] The ionization potential of the appropriate polymer segment can be deduced from values for "model" compounds. Most ionization potential data are determined in the gas phase. There is evidence that, because of the polarizability forces within a crystal, solid state ionization potentials are about 1 to 2eV lower than those in the gaseous state, especially for unsaturated organic solids.[143] Fortunately, this is already accounted for in equation (13). When the $\bar{C}$ is about 2.2 I approximation was made, it was using a conventional value of I, i.e., determined in the gas or in an apolar liquid. Thus, I values directly from the tables can be used (Table IV). Ionization potential for ions are not readily available and certain assumptions must be made to deduce values for charged groups.

Permanent dipole moments of molecules are well-known, and extensive tabulations are available.[61, 133, 144] In most cases, group dipole moments will be important, some of which are also tabulated.[74 133] Values for proteins and amino acids are listed in Cohn and Edsall's book.[134]

f. Medium Effects

The basic equations for the calculation of intermolecular forces between molecules separated by a vacuum have been presented. Now the expressions must be modified to account for the effect of the water medium. This is easily done for the electrostatic expressions by including the static dielectric constant of water in the Coulomb's Law expressions. The result is a D or D^2 term in the expression, depending on the type of interaction.[110, 121]

The modification of the London equation is a bit more subtle. As the London interactions are due to high frequency fluctuations, Setlow and Pollard[121] reasoned that one could use the $\epsilon \cong n^2$ approximations, which results in an n^4 term in the denominator (because the electrostatic analogy to the London forces, a dipole-induced dipole interaction, has a D^2 term in the denominator). This approach has been criticized,[145] as it leads to too great a decrease as compared to other more rigorous evaluations.

Fowkes[146] assumed one could use the high frequency dielectric

constant directly in the denominator ($\epsilon = n^2 = 1.76$ for water) and simply divided his expression by ϵ (as opposed to Setlow and Pollard's ϵ^2, above).

A rigorous analysis of this problem has been carried out.[128-9] Kestner and Sinanoglu[128, 145] concluded that the reduction between two polymer chains in water is 15 to 30%. Salem[110] has accepted their results and assumed a constant 30% reduction in water. Thus, the equations to be used later will include a 0.7 correction term. In addition, it will be assumed that the water medium correction is approximately the same as that for an apolar polymer medium, so the same corrected equation will be used for all calculations. This assumption is justified, as n^2 for water is 1.83, while n^2 for polyethylene is 2.4; any error resulting from such an assumption would easily lie withint the limits of error of the equations.

g. Additivity and Summary

When the dialectric constant is included, the important energy and force ($F = -dU/dr$) equations are:

$$\bar{U}\,(Q^2 - \mu) = -Q_1^2\,\mu_2^2\,/(3kT\,D^2\,r^4) \qquad (16a)$$

$$\bar{F}\,(Q - \mu) = -4Q_1^2\,\mu_2^2/(3kTD^2r^5) \qquad (16b)$$

$$\bar{U}\,(\mu - \mu) = -2\mu_1^2\,\mu_2^2\,/(3kTD^2\,r^6) \qquad (17a)$$

$$\bar{F}\,(\mu - \mu) = -4\mu_1^2\,\mu_2^2\,/(kT\,D^2\,r^7) \qquad (17b)$$

$$U(\mu - \mu) = -2\mu_1\mu_2/(D^2 r^3) \tag{18a}$$

for optimum orientation;

$$F(\mu - \mu) = -6\mu_1\mu_2/(D^2 r^4) \tag{18b}$$

for optimum orientation;

$$U(\text{Q-ind.}\ \mu) = -Q_1^2\alpha_2/(2r^4 D^2) \tag{19a}$$

$$F(\text{Q-ind.}\ \mu) = -2Q_1^2\alpha_2/(D^2 r^5)\cdot \tag{19b}$$

The final form of the London equation is:

$$U(d) = -(1.5)(2.2)(0.7)\, I_1 I_2 \alpha_1\alpha_2/[(I_1 + I_2) r^6] \tag{20a}$$

$$F(d) = -9(2.2)(0.7)\, I_1 I_2 \alpha_1\alpha_2/[I_1 + I_2) r^7], \tag{20b}$$

where the 2.2 is the ionization potential correction and the 0.7 is the water medium correction. All of the interactions are attractive (negative sign). The negative signs will be dropped for convenience, as attraction is understood.

Note that in the absence of charges, the two relevant expressions are functions of r^{-6} if random dipoles can be assumed. If one considers only the constant due to medium effects, i.e., $1/D^2$ in equation (17) and 0.7 in equation (20), then London forces have a 4500-fold advantage in water (as D^2 for water is about 6400). In the absence of water, such an advantage would not exist.

All the expressions necessary for calculating long-range inter-

actions between molecules and particles in water are now available. Using the general principle of additivity of intermolecular forces,[74,83,112] all the contributions can be summed to obtain the total effect.

Values of α and I are given in Table IV.

TABLE III

BOND POLARIZABILITIES AND BOND LENGTHS[111,137]

Bond	$\alpha_{\parallel} \times 0^{25}\,cm^{-3}$	$\alpha_{\perp} \times 10^{25}\,cm^{3}$	$\alpha_{\perp}/\alpha_{\parallel}$	Bond Length (Angstroms)
C-C (Aliphatic)	18.8	0.2	0.01	1.54
C-C (Aromatic)	22.5	4.8	0.21	1.39
C=C	28.6	10.6	0.37	1.34
C≡C	35.4	12.7	0.36	1.20
C-H	7.9	5.8	0.73	1.09
C=O	19.9	7.5	0.38	1.24
C-Cl	36.7	20.8	0.57	1.76
C-Br	50.4	28.8	0.57	1.90
C=S	75.7	27.7	0.37	1.62
C≡N	31	14	0.45	1.15
N-H	5.8	8.4	1.45	1.02
N≡N	24.3	14.3	0.59	1.09
Cl-Cl	66.0	36.2	0.55	1.98
H-H	6.8	8.9	1.31	0.74
H-Cl	31.3	23.9	0.76	1.27
H-Br	42.3	33.2	0.78	1.41
H-I	65.8	48.9	0.74	1.61
H-S	23.0	17.2	0.75	1.35

TABLE IV*

SELECTED PROPERTIES OF SOME GROUPS AND MOLECULES

Molecule or Group	α ($\times 10^{-24}$ cm^3)	I ($\times 10^{-11}$ erg)	ρ (gr/cc)	M	$n^2 = \epsilon$
$-CH_2-$	1.94 (a) 1.80 (b)	1.63 (c)	----	14	---
Ethane	4.54 (a)	1.87 [142]	----	30	---
n-Butane	8.42 (a)	1.70 [142]	----	58	---
n-Hexane	12.3 (a)	1.63 [142]	0.66 [150]	86	1.89 [150]
Polyethylene (High Density)	1.80 (b)	1.63 (c)	0.96 [149]	14	2.37 [150] **
Polypropylene	5.36 (b)	1.62 (d) [142]	0.90 [149]	42	2.22 [149]
Polystyrene	13.4 (b)	1.36 (e) [147]	1.05 [136]	104	2.54 [149] 2.46 (e)
Polytetrafluorethylene (PTFE)	2.00 (b, f)	2.4 (f) [148]	2.15 [149]	50	1.82 [149]
Water	1.48 [74]	2.02 [141]	1.0	18	1.77 (c)

* Parentheses refer to footnotes below

** 2.31 in α, β and 2.50 in γ crystallographic directions. [136]

a. Calculated from bond polarizabilty data (Table III and equation (15b).

b. Calculated from equation (14b).

c. Model compound: n-decane.

d. Model Compound: 3-methyl pentane.

e. Model compound: toluene.

f. Model compound: $CF_3(CF_2)_3CF_3$.

B. The Adsorption Model

1. Conceptual:

a. The Adsorption Force

The brief discussion of adsorption presented in the last chapter showed that, although many generalizations and thermodynamic treatments have been given, a general mechanistic model of adsorption is not available. The purpose of this chapter is to deduce such a model and then to place it on a physical foundation by considering intermolecular interactions. The general treatment must consider solute-water, water-water, water-surface, and solute-surface interactions. All of the discussions, derivations, and calculations will be for water as a solvent. The treatment can easily be generalized to consider other pure solvents if the appropriate physical constants are known.

The simplest example of adsorption from aqueous solution is the water/air interface. Figure 6a shows the forces on an apolar solute molecule in bulk water and the forces on an equivalent volume of water (the excluded volume). The superscript s indicates forces or energies due to interactions with the solute molecule, while the superscript w indicates interactions with the equivalent volumes of water. The subscript u (up) indicates that the interaction is with the finite water slab between the volume and the interface, while d (down) indicates that the interaction is with the infinite volume of bulk water. These notations

are merely for convenience. All of the forces are attractive, but a sign convention must be set up in order to consider directions and determine the net forces or energies. Thus, interactions in the direction toward the interface are negative, while those in the opposite direction are taken as positive.

The solute water forces are due to dispersion interactions only if the solute is a saturated hydrocarbon. The water-water forces are due to both dispersion and dipole-dipole (hydrogen-bonding) effects. In the vicinity of the surface (Figure 6b), there is an asymmetry of forces, since the finite slab of water cannot interact as strongly with both volumes as the infinite bulk water. If both volumes are on the surface (Figure 6c), there is only one force acting on each volume. This is the force responsible for the surface tension. The net force ($F_n = F_d - F_u$) or energy ($E_n = E_d - E_u$) due to water interactions is thus a maximum at the surface and rapidly approaches zero with increasing distance from the surface. A hypothetical plot of the energies as a function of distance is given in Figure 7a for water (the center curve), for a low energy solute (the lower curve), and for a high energy solute (the upper curve).

Consider a solute volume at a distance x_2 from the surface (Figure 7b) and an equivalent volume of water directly above it at a distance x_1 from the surface. Examine Figure 7a to see what energies are required to move the solute from x_2 to x_1 and simultaneously move the water volume

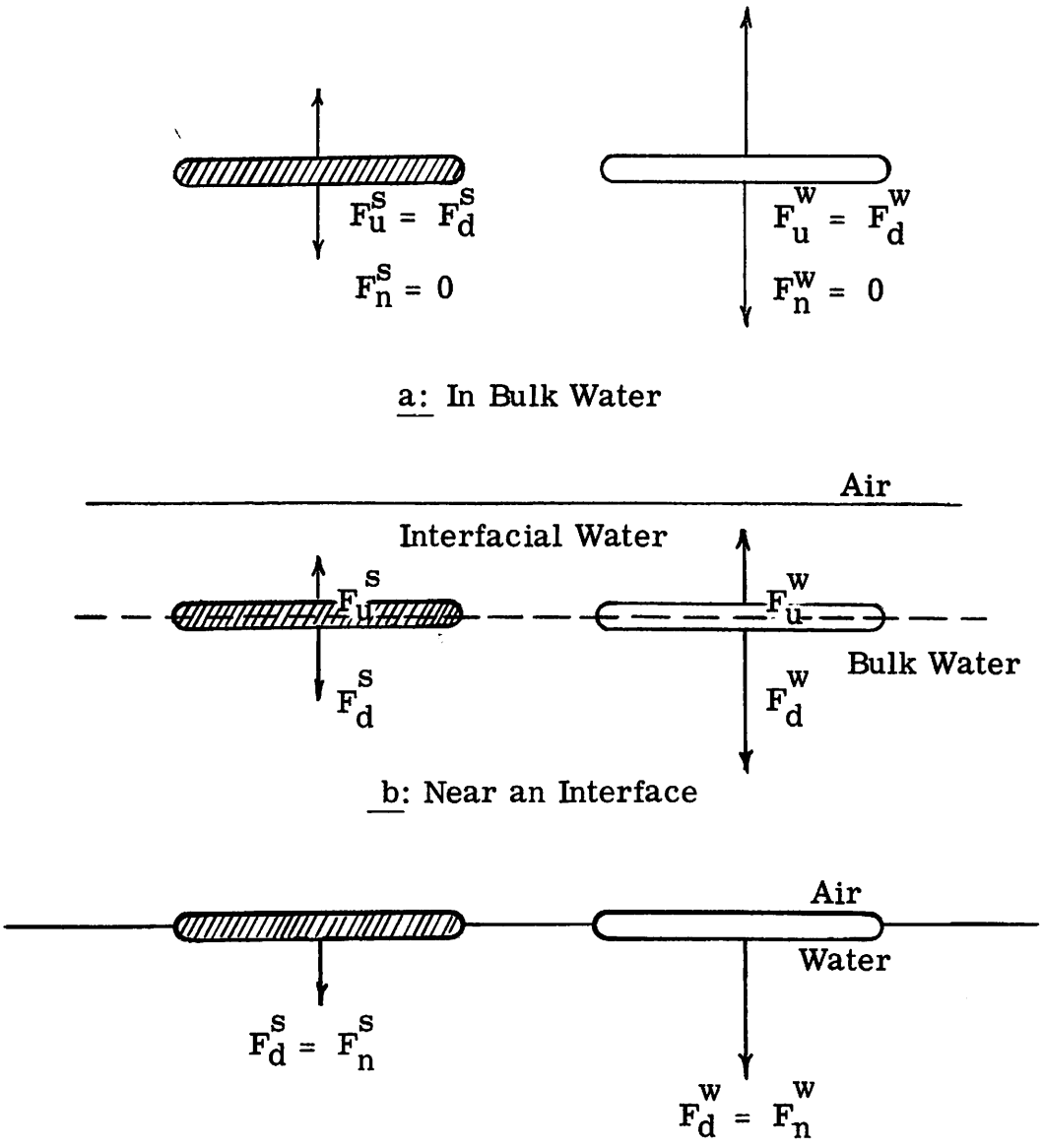
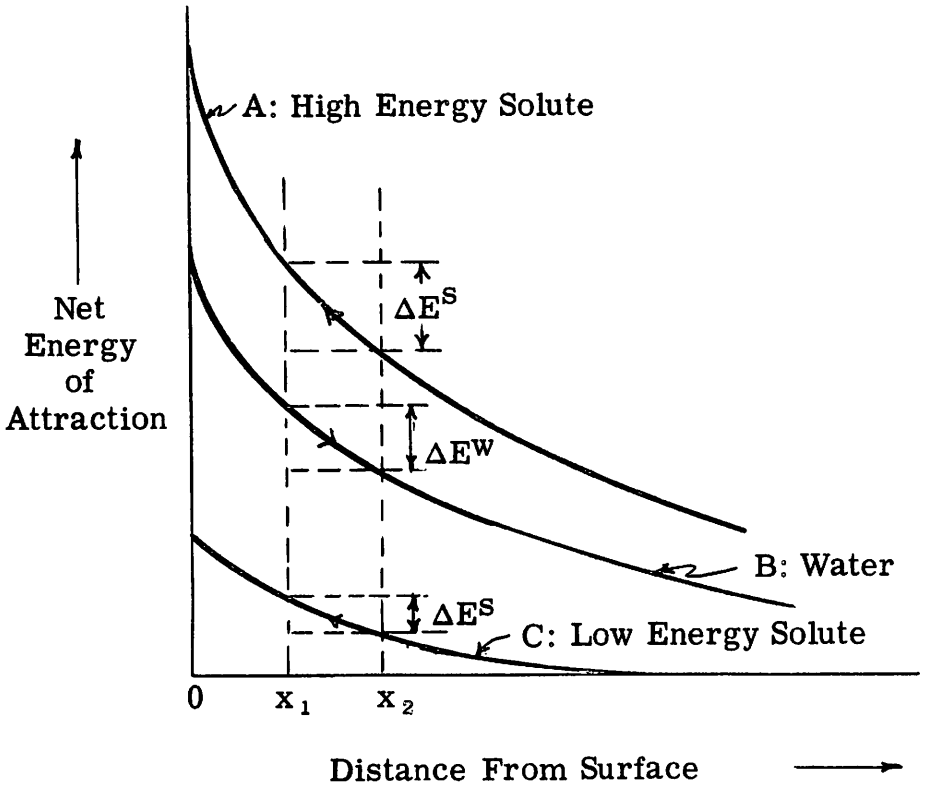
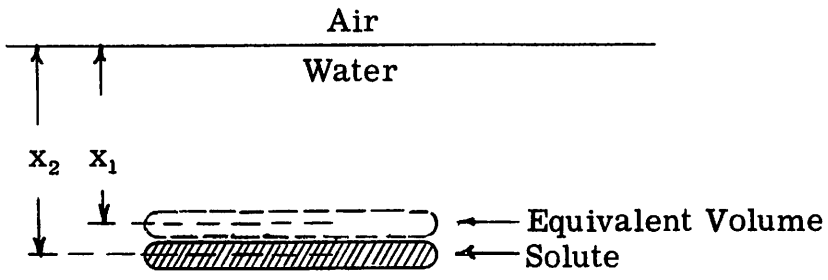


Figure 6. The Forces on a Solute Molecule and on the Equivalent Volume of Water Under Different Conditions: a. In Bulk Water; b. In the Vicinity of a Water/Air Interface; c. At the Water/Air Interface. The cross-hatched volume represents the solute molecule; the uncrosshatched volume is the equivalent volume of water. The notation is defined in the text.



a.



b.

Figure 7. a: Hypothetical Energy-Distance Curves for a High-Energy Solute, a Low-Energy Solute, and for an Equivalent Volume of Water.
b: The geometry corresponding to the arrows in a (see text).

from x_1 to x_2. These processes are indicated by the arrows in Figure 7a. To move the solute towards the interface required an expenditure of energy, ΔE^S, while the movement of the equivalent volume of water generates an amount of energy, ΔE^W. Clearly, $| \Delta E^W | > | \Delta E^S |$, and the process is favorable. The net energy difference can be considered as the driving force for adsorption. The result can be viewed as a virtual force acting on the system:

$$F^{virtual} \equiv \frac{| \Delta E^S | - | \Delta E^W |}{\Delta x} \equiv F^{ads} \quad . \qquad (21)$$

The virtual force is the force responsible for adsorption at the water/air interface. If $| \Delta E^S | > | \Delta E^W |$, then $F^{ads} > 0$ and adsorption cannot occur (curves A and B in Figure 7a). This is the case of negative adsorption, that is, there will be a deficiency of solute at the surface. If $| \Delta E^S | < | \Delta E^W |$, then $F^{ads} < 0$, and adsorption does occur (curves B and C in Figure 7a). These conclusions are in agreement with the results of Figure 4, discussed earlier. The same analysis will hold for any solvent if curve B is shifted appropriately. The high cohesive energy of water leads to greater adsorption tendencies than most common solvents.

The adsorption force is the difference between the net force on the solute and on the equivalent volume of water. It can be calculated by the expression

$$F^{ads} = | F_n{}^S | - | F_n{}^W |, \text{ where } F_n = F_d - F_u . \qquad (22)$$

If the solute molecule is apolar, as for a saturated hydrocarbon, then the solute-water interaction (F_n^S) can only be due to dispersion forces,[73] as dipole-induced dipole forces are negligible.[74] The dispersion force can be easily calculated with the London expression derived earlier; this will be done later. The calculation of the water-water interaction is less straight-forward, as there are large dipole-dipole as well as dispersion interactions. The calculation of dipole-dipole interactions requires that either the exact orientation be known, equations (2) and (3), or that random orientation can be assumed, equation (6) or (17). As the dipole-dipole interactions in water are certainly not random, and as the exact orientations are not known, the dipole-dipole interactions cannot be exactly calculated. They can, however, be estimated to a reasonable degree of accuracy using Fowkes' data.[73] The total surface energy of water at 20 C is about 73 ergs/cm^2 ; Fowkes has shown[73] that the dispersion force component of the surface energy of water is about 22 ergs/cm^2. Thus, the surface energy of water is to a good approximation equal to 3.3 times the dispersion energy. The force of adsorption at water/air interfaces is given by equation (22) where F_n is assumed to be 3.3 times the dispersion force.

The following assumptions are, therefore, important to the semi-quantitative development of the model which will follow:

1. The solute molecule can only interact with water by dispersion forces.

2. The total equivalent volume interaction is 3.3 times its dispersion interaction.

3. The polymer surface can only interact with water and with the solute molecule by dispersion interactions.

The first assumption limits the quantitative development to hydrocarbons, though polar and charged molecules will be discussed qualitatively.

The second assumption is only valid for water. In apolar solvents, the 3.3 term would net exist and F_n^w would be roughly of the same magnitude as F_n^s; adsorption tendencies would therefore be much lower than in a cohesive solvent.

The third assumption limits the analysis to apolar surfaces. If the solute is capable of dipole-dipole interactions, such as an alcohol or carboxylic acid, then F_n^s would be significantly greater and the net force of adsorption would be decreased. A polar compound would also be much more easily desorbed, as occasionally a cluster could form which might optimize the dipole-dipole interactions and return the molecule to the solution.

The case of a polymer/water interface is only slightly more complicated and is shown in Figure 8. In this case the polymer interaction forces, F_p^s and F_p^w, will modify the curves in Figure 7a. Curve B will start at a lower value, corresponding to the polymer-water interfacial energy; curve A will also be lower and will start at the polymer-solute

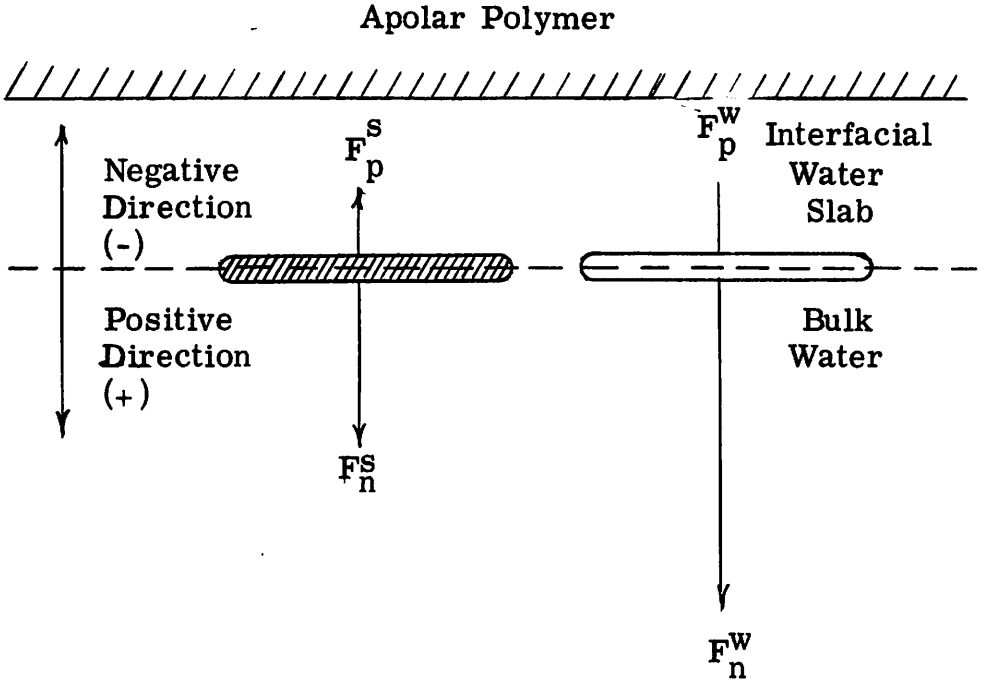


Figure 8. The Forces on a Solute Molecule and on the Equivalent Volume of Water in the Vicinity of a Water/Polymer Interface.

interfacial energy. The slopes will thus be decreased, resulting in lower ΔE values and in a lower value for the adsorption force. For the more general case of a polymer/water interface, the force of adsorption is:

$$F^{ads} = (F_n^s - F_p^s) - (F_n^w - F_p^w) \quad \text{or}$$
$$F^{ads} = (F_n^s - F_n^w) - (F_p^w - F_p^s). \qquad (23)$$

Only apolar polymers will be considered, thus, F_p^s and F_p^w can be calculated by dispersion equations. It is clear that the adsorption force may be lower at polymer/water than at air/water interfaces. If $F_p^s = F_p^w$, then the interactions with the polymer cancel out, and the adsorption

tendency is the same for air/water and polymer/water interfaces. If F_p^s is less than F_p^w, then water/polymer interactions are greater than solute/polymer interactions, and solute adsorption decreases. If F_p^s is greater than F_p^w, solute adsorption is greater than at the air/water interface.

It is evident that for apolar solutes and apolar polymers adsorption can not be prevented, though it can be maximized and minimized. This conclusion will be demonstrated later in the chapter when the calculations are performed and the results plotted. It will also be seen that different orientations of a solute molecule with respect to the interface will lead to very different adsorption tendencies, thus conclusions as to orientation of adsorbed species will be made. The orientation of the chains in the polymer surface, i.e., the crystallinity of the polymer surface, will also be discussed in relation to its effect on adsorption.

The above discussion provides a mechanism for adsorption from aqueous solution which is compatible with thermodynamic considerations and which will be used to explain several previously unexplainable phenomena in surface science.

In summary,

$$F^{ads} = (F_n^s - F_n^w) + (F_p^w - F_p^s), \qquad (24)$$

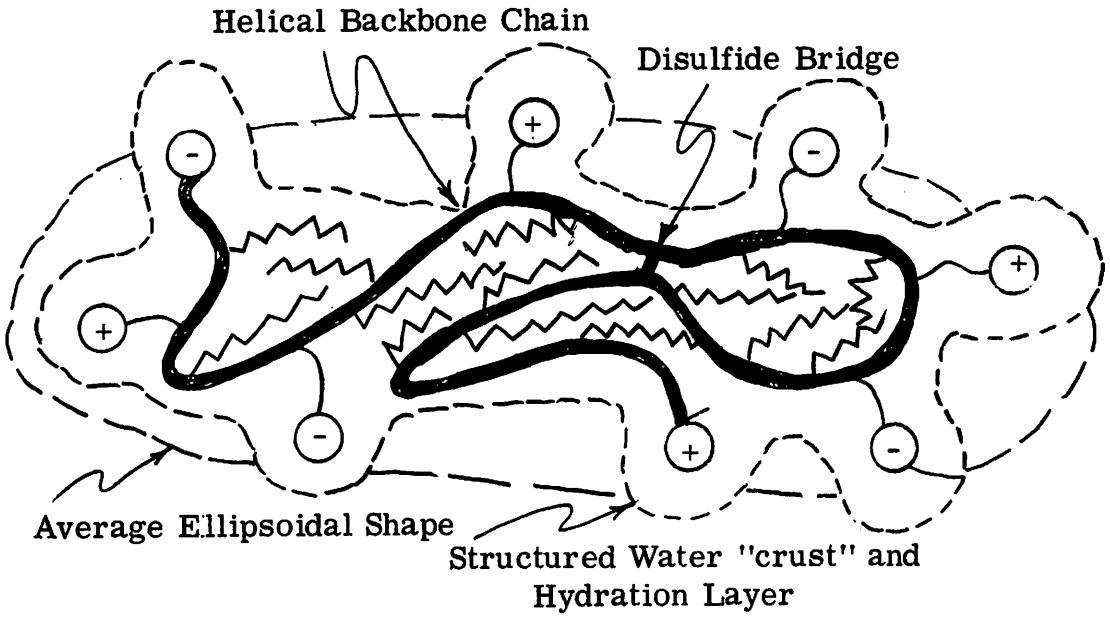
when F^{ads} is less than 0, adsorption occurs; when F^{ads} is greater than 0, negative adsorption occurs, i.e., a surface deficiency is produced. Forces are negative if they point towards the interface and positive if

they point away from the interface. The analogous expressions for the energy are straight-forward:

$$U^{ads} = (U_n^s - U_n^w) + (U_p^w - U_p^s). \qquad (25)$$

b. Protein Adsorption

Consider the hypothetical two-dimensional, ellipsoidal protein of Figure 9. Assume it has no net charge at pH 7; this is the case for some gamma globulins.[63] What happens when such a molecule approaches an interface? If the interface is charged or relatively polar, F_p^s can be treated in terms of electrical double layer theory,[4] at least for relatively large separations. At close separations the surface cannot see a net protein charge, but will feel the local charge and dipole distributions on the protein. If a protein has a locally charged region somewhere on its surface, which is not unreasonable as many proteins have large dipole moments;[133] then some of the proteins could be expected to adsorb in the manner sketched in Figure 10. A protein with a net negative or positive charge might be capable of adsorbing on both negatively and positively charged surfaces, if it contained a negatively charged region at one end of the molecule and a positive center at the other end. Albumin, which is highly negatively charged at pH 7, is known to neutralize both positive and negative ion-exchange resins by adsorption.[151] At large distances the net charges on the protein and the surface would probably interact, and,



Indicates Hydrophobic (Apolar) Side Chains

Indicates Negatively Charged Groups (COO^-)

Indicates Positively Charged Groups (NH_3^+)

Figure 9. A Hypothetical Two-Dimensional Protein With a Uniform Charge Distribution.

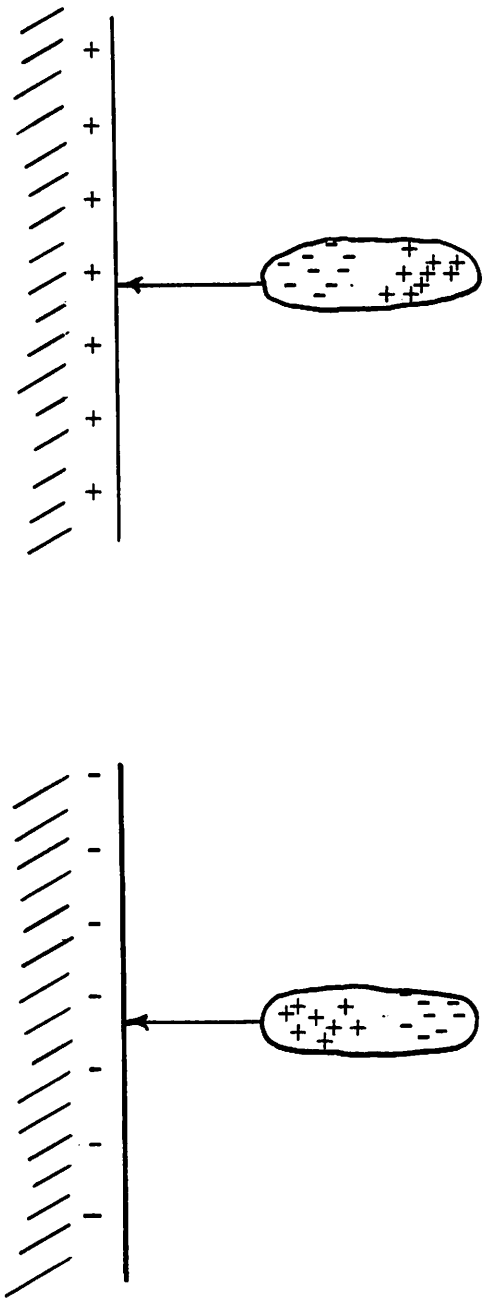


Figure 10. Neutral Proteins with Localized Charge Distributions Interacting with Charged of Highly Polar Surfaces.

if the interaction is strong enough, it could simply overwhelm the other interactions present.* Thus, the observations of Sawyer and his group[152] with metals are not necessarily in conflict with the negative surface charge concept voiced by Margolis.[100] Just as one cannot think of water as having a definite structure, one cannot naively assume that proteins can adsorb by only one mechanism. A protein is a macro molecule with a macroscopic surface of its own. It contains hydrophobic groups, dipoles, and charges, no doubt distributed and concentrated in various parts of the molecule (this will be shown later for ribonuclease), giving the particular protein its own unique properties. Thus, rather than expect all proteins to have the same adsorption properties, they should all be expected to adsorb differently, and to adsorb by different mechanisms on different surfaces.

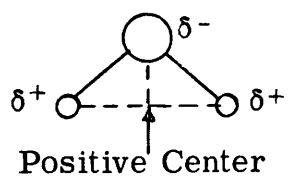
Electrostatic interactions may occur even if the surface is not polarized or charged. As a highly charged molecule such as albumin (net charge about -50 at pH 7) or fibrinogen (about -300 at pH 7) approaches a polymer surface, it can induce dipoles on the polymer surface; thus attraction is almost inevitable at small separation distances.

At large separations there is probably no protein-surface inter-

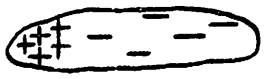
*This concept was suggested by Dr. James Bougas of Boston University in a lecture given to the Denver Conference on Biomedical Materials, Denver, Colorado, July, 1968.

action (F_p^s) unless the surface has a relatively high charge density. If it has a high charge density, then it will probably interact with the net charge on the protein, producing a repulsion or attraction. At nearer distances, the surface and proteins will interact by their own specific charge distributions. Proteins oriented so that attraction is maximized will tend to be adsorbed, while those oriented differently will not, though they, too, will eventually be adsorbed as electrical torques and thermal vibrations bring them into the necessary orientation. When the molecule is close to the polymer surface (of the order of 10 or 100 A), the interaction is due to dispersion forces as well. Finally, there may be dipoles induced in the polymer by charges on the protein, resulting in a net electrostatic interaction. Because of the increase in static dielectric constant with distance (to be discussed later), induced dipole effects will only be significant at very close separations (up to 5 A). These effects are aided by the ever-present adsorption force due to the cohesion forces of the water molecules themselves (equation (22)).

Figure 10 showed the proteins as having both negative and positive regions; this may be misleading. Such separations probably occur with some proteins, but they are not necessary to explain adsorption or dipole moments. Consider the water molecule:



The positive center of the dipole interacts less strongly than the negative seat as it is less concentrated. The same may be true in a protein; the protein below is neutral, yet it will interact much more efficiently with a negatively charged surface than with a positively charged one:



If these concepts of protein adsorption are accepted as valid, then the experimental results on many different types of surfaces begin to make some sense. If a surface is placed in blood, with its many protein constituents, it makes relatively little difference what its surface characteristics are (unless it has a gross charge on it), because sooner or later it will find proteins or other molecules with which in can interact. It is, therefore, no surprise that the zeta potential of most surfaces goes to zero when placed in contact with blood.[153]

It is also clear that adsorption need not stop after a monolayer is formed, though it will be shown later why this is often the case. The actual behavior will again depend on the surface-solute interactions, on the nature of the adsorbed layer, and on its interaction with both solute and solvent. A surface composed of an adsorbed monolayer may also have characteristic properties which could produce additional interactions, as so beautifully demonstrated by Vroman's model of coagulation as a series of hydrophilic-hydrophilic and hydrophobic-hydrophobic bonds.[1,99] If one molecule (A) has a greater interaction for a particular

surface than another (B), it is conceivable that A may displace B from the surface. Such competitive adsorption is the basis of adsorption chromatography.[154] Some of Vroman's work indicates that such a process may occur with Hageman Factor and adsorbed fibrinogen.[1] It was noted earlier that competitive adsorption occurs with synthetic polymers.

In the development that follows, only polymeric, hydrophobic, low-energy surfaces will be considered. For such surfaces, charge and dipole interactions will not be important, though charge-induced dipole interactions may be important at very close separations. The equations developed earlier, especially the dispersion expressions, will be used in an attempt to predict the magnitude of the forces responsible for adsorption. The expressions will be examined to see what surface characteristics are necessary to minimize those forces.

2. Preliminary Calculations

The various intermolecular forces and energies which may act on a body in the vicinity of an interface will now be estimated. The equations are listed below with the constants evaluated to two significant digits (using the sign convention of page 77):

$$\bar{U}\,(Q - \mu) = 8.0 \times 10^{13}\, Q^2 \mu^2 / (D^2 r^4) \qquad (26a)$$

$$\bar{F}\,(Q - \mu) = 3.2 \times 10^{13}\, Q^2 \mu^2 / (D^2 r^5) \qquad (26b)$$

$$\bar{U}\,(\mu - \mu) = 1.6 \times 10^{13}\, \mu_1^2 \mu_2^2 / (D^2 r^6) \qquad (27a)$$

$$\bar{F}\,(\mu - \mu) = \quad 9.7 \times 10^{13}\,\mu_1^2\mu_2^2/(D^2\,r^7) \qquad (27b)$$

$$U\,(\mu - \mu) = \quad 2.0\,\mu_1\,\mu_2/(D^2r^3) \qquad (28a)$$

for optimum orientation,

$$F\,(\mu - \mu) = \quad 6.0\;\mu_1\,\mu_2/(D^2r^4) \qquad (28b)$$

for optimum orientation,

$$U\,(Q\text{-ind }\mu) = \quad 0.5\,Q^2\alpha/(D^2\,r^4) \qquad (29a)$$

$$F\,(Q\text{-ind }\mu) = \quad 2.0\,Q^2\alpha/(D^2\,r^5) \qquad (29b)$$

$$U\,(d) = \quad 2.3\,A_0/r^6 \qquad (30a)$$

$$F(d) = \quad 14\,A_0/\,r^7,$$

where

$$A_0 = \frac{\alpha_1\alpha_2 I_1 I_2}{(I_1 + I_2)} \quad .$$

These equations yield the force in dynes and the energy in ergs when Q is expressed in esu units (statcoulombs), r in centimeters, I in ergs, α in cm^3, and μ in esu–cm. To get an approximate idea of the magnitudes involved, some values will be put in the equations (see Table IV):

Q = electron charge $\simeq 4.8 \times 10^{-10}$ esu

μ = water dipole moment $\simeq 1.8 \times 10^{-18}$ esu-cm

α = water polarizability $\simeq 1.5 \times 10^{-24}\ cm^3$

I = water ionization energy $\simeq 2.0 \times 10^{-11}$ erg.

Salem estimates[119] that the dielectric constant in water for a five angstrom separation distance is about fifteen. At ten angstroms it is most likely near the full value of 80, possibly 70. If two water molecules are interacting with no other molecules between them, then the dielectric constant is one; if the intervening medium is apolar, then a good approximation is two.[119] The results of the calculations are given in Table V for three different values of r and for the appropriate dielectric constants.

If one sums the $U(\mu-\mu)$ and U(d) energies at r = 3A, one gets a number for the hydrogen bond energy in water of about 5 kcal/mole, which is in agreement with the accepted values of 3.4 to 5.0 kcal/mole.[74] It is also clear that at three angstroms $U(d) \simeq 0.3$ U (total), in excellent agreement with Fowkes' results[73] that dispersion forces account for about 30% of the surface tension of water. It is clear that the dispersion term is very significant at close separations and becomes dominant at larger separations. The dipole-dipole energy will be negligible with respect to the dispersion energy at a distance of five angstroms or more. Therefore, making the good assumption that the total water interaction is about 3.3 times the dispersion interaction, one need only calculate dispersion forces for the case of hydrocarbons interacting with apolar polymers.

The interactions between two water molecules are only important at relatively close separations. At 10 A separations the dispersion

TABLE V

APPROXIMATE CALCULATIONS FOR THE FORCES AND ENERGIES BETWEEN TWO WATER MOLECULES AND BETWEEN WATER AND AN ELECTRON CHARGE

Quantity	r = 3A (D = 1)	r = 5A (D = 15)	r = 10A (D = 70)
$\bar{F}$ (Q - μ)	1.0×10^{-3}	3.6×10^{-7}	5.1×10^{-10}
F (Q-ind μ)	2.8×10^{-5}	9.7×10^{-9}	1.4×10^{-11}
$\bar{F}$ (μ - μ)	5.0×10^{-6}	6.3×10^{-10}	2.2×10^{-13}
F (μ - μ)	2.6×10^{-5}	1.5×10^{-8}	4.3×10^{-11}
optimum F (d)	2.0×10^{-5} *	5.7×10^{-7} *	3.1×10^{-9}
$\bar{U}$ (Q- μ)	7.9×10^{-12}	4.6×10^{-15}	1.3×10^{-17}
U (Q-ind μ)	2.1×10^{-13}	1.2×10^{-16}	3.5×10^{-19}
$\bar{U}$ (μ - μ)	2.5×10^{-14}	5.1×10^{-18}	3.7×10^{-21}
U (μ - μ)	2.5×10^{-13}	2.4×10^{-16}	1.0×10^{-18}
optimum U (d)	1.0×10^{-13} *	4.7×10^{-15} *	5.1×10^{-17}

kT energy is about 4.0×10^{-14} erg.

*These values do not include the 0.7 water medium correction as it is probably unnecessary at these separation distances.

energy is roughly 1/1000kT; such an energy is negligible. Energies (and the equivalent forces) which are of the order of 10^{-15} erg or less will be ignored. An energy 10^{-15} erg is about 2.5% of kT. When the interactions of thousands of groups with a single molecule are considered, the interaction energy becomes significant, even at 10 A and beyond.

3. Derivations and Calculations for Simple Models:

a. A Group Interacting with a Flat Plate

Consider a disc of radius ℓ and cross-sectional area of $d\ell dx$ in the slab of thickness δ (Figure 11a). The volume of the disc is

$$dV = 2\pi \ell \, d\ell dx$$

If R is greater than a few molecular diameters, it is said that the force can be computed by integrating.[124] If R is of the order of molecular dimensions, a summation must be used. Approximate summations are available[70, 155] which greatly simplify the computations. The expressions for both the energy and the force of interaction will now be developed. The starting equations are:

$$\begin{aligned} dU &= 2\pi N \ell \, d\ell dx \, f(r) \text{ and} \\ dF &= 2\pi N \ell \, d\ell dx \, f(r) \cos\theta, \end{aligned} \tag{31}$$

where f(r) is the appropriate interaction term and $\cos\theta$ gives the resultant force in the horizontal direction. For dispersion interactions equation (30) is used, and the appropriate expressions become:

$$U(d) = 4.6\pi\, NA_0 \int_0^{\delta} dx \int_0^{L} \ell\, d\ell \Big/ [(R + x)^2 + \ell^2]^3 \text{ and}$$

$$F(d) = 28\,\pi\; NA_0 \int_0^{L} \ell\, d\ell \int_0^{\delta} (R + x)dx \Big/ [(R + x)^2 + \ell^2]^4 \,. \tag{32}$$

The integrations are straight-forward when taken in the order given above. Letting L go to infinity, the terms in L go to zero; when δ is allowed to go to infinity, the expressions reduce to the simple forms:

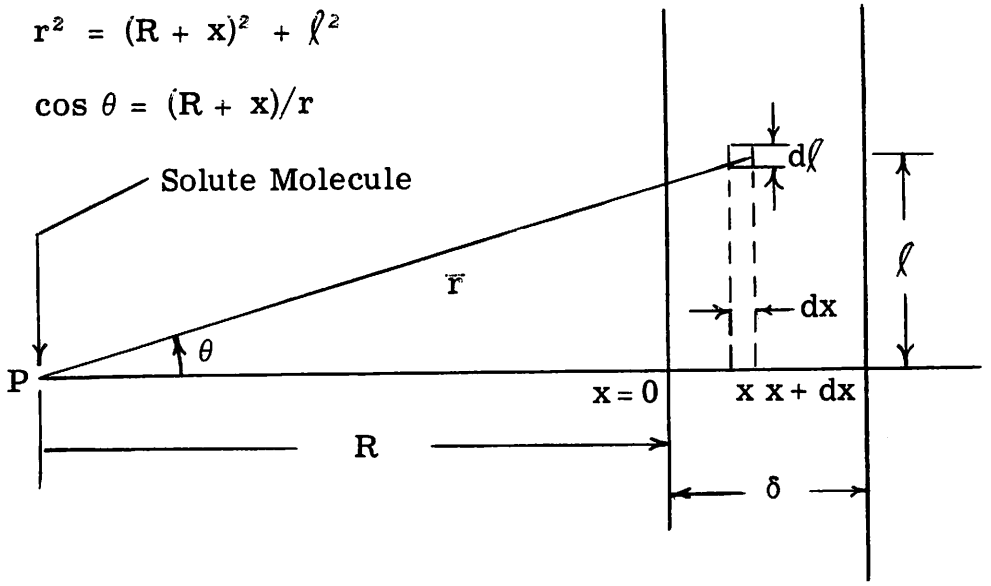
$$U(d) = 1.2\ N\ A_0/R^3 \text{ and}$$

$$F(d) = 3.7\ NA_0/R^4 \,. \tag{33}$$

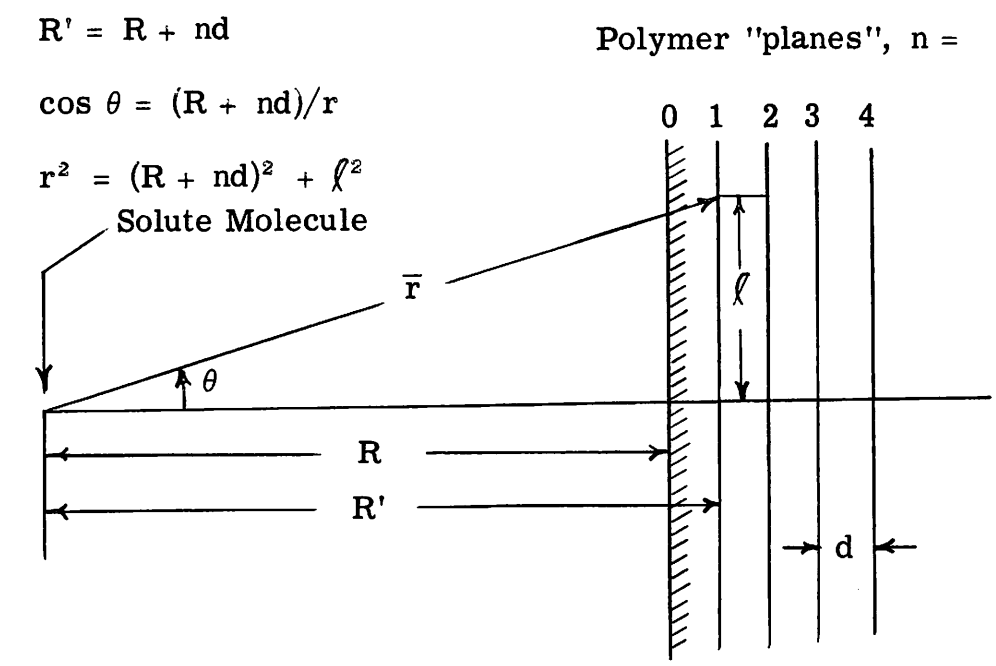
If the molecule is 10 A or farther from the surface, one may be justified in using equations (33) but for closer distances the triple integration is not valid and Crowell's summation method[155] will be used. It is assumed that the polymer units comprising the slab of Figure 11b are uniformly distributed on parallel planes a distance d apart. An integration over each plane is performed, and then a summation over the various planes. This technique has been used by Fowkes.[70] This means that the integration over x in equation (32) is replaced by a summation. The geometry is given in Figure 11b. The set-up and integrations are again straight-forward, giving

$$F(d) = 14.5\sigma A_0 \sum_{n=0}^{m} 1/(R + nd)^5 \text{ and} \tag{34a}$$

$$U(d) = 3.6\sigma A_0 \sum_{n=0}^{m} 1/(R + nd)^4 \,, \tag{34b}$$



a.



b.

Figure 11. The Geometries for a Molecule Interacting With a Flat Plate. a. For a Triple Integration; b. for Crowell's[155] summation method.

where σ is the number of groups per cm^2. The planar separation distance, d, is given as the cube edge of the average volume occupied by a group:

$$d = (M/N_0 \rho)^{0.33} = 1.18(M/\rho)^{0.33} \text{ A}, \qquad (35)$$

where M is the molecular weight and ρ is the density of the polymer. Values of d for several polymers and for water are given in Table VII.

Equations (33) and (34) were programmed and evaluated on the Burroughs 5500 computer at the University of Denver. The results (Table VI) clearly show that there is a significant difference in the forces calculated by equations (33) and (34), even when R is as large as 30 A. The same program also indicated that one need not consider more than ten polymer planes in the summation. In fact, considering only four to six planes is sufficient for an accuracy of three significant digits. It is also clear that forces beyond ten angstroms are probably not significant, as the interaction would be overwhelmed by kT energy.

Equation (34) is sufficient to calculate dispersion interactions between a molecule and a medium which can be approximated as flat planes. If only dispersion forces can act, then all the terms in equation (23) can be calculated using the assumption that F_n^W is about 3.3 times the dispersion interaction.

TABLE VI

THE DISPERSION FORCES CALCULATED BY INTEGRATION AND BY SUMMATION FOR A SINGLE WATER MOLECULE INTERACTING WITH A HIGH DENSITY POLYETHYLENE SURFACE

Force by Integration (Equation 33)	Force by Summation (Equation 34 for m = 5)	R, A
3.91×10^{-6}	15.4×10^{-6}	3.1
2.45×10^{-7}	5.57×10^{-7}	6.2
4.83×10^{-8}	8.65×10^{-8}	9.3
1.53×10^{-8}	2.39×10^{-8}	12.4
6.26×10^{-9}	8.98×10^{-9}	15.5
3.02×10^{-9}	4.07×10^{-9}	18.6
1.63×10^{-9}	2.09×10^{-9}	21.7
9.55×10^{-10}	1.18×10^{-9}	24.8
5.96×10^{-10}	7.08×10^{-10}	27.9
3.91×10^{-10}	4.50×10^{-10}	31.0
2.67×10^{-10}	2.99×10^{-10}	34.1

TABLE VII

DATA FOR THE EVALUATION OF

DISPERSION INTERACTIONS

Substance	α*	I**	d(A)	σ***
$-CH_2-$	1.80	1.63		
n-Ethane	4.54	1.875		
n-Butane	8.42	1.70		
n-Hexane	12.3	1.63		
Polyethylene (density = 0.96)	1.80	1.63	2.88	11.93
Polystyrene	13.4	1.36	5.45	3.33
Polytetrafluoroethylene	2.00	2.42	3.36	8.75
Polypropylene	5.36	1.62	4.24	5.50
Water	1.48	2.02	3.10	10.3

* $\times 10^{-24}$ cm^3.

** $\times 10^{-11}$ erg.

*** $\times 10^{14}$ Molecules/cm^2.

b. A Group Interacting with Its Environment Near a Flat Plate

Consider a volume of solute of water at Point P surrounded by water (Figure 12); the situation corresponds to Figure 6b. The force due to the infinite slab of water is F_d; the force due to the finite slab or water is F_u; and the force due to the infinite polymer slab is F_p. The water in the plane containing the molecule is ignored, as it produces an interaction which cancels out.

Using the sign conventions of Figure 8 and of equation (24) and the geometry of Figure 12, the expressions for F_u, F_d, and F_n become:

$$F_u = 14.5\ \sigma_w A_0 \sum_{m_1=1}^{m_1} (3.1m_1)^{-5}$$

$$F_d = 14.5\ \sigma_w A_0 \sum_{m=1}^{m} (3.1m)^{-5}$$

$$F_n = F_d - F_u = 14.5\ \sigma_w A_0 \sum_{m=m_1+1}^{m_1+5} (3.1m)^{-5}; \qquad (35)$$

while the expression for the polymer interaction is:

$$F_p = 14.5\ \sigma_p A_0 \sum_{n=}^{5} (R + nd)^{-5} \qquad (36)$$

where $R = 3.1\ (m_1 + 1)$.

The analogous expressions for the energies are:

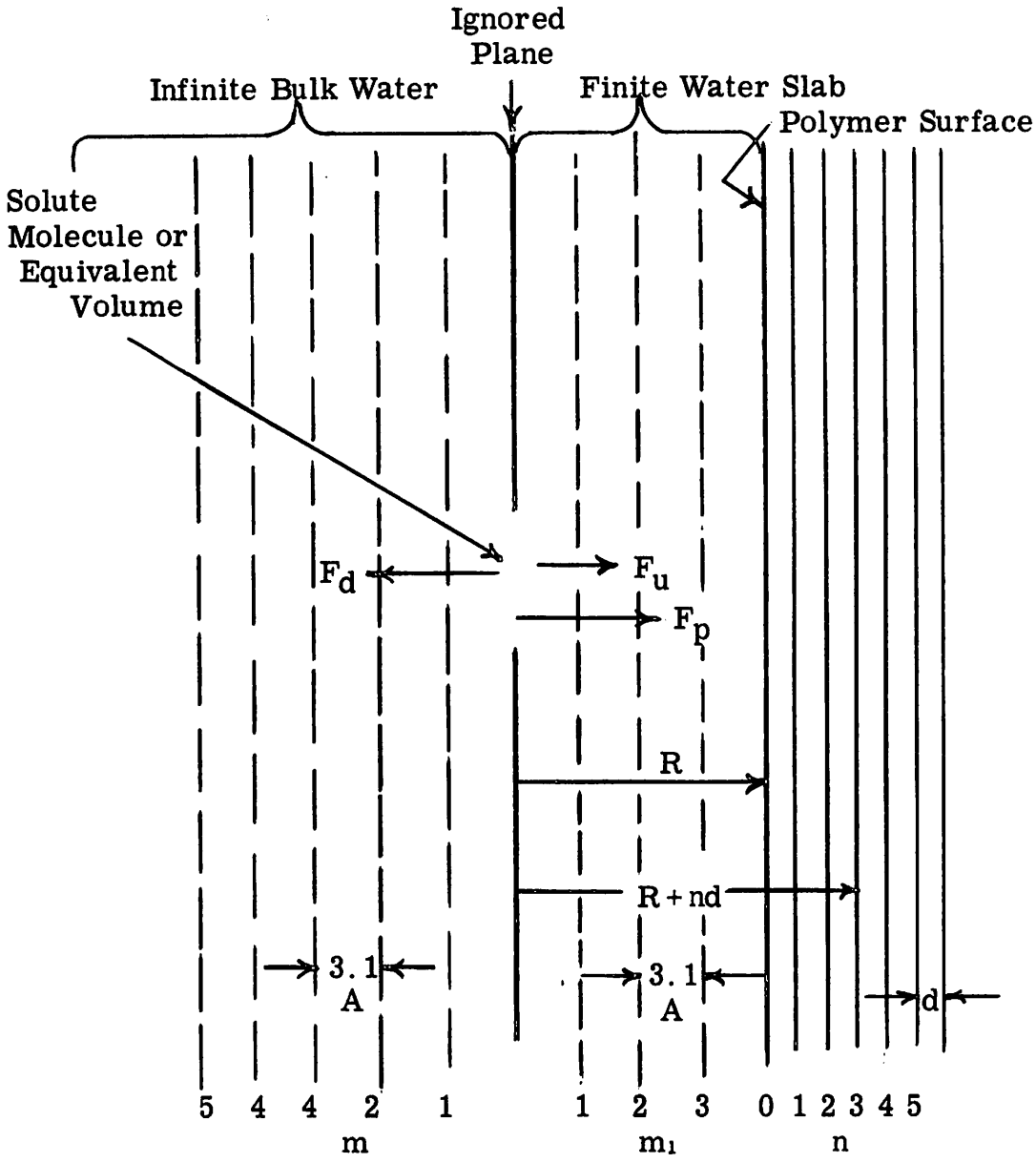


Figure 12. A Molecule Interacting with a Polymer Surface Through a Water Slab. See Figure 6 for Force Notations. The Separation of the Water Planes is 3.1 A (equation 35).

$$U_n = 3.6\,\sigma_w\,A_0 \sum_{m=m_1+1}^{m_1+5} (3.1\,m)^{-4} \quad \text{and} \tag{37}$$

$$U_p = 3.6\,\sigma_p\,A_0 \sum_{n=0}^{5} (R + nd)^{-4}\,. \tag{38}$$

The total force of adsorption is given by equation (24). Equation (35) will give both F_n^s and F_n^w; the only difference is that A_0 terms are different and that the expression for F_n^w must be multipled by 3.3. Equation (36) will give both F_p^w and F_p^s if the appropriate A_0 terms are used.

Though a $-CH_2-$ group and a water molecule are about the same size,[73] they do not occupy the same volume in solution. Water is a relatively loosely packed structure wherein each water molecule occupies a volume of about 30 A^3. If one examines scale molecular models of chains of $-CH_2-$ groups and allows for some vibration and rotation of the groups, the volume occupied by a $-CH_2-$ in an extended chain is about 20 A^3. If a long chain assumes a globular compact structure, the volume per $-CH_2-$ is closer to 30 A^3. The molecules to be considered here are all relatively small or short chains, thus the extended chain arrangement is more reasonable. The assumption is therefore made that a $-CH_2-$ group occupies about two-thirds the volume of a water molecule. Thus, the equivalent volume of water contains two-thirds the number of molecules contained in the solute volume. This can be

accounted for in the equations previously derived by incorporating an 0.67 X term in the A_0 expressions for excluded volume interactions, where X is the number of $-CH_2-$ groups in the solute molecule. Incorporating this correction, placing equations (35) and (36) in equation (24), and consolidating terms, the results are:

$$(F_n^S - F_n^W) = 14.5\,\sigma_W\,(A2 - 3.3\,A1)\sum_{m=m_1+1}^{m_1+5}(3.1m)^{-5} \qquad (39)$$

$$(F_p^W - F_p^S) = 14.5\;\sigma_p\,(A4 - A3)\sum_{n=0}^{5}(R + nd)^{-5} \qquad (40)$$

where A1 ≡ 0.67 X A_0, for water-water interactions;

A2 ≡ A_0, for water-solute interactions;

A3 ≡ A_0, for polymer-solute interactions;

A4 ≡ 0.67 X A_0, for polymer-water interactions;

$$A_0 \equiv \frac{\alpha_1 \alpha_2 I_1 I_2}{(I_1 + I_2)}\text{; and}$$

X = is the number of $-CH_2-$ groups in the solute. Values for α, I, σ, and d were given in Table VII. The sum of equations (39) and (40) gives the total force of adsorption.

Equations (39) and (40) and the analogous expression for the energies of adsorption were programmed and evaluated for the interaction

of a $-CH_2-$ group, ethane, butane, and hexane with some common polymers. The data for the $-CH_2-$ group are given in Table VIII and are plotted in Figure 13. The adsorption force in the absence of a polymer surface is given by equation (39) and is indicated in Figure 13. The adsorption forces due solely to the presence of the polymer phase are calculated by equation (40); the four lower curves in Figure 13 represent the various polymer contributions, decreasing in this order: polystyrene, polypropylene, polyethylene, and PTFE. The interaction roughly follows the trend in the values of the dispersion force component of the surface tension,[73] as expected. The total force of adsorption (equation 24) is given for the PTFE and polystyrene cases in Figure 13. These are the two upper curves in the figure and essentially give the range of interactions, as the curves for the other polymers fall between the two given. It is thus clear that for hydrophobic polymers, the difference in interactions between different polymers surfaces is minimal and never exceeds a spread of 7 to 10%.

The data for ethane, butane, and hexane are given in Tables IX, X, and XI, respectively. The trends are the same as for the $-CH_2-$ case. It is interesting to compare the interactions of the three solute molecules with the same polymer. This is done in Figure 14, where the net force of adsorption between all three alkanes and high density polyethylene is plotted. The differences between the three molecules is quite significant and roughly related to the size of the molecule.

TABLE VIII

THE ADSORPTION FORCES* AND ENERGIES*
BETWEEN A $-CH_2-$ GROUP AND SOME COMMON POLYMERS

R.A.	Water Force $F_n^S - F_n^W$	Water Energy $U_n^S - U_n^W$
3.1	$1.35\ 10^{-5}$	$1.08\ 10^{-13}$
6.2	$4.78\ 10^{-7}$	$8.11\ 10^{-15}$
9.3	$7.27\ 10^{-8}$	$1.90\ 10^{-15}$
12.4	$1.97\ 10^{-8}$	$6.94\ 10^{-16}$
15.5	$7.22\ 10^{-9}$	$3.18\ 10^{-16}$
18.6	$3.19\ 10^{-9}$	$1.68\ 10^{-16}$

R.A.	$F_p^W - F_p^S$	F^{ads}	$U_p^W - U_p^S$	U^{ads}
	High Density Polyethylene			
3.1	$0.65\ 10^{-5}$	$2.00\ 10^{-5}$	$0.53\ 10^{-13}$	$1.61\ 10^{-13}$
6.2	$2.36\ 10^{-7}$	$7.14\ 10^{-7}$	$4.04\ 10^{-15}$	$1.22\ 10^{-14}$
9.3	$3.64\ 10^{-8}$	$1.09\ 10^{-7}$	$0.96\ 10^{-15}$	$2.87\ 10^{-15}$
12.4	$1.00\ 10^{-8}$	$2.97\ 10^{-7}$	$3.56\ 10^{-16}$	$1.05\ 10^{-15}$
15.5	$3.69\ 10^{-9}$	$1.09\ 10^{-8}$	$1.65\ 10^{-16}$	$4.83\ 10^{-16}$
18.6	$1.64\ 10^{-9}$	$4.83\ 10^{-8}$	$0.88\ 10^{-16}$	$2.56\ 10^{-16}$
	Polystyrene			
3.1	$1.21\ 10^{-5}$	$2.56\ 10^{-5}$	$0.94\ 10^{-13}$	$2.02\ 10^{-13}$
6.2	$3.94\ 10^{-7}$	$8.74\ 10^{-7}$	$6.39\ 10^{-15}$	$1.45\ 10^{-14}$
9.3	$5.58\ 10^{-8}$	$1.29\ 10^{-7}$	$1.41\ 10^{-15}$	$3.31\ 10^{-15}$
12.4	$1.44\ 10^{-8}$	$3.41\ 10^{-8}$	$4.96\ 10^{-16}$	$1.19\ 10^{-15}$
15.5	$5.14\ 10^{-9}$	$1.24\ 10^{-8}$	$2.25\ 10^{-16}$	$5.43\ 10^{-16}$
18.6	$2.24\ 10^{-9}$	$5.43\ 10^{-9}$	$1.19\ 10^{-16}$	$2.87\ 10^{-16}$
	Polytetrafluoroethylene (PTFE)			
3.1	$0.61\ 10^{-5}$	$1.95\ 10^{-5}$	$0.48\ 10^{-13}$	$1.56\ 10^{-13}$
0.2	$2.12\ 10^{-7}$	$6.90\ 10^{-7}$	$3.59\ 10^{-15}$	$1.17\ 10^{-14}$
9.3	$3.21\ 10^{-8}$	$1.05\ 10^{-7}$	$0.84\ 10^{-15}$	$2.75\ 10^{-15}$
12.4	$0.87\ 10^{-8}$	$2.83\ 10^{-8}$	$3.08\ 10^{-16}$	$1.00\ 10^{-15}$
15.5	$3.19\ 10^{-9}$	$1.04\ 10^{-8}$	$1.42\ 10^{-16}$	$4.61\ 10^{-16}$
18.6	$1.42\ 10^{-9}$	$4.61\ 10^{-9}$	$0.76\ 10^{-16}$	$2.45\ 10^{-16}$
	Polypropylene			
3.1	$0.87\ 10^{-5}$	$2.22\ 10^{-5}$	$0.69\ 10^{-13}$	$1.77\ 10^{-13}$
6.2	$2.93\ 10^{-7}$	$7.71\ 10^{-7}$	$4.85\ 10^{-15}$	$1.30\ 10^{-14}$
9.3	$4.28\ 10^{-8}$	$1.16\ 10^{-7}$	$1.10\ 10^{-15}$	$3.01\ 10^{-15}$
12.4	$1.13\ 10^{-8}$	$3.10\ 10^{-8}$	$3.97\ 10^{-16}$	$1.09\ 10^{-15}$
15.5	$4.11\ 10^{-9}$	$1.13\ 10^{-8}$	$1.82\ 10^{-16}$	$5.01\ 10^{-16}$
18.6	$1.81\ 10^{-9}$	$5.00\ 10^{-9}$	$0.97\ 10^{-16}$	$2.66\ 10^{-16}$

*All forces and energies are negative, indicating adsorption.

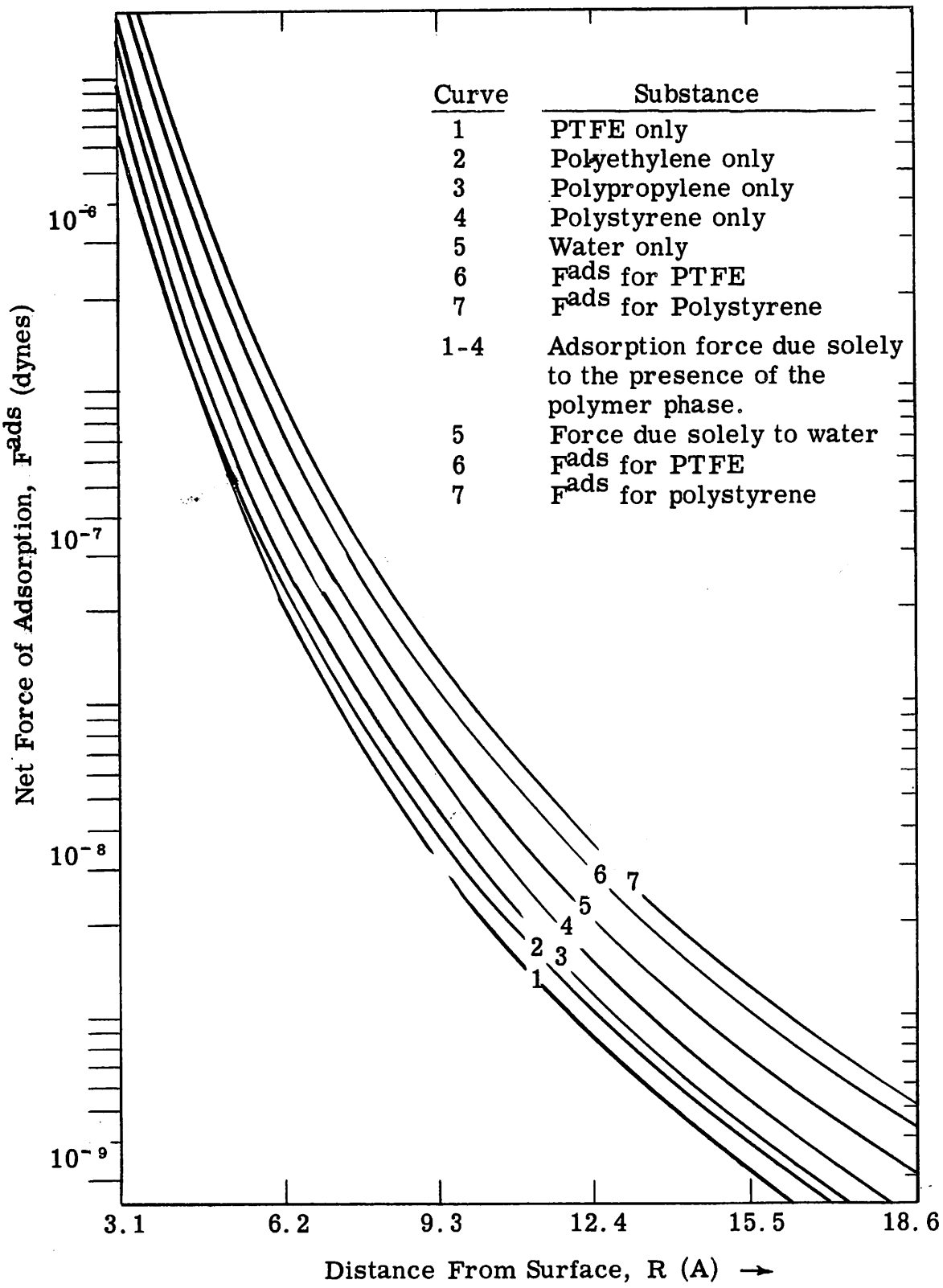


Figure 13. The Adsorption Forces Between a $-CH_2-$ Group and Some Common Polymers (see Text and Table VIII).

TABLE IX

THE ADSORPTION FORCES* AND ENERGIES* BETWEEN AN ETHANE MOLECULE AND SOME COMMON POLYMERS

R. A.	Water Force, $F_n^s - F_n^w$	Water Energy, $U_n^s - U_n^w$
3.1	$1.76\ 10^{-5}$	$1.41\ 10^{-13}$
6.2	$6.26\ 10^{-7}$	$1.06\ 10^{-14}$
9.2	$0.95\ 10^{-7}$	$2.49\ 10^{-15}$
12.4	$2.58\ 10^{-8}$	$0.91\ 10^{-15}$
15.5	$0.94\ 10^{-8}$	$4.17\ 10^{-16}$
18.6	$4.18\ 10^{-9}$	$2.20\ 10^{-16}$

R, A	$F_p^w - F_p^s$	F^{ads}	$U_p^w - U_p^s$	U^{ads}
		High Density Polyethylene		
3.1	$2.47\ 10^{-5}$	$4.23\ 10^{-5}$	$1.99\ 10^{-13}$	$3.41\ 10^{-13}$
6.1	$8.92\ 10^{-7}$	$1.52\ 10^{-6}$	$1.53\ 10^{-14}$	$2.59\ 10^{-14}$
9.3	$1.38\ 10^{-7}$	$2.33\ 10^{-7}$	$3.64\ 10^{-15}$	$6.14\ 10^{-15}$
12.4	$3.77\ 10^{-8}$	$6.34\ 10^{-8}$	$1.34\ 10^{-15}$	$2.25\ 10^{-15}$
15.5	$1.39\ 10^{-8}$	$2.34\ 10^{-8}$	$6.23\ 10^{-16}$	$1.04\ 10^{-15}$
18.6	$6.21\ 10^{-9}$	$1.04\ 10^{-8}$	$3.32\ 10^{-16}$	$5.53\ 10^{-16}$
		Polystyrene		
3.1	$4.49\ 10^{-5}$	$6.25\ 10^{-5}$	$3.50\ 10^{-13}$	$4.91\ 10^{-13}$
6.2	$1.46\ 10^{-6}$	$2.09\ 10^{-6}$	$2.37\ 10^{-14}$	$3.43\ 10^{-14}$
9.3	$2.07\ 10^{-7}$	$3.02\ 10^{-7}$	$5.22\ 10^{-15}$	$7.71\ 10^{-15}$
12.4	$5.35\ 10^{-8}$	$7.92\ 10^{-8}$	$1.84\ 10^{-15}$	$2.75\ 10^{-15}$
15.5	$1.91\ 10^{-8}$	$2.85\ 10^{-8}$	$8.35\ 10^{-16}$	$1.25\ 10^{-15}$
18.6	$8.31\ 10^{-9}$	$1.25\ 10^{-8}$	$4.41\ 10^{-16}$	$6.62\ 10^{-16}$
		Polytetrafluoroethylene (PTFE)		
3.1	$2.39\ 10^{-5}$	$4.15\ 10^{-5}$	$1.91\ 10^{-13}$	$3.32\ 10^{-13}$
6.2	$8.36\ 10^{-7}$	$1.46\ 10^{-6}$	$1.41\ 10^{-14}$	$2.48\ 10^{-14}$
9.3	$1.26\ 10^{-7}$	$2.21\ 10^{-7}$	$3.31\ 10^{-15}$	$5.81\ 10^{-15}$
12.4	$3.42\ 10^{-8}$	$5.99\ 10^{-8}$	$1.21\ 10^{-15}$	$2.12\ 10^{-15}$
15.5	$1.26\ 10^{-8}$	$2.20\ 10^{-8}$	$5.61\ 10^{-16}$	$9.78\ 10^{-16}$
18.6	$5.59\ 10^{-9}$	$9.76\ 10^{-9}$	$3.00\ 10^{-16}$	$5.20\ 10^{-16}$
		Polypropylene		
3.1	$3.29\ 10^{-5}$	$5.05\ 10^{-5}$	$2.59\ 10^{-13}$	$4.00\ 10^{-13}$
6.2	$1.11\ 10^{-6}$	$1.73\ 10^{-6}$	$1.83\ 10^{-14}$	$2.89\ 10^{-14}$
9.3	$1.62\ 10^{-7}$	$2.57\ 10^{-7}$	$4.16\ 10^{-15}$	$6.66\ 10^{-15}$
12.4	$4.28\ 10^{-8}$	$6.86\ 10^{-8}$	$1.50\ 10^{-15}$	$2.41\ 10^{-15}$
15.5	$1.55\ 10^{-8}$	$2.50\ 10^{-8}$	$6.90\ 10^{-16}$	$1.11\ 10^{-15}$
18.6	$6.86\ 10^{-9}$	$1.10\ 10^{-8}$	$3.67\ 10^{-16}$	$5.88\ 10^{-16}$

* All forces and energies are negative, indicating adsorption.

TABLE X

THE ADSORPTION FORCES* AND ENERGIES* BETWEEN A BUTANE MOLECULE AND SOME COMMON POLYMERS

R.A.	Water Force, $F_n^s - F_n^w$	Water Energy, $U_n^s - U_n^w$
3.1	$4.37\ 10^{-5}$	$3.50\ 10^{-13}$
6.2	$1.55\ 10^{-6}$	$2.63\ 10^{-14}$
9.3	$2.35\ 10^{-7}$	$6.18\ 10^{-15}$
12.4	$6.39\ 10^{-8}$	$2.25\ 10^{-15}$
15.5	$2.34\ 10^{-8}$	$1.03\ 10^{-15}$
18.6	$1.03\ 10^{-8}$	$5.46\ 10^{-16}$

R.A.	$F_p^w - F_p^s$	F^{ads}	$U_p^w - U_p^s$	U^{ads}
		High Density Polyethylene		
3.1	$3.91\ 10^{-5}$	$8.28\ 10^{-5}$	$3.15\ 10^{-13}$	$6.66\ 10^{-13}$
6.2	$1.41\ 10^{-6}$	$2.96\ 10^{-6}$	$2.42\ 10^{-14}$	$5.05\ 10^{-14}$
9.3	$2.18\ 10^{-7}$	$4.54\ 10^{-7}$	$5.76\ 10^{-15}$	$1.19\ 10^{-14}$
12.4	$5.96\ 10^{-8}$	$1.23\ 10^{-7}$	$2.13\ 10^{-15}$	$4.38\ 10^{-15}$
15.5	$2.20\ 10^{-8}$	$4.55\ 10^{-8}$	$9.85\ 10^{-16}$	$2.01\ 10^{-15}$
18.6	$9.82\ 10^{-9}$	$2.02\ 10^{-8}$	$5.26\ 10^{-16}$	$1.07\ 10^{-15}$
		Polystyrene		
3.1	$7.16\ 10^{-5}$	$1.15\ 10^{-4}$	$5.59\ 10^{-13}$	$9.09\ 10^{-13}$
6.2	$2.34\ 10^{-6}$	$3.89\ 10^{-6}$	$3.79\ 10^{-14}$	$6.42\ 10^{-14}$
9.3	$3.31\ 10^{-7}$	$5.6\ 10^{-7}$	$8.33\ 10^{-15}$	$1.45\ 10^{-14}$
12.4	$8.54\ 10^{-8}$	$1.49\ 10^{-7}$	$2.94\ 10^{-15}$	$5.19\ 10^{-15}$
15.5	$3.15\ 10^{-8}$	$5.39\ 10^{-8}$	$1.33\ 10^{-15}$	$2.37\ 10^{-15}$
18.6	$1.33\ 10^{-8}$	$2.36\ 10^{-8}$	$7.05\ 10^{-16}$	$1.25\ 10^{-15}$
		Polytetrafluoroethylene (PTFE)		
3.1	$3.70\ 10^{-5}$	$8.07\ 10^{-5}$	$2.95\ 10^{-13}$	$6.46\ 10^{-13}$
6.2	$1.29\ 10^{-6}$	$2.85\ 10^{-6}$	$2.19\ 10^{-14}$	$4.82\ 10^{-14}$
9.3	$1.96\ 10^{-7}$	$4.32\ 10^{-7}$	$5.13\ 10^{-15}$	$1.13\ 10^{-14}$
12.4	$5.29\ 10^{-8}$	$1.17\ 10^{-7}$	$1.88\ 10^{-15}$	$4.13\ 10^{-15}$
15.5	$1.95\ 10^{-8}$	$4.29\ 10^{-8}$	$8.69\ 10^{-16}$	$1.90\ 10^{-15}$
18.6	$8.65\ 10^{-9}$	$1.90\ 10^{-8}$	$4.64\ 10^{-16}$	$1.01\ 10^{-15}$
		Polypropylene		
3.1	$5.20\ 10^{-5}$	$9.57\ 10^{-5}$	$4.10\ 10^{-13}$	$7.60\ 10^{-13}$
6.2	$1.75\ 10^{-6}$	$3.30\ 10^{-6}$	$2.90\ 10^{-14}$	$5.53\ 10^{-14}$
9.3	$2.56\ 10^{-7}$	$4.92\ 10^{-7}$	$6.59\ 10^{-15}$	$1.28\ 10^{-14}$
12.4	$6.77\ 10^{-8}$	$1.32\ 10^{-7}$	$2.38\ 10^{-15}$	$4.63\ 10^{-15}$
15.5	$2.46\ 10^{-8}$	$4.80\ 10^{-8}$	$1.03\ 10^{-15}$	$2.12\ 10^{-15}$
18.5	$1.08\ 10^{-8}$	$2.12\ 10^{-8}$	$5.81\ 10^{-16}$	$1.13\ 10^{-15}$

*All forces and energies are negative, indicating adsorptions.

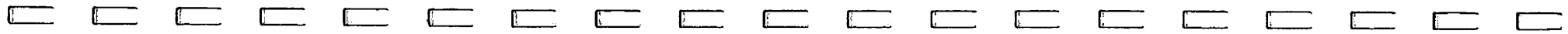


TABLE XI

THE ADSORPTION FORCES* AND ENERGIES* BETWEEN A HEXANE MOLECULE AND SOME COMMON POLYMERS

R, A	Water Force, $F_n^s - F_n^w$	Water Energy, $U_n^s - U_n^w$
3.1	$7.00\ 10^{-5}$	$5.62\ 10^{-13}$
6.2	$2.49\ 10^{-6}$	$4.22\ 10^{-14}$
9.3	$3.78\ 10^{-7}$	$9.90\ 10^{-15}$
12.4	$1.02\ 10^{-7}$	$3.61\ 10^{-15}$
15.5	$3.74\ 10^{-8}$	$1.66\ 10^{-15}$
18.6	$1.66\ 10^{-8}$	$8.76\ 10^{-16}$

R, A	$F_p^w - F_p^s$	F^{ads}	$U_p^w - U_p^s$	U^{ads}
	High Density Polyethylene			
3.1	$5.31\ 10^{-5}$	$1.23\ 10^{-4}$	$4.29\ 10^{-13}$	$9.90\ 10^{-13}$
6.2	$1.92\ 10^{-6}$	$4.40\ 10^{-6}$	$3.29\ 10^{-14}$	$7.50\ 10^{-14}$
9.3	$2.96\ 10^{-7}$	$6.74\ 10^{-7}$	$7.83\ 10^{-15}$	$1.77\ 10^{-14}$
12.4	$0.81\ 10^{-7}$	$1.83\ 10^{-7}$	$2.89\ 10^{-15}$	$6.50\ 10^{-15}$
15.5	$3.00\ 10^{-8}$	$6.75\ 10^{-8}$	$1.34\ 10^{-15}$	$2.99\ 10^{-15}$
18.6	$1.33\ 10^{-8}$	$2.99\ 10^{-8}$	$7.14\ 10^{-16}$	$1.59\ 10^{-15}$
	Polystyrene			
3.1	$9.78\ 10^{-5}$	$1.68\ 10^{-4}$	$7.63\ 10^{-13}$	$1.32\ 10^{-12}$
6.2	$3.19\ 10^{-6}$	$5.68\ 10^{-6}$	$5.17\ 10^{-14}$	$9.39\ 10^{-14}$
9.3	$4.51\ 10^{-7}$	$8.30\ 10^{-7}$	$1.14\ 10^{-14}$	$2.13\ 10^{-14}$
12.4	$1.17\ 10^{-7}$	$2.19\ 10^{-7}$	$4.02\ 10^{-15}$	$7.62\ 10^{-15}$
15.5	$4.16\ 10^{-8}$	$7.91\ 10^{-8}$	$1.82\ 10^{-15}$	$3.48\ 10^{-15}$
18.6	$1.81\ 10^{-8}$	$3.47\ 10^{-8}$	$9.62\ 10^{-16}$	$1.84\ 10^{-15}$
	Polytetrafluoroethylene (PTFE)			
3.1	$4.97\ 10^{-5}$	$1.20\ 10^{-4}$	$3.97\ 10^{-13}$	$9.57\ 10^{-13}$
6.2	$1.74\ 10^{-6}$	$4.23\ 10^{-6}$	$2.94\ 10^{-14}$	$7.16\ 10^{-14}$
9.3	$2.63\ 10^{-7}$	$6.41\ 10^{-7}$	$6.89\ 10^{-15}$	$1.68\ 10^{-14}$
12.4	$7.11\ 10^{-8}$	$1.73\ 10^{-7}$	$2.52\ 10^{-15}$	$6.13\ 10^{-15}$
15.5	$2.61\ 10^{-8}$	$6.37\ 10^{-8}$	$1.17\ 10^{-15}$	$2.82\ 10^{-15}$
18.6	$1.16\ 10^{-8}$	$2.82\ 10^{-8}$	$6.23\ 10^{-16}$	$1.50\ 10^{-15}$
	Polypropylene			
3.1	$7.07\ 10^{-5}$	$1.41\ 10^{-4}$	$5.57\ 10^{-13}$	$1.12\ 10^{-12}$
6.2	$2.38\ 10^{-6}$	$4.86\ 10^{-6}$	$3.94\ 10^{-14}$	$8.15\ 10^{-14}$
9.3	$3.48\ 10^{-7}$	$7.26\ 10^{-7}$	$8.95\ 10^{-15}$	$1.89\ 10^{-14}$
12.4	$9.20\ 10^{-8}$	$1.94\ 10^{-7}$	$3.23\ 10^{-15}$	$6.48\ 10^{-15}$
15.5	$3.34\ 10^{-8}$	$7.09\ 10^{-8}$	$1.48\ 10^{-15}$	$3.24\ 10^{-15}$
18.6	$1.47\ 10^{-8}$	$3.13\ 10^{-8}$	$7.89\ 10^{-16}$	$1.67\ 10^{-15}$

* All forces and energies are negative, indicating adsorption.

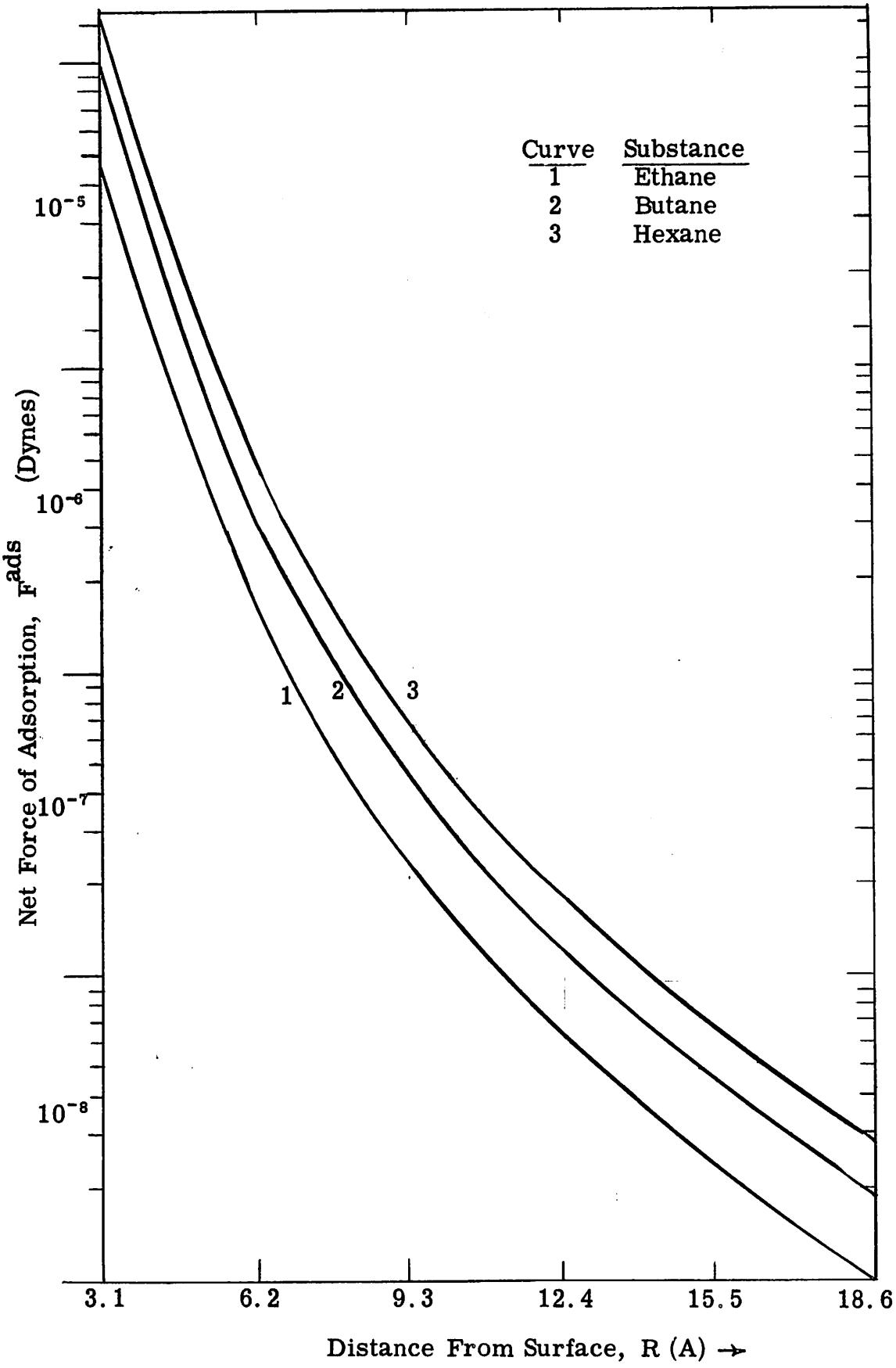


Figure 14. The Adsorption Force Between Some n-Alkanes and High Density Polyethylene.

Figure 13 shows that maximum interaction occurs with polystyrene and minimum interaction with polytetrofluoroethylene (PTFE). The data for polystyrene and PTFE are plotted in Figure 15 for the three alkanes and for a $-CH_2-$ group. The range of interactions for a given solute molecule does not overlap with those of the other solutes considered. If the odd alkanes, e.g., pentane, had also been considered, there would have been some overlap with the curves for the adjacent even-alkanes. It is, therefore, quite clear that the adsorption force is more dependent on the solute molecule than on the hydrophobic polymer surface.

These results all assume that the molecule can be located at a point and that the polarizability of the solute is a constant, independent of orientation. The case of orientation-dependent polarizability will be discussed later.

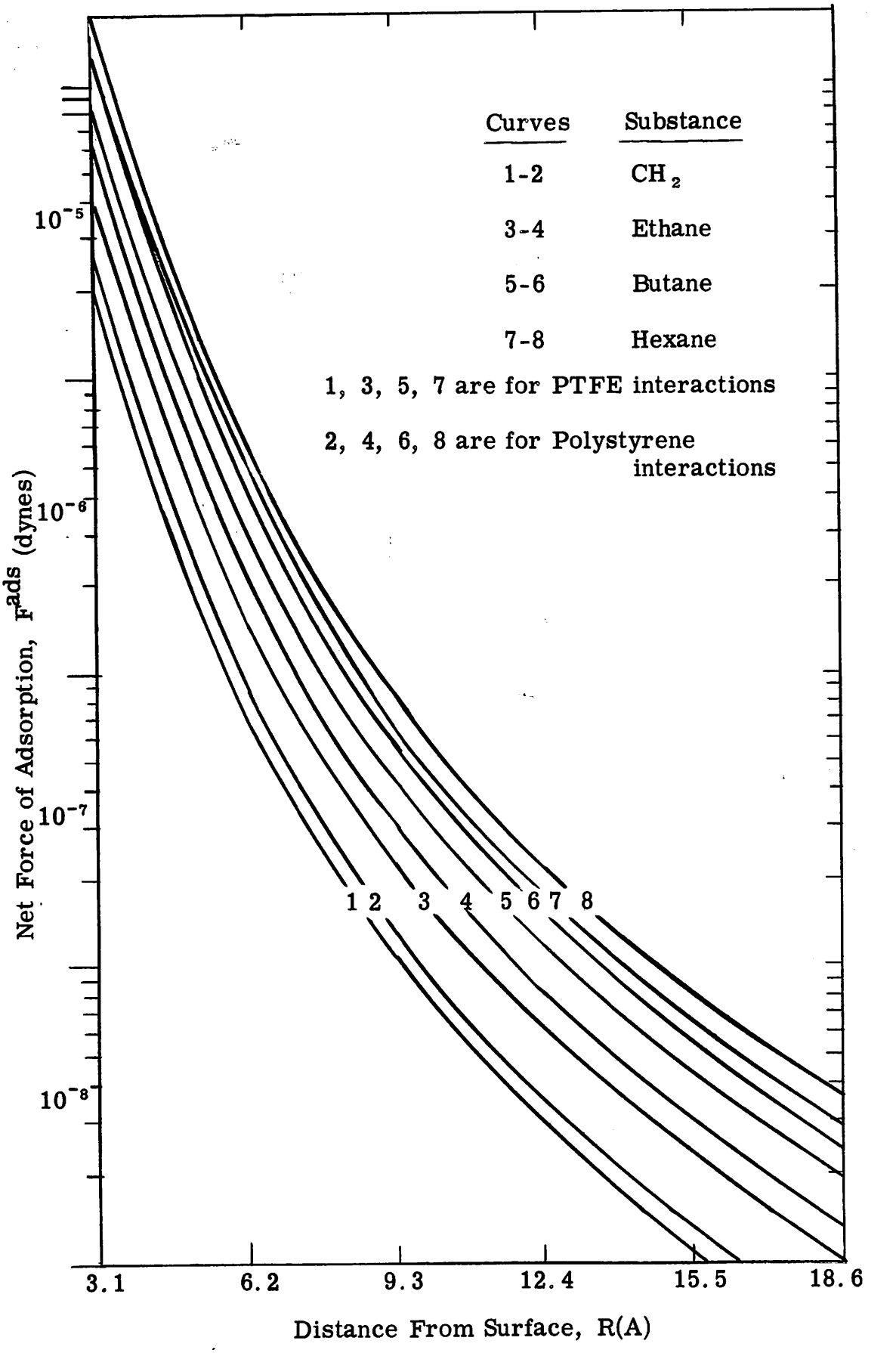


Figure 15. The Spread of Adsorption Forces for Some Alkanes Interacting With Some Common Polymers (see text).

c. Effect of Polymer Density and Water Content

The case where the polymer surface is porous and permeable to water will now be considered. This analysis and the calculations derived from it will be used in Section C to explain monolayer adsorption. The case where the polymer is impermeable (contains zero per cent water) has already been treated. The case where there is no polymer ("it" contains one hundred per cent water) corresponds to that of a solute molecule surrounded by bulk water, and the adsorption force must be zero. The case where the polymer density is variable, but it is impermeable to water, will also be treated. For this latter case, when the polymer density is zero, the situation must be the same as at an air/water interface.

Let the weight fraction of polymer be Y and the weight fraction of uniformly dispersed water be 1-Y. Only the case of high density (0.96) polyethylene will be treated. Thus, the polymer and water density are approximately the same, and the treatment is simplified. The effective polymer density is Y grams/cm^3 and the effective water density is (1-Y) grams/cm^3. The equivalent planar spacings of the water and polymer components can be calculated with equation (35). The value for polyethylene is

$$d_{PE} = 1.18\ (14/Y)^{0.33}\ A, \qquad (41)$$

and the value for dispersed water is

$$d_{dw} = 1.18\,[18/(1 - Y)]^{0.33}\,A. \tag{42}$$

The subscript PE denotes polyethylene and dw denotes "dispersed water." The number of molecules/cm^2, σ, is given by the equation,

$$\sigma = (\rho N_0/M)^{0.67},$$

where ρ is the density, N_0 is Avogadro's number, and M is the group molecular weight. The value for polyethylene is

$$\sigma_{PE} = (11.9 \times 10^{14})(Y)^{0.67}, \tag{43}$$

and the value for the dispersed water is

$$\sigma_{dw} = (10.3 \times 10^{14})(1 - Y)^{0.67}. \tag{44}$$

The polymer interaction, F_p (equation 36), is now composed of two terms. The first is due to the interaction with the actual polymer present, and the second is due to the interaction with the water dispersed in the polymer phase. Therefore, F_p becomes:

$$F_p = 14.5\left[\left(\sigma A_0 \sum_{n=0}^{5} (R + nd)^{-5}\right)_{PE} + \left(\sigma A_0 \sum_{n=0}^{5} (R + nd)^{-5}\right)_{dw}\right] \tag{45}$$

The adsorption force due to the presence of a water-permeable polymer now becomes:

$$F_p^w - F_p^s = 14.5\sigma_{PE}(A4 - A3) \sum_{n=0}^{5} (R + nd_{PE})^{-5} + 14.5\sigma_{dw}(3.3A1 - A2) \sum_{n=0}^{5} (R + nd_{dw})^{-5} \tag{46}$$

where σ and d terms are given by equations (41) to (44). Equation (39) is not affected by the presence of water in the polymer phase.

The case where the polymer density varies but no water is present in the polymer is also given by equations (39) and (46) if the second term in equation (46) is set equal to zero.

Equations (39) and (46) were programmed and evaluated for polymer densities varying from 0 to 1.0. The data are given in Table XII and plotted in Figures 16 and 17. The results are very interesting. Figure 16 clearly shows that the force of adsorption tends to zero as Y decreases (as the water content increases), as expected. If the polymer density decreases but the polymer is not allowed to take on water, the interaction force decreases with Y until at $Y = 0$ the force is the same as for an air/water interface (Figure 17).

TABLE XII

THE EFFECT OF POLYMER DENSITY AND WATER CONTENT ON DISPERSION INTERACTIONS BETWEEN A $-CH_2-$ GROUP AND POLYETHYLENE*

R, A	Water Force, $F_n^s - F_n^w$
3.1	$1.25\ 10^{-5}$
6.2	$4.42\ 10^{-7}$
9.3	$6.73\ 10^{-8}$
12.4	$1.82\ 10^{-8}$
15.5	$6.68\ 10^{-9}$
18.6	$2.95\ 10^{-9}$

Y	R, A	Impermeable Case $F_p^w - F_p^s$	Impermeable Case F^{ads}	Permeable Case $F_p^w - F_p^s$	Permeable Case F^{ads}
1.0	3.1	$7.79\ 10^{-6}$	$2.03\ 10^{-5}$	$7.79\ 10^{-6}$	$2.03\ 10^{-5}$
	6.2	$2.82\ 10^{-7}$	$7.25\ 10^{-7}$	$2.82\ 10^{-7}$	$7.25\ 10^{-7}$
	9.3	$4.37\ 10^{-8}$	$1.11\ 10^{-8}$	$4.37\ 10^{-8}$	$1.11\ 10^{-8}$
	12.4	$1.20\ 10^{-8}$	$3.02\ 10^{-8}$	$1.20\ 10^{-8}$	$3.02\ 10^{-8}$
	15.5	$4.44\ 10^{-9}$	$1.11\ 10^{-9}$	$4.44\ 10^{-9}$	$1.11\ 10^{-9}$
	18.6	$1.98\ 10^{-9}$	$4.93\ 10^{-9}$	$1.98\ 10^{-9}$	$4.93\ 10^{-9}$
0.8	3.1	$6.65\ 10^{-6}$	$1.91\ 10^{-5}$	$2.53\ 10^{-6}$	$1.50\ 10^{-5}$
	6.2	$2.38\ 10^{-7}$	$6.80\ 10^{-7}$	$1.02\ 10^{-7}$	$5.45\ 10^{-7}$
	9.3	$3.64\ 10^{-8}$	$1.04\ 10^{-7}$	$1.72\ 10^{-8}$	$8.45\ 10^{-8}$
	12.4	$9.92\ 10^{-9}$	$2.81\ 10^{-8}$	$4.94\ 10^{-9}$	$2.32\ 10^{-8}$
	15.5	$3.66\ 10^{-9}$	$1.02\ 10^{-8}$	$1.88\ 10^{-9}$	$8.56\ 10^{-9}$
	18.6	$1.63\ 10^{-9}$	$4.58\ 10^{-9}$	$8.53\ 10^{-10}$	$3.80\ 10^{-9}$
0.6	3.1	$5.44\ 10^{-6}$	$1.79\ 10^{-5}$	$1.17\ 10^{-6}$	$1.13\ 10^{-5}$
	6.3	$1.91\ 10^{-7}$	$6.33\ 10^{-7}$	$3.26\ 10^{-8}$	$4.10\ 10^{-7}$
	9.3	$2.89\ 10^{-8}$	$9.62\ 10^{-8}$	$3.92\ 10^{-9}$	$6.34\ 10^{-8}$
	12.4	$7.80\ 10^{-9}$	$2.60\ 10^{-8}$	$8.86\ 10^{-10}$	$1.73\ 10^{-8}$
	15.5	$2.87\ 10^{-9}$	$9.55\ 10^{-9}$	$2.88\ 10^{-10}$	$6.39\ 10^{-9}$
	18.6	$1.28\ 10^{-9}$	$4.23\ 10^{-9}$	$1.19\ 10^{-10}$	$2.83\ 10^{-9}$
0.4	3.1	$4.11\ 10^{-6}$	$1.66\ 10^{-5}$	$+4.63\ 10^{-6}$	$7.83\ 10^{-6}$
	6.2	$1.41\ 10^{-7}$	$5.83\ 10^{-7}$	$+1.61\ 10^{-7}$	$2.81\ 10^{-7}$
	9.3	$2.09\ 10^{-8}$	$8.82\ 10^{-8}$	$+2.43\ 10^{-8}$	$4.31\ 10^{-8}$
	12.4	$5.58\ 10^{-9}$	$2.38\ 10^{-8}$	$+6.53\ 10^{-9}$	$1.17\ 10^{-8}$
	15.5	$2.04\ 10^{-9}$	$8.72\ 10^{-9}$	$+2.40\ 10^{-9}$	$4.28\ 10^{-9}$
	18.6	$9.02\ 10^{-10}$	$3.85\ 10^{-9}$	$+1.07\ 10^{-9}$	$1.89\ 10^{-9}$

* All forces are negative, indicating adsorption, EXCEPT those specifically written with positive (+) signs.

Table Continued...

TABLE XII*

(Continued)

Y	R, A	$F_p^w - F_p^s$	F^{ads}	$F_p^w - F_p^s$	F^{ads}
		Impermeable Case		Permeable Case	
0.2	3.1	$2.56\ 10^{-6}$	$1.51\ 10^{-5}$	$+8.12\ 10^{-6}$	$4.34\ 10^{-6}$
	6.2	$8.46\ 10^{-8}$	$5.27\ 10^{-7}$	$+2.90\ 10^{-7}$	$1.52\ 10^{-7}$
	9.3	$1.22\ 10^{-8}$	$7.95\ 10^{-8}$	$+4.46\ 10^{-8}$	$2.27\ 10^{-8}$
	12.4	$3.18\ 10^{-9}$	$2.14\ 10^{-8}$	$+1.22\ 10^{-8}$	$6.02\ 10^{-9}$
	15.5	$1.15\ 10^{-9}$	$7.82\ 10^{-9}$	$+4.52\ 10^{-9}$	$2.16\ 10^{-9}$
	18.6	$5.03\ 10^{-10}$	$3.45\ 10^{-9}$	$+2.01\ 10^{-9}$	$9.38\ 10^{-10}$
0.1	3.1	$1.60\ 10^{-6}$	$1.41\ 10^{-5}$	$+1.00\ 10^{-5}$	$2.47\ 10^{-6}$
	6.2	$5.17\ 10^{-8}$	$4.94\ 10^{-7}$	$+3.58\ 10^{-7}$	$8.39\ 10^{-8}$
	9.3	$7.23\ 10^{-9}$	$7.46\ 10^{-8}$	$+5.52\ 10^{-8}$	$1.21\ 10^{-9}$
	12.4	$1.85\ 10^{-9}$	$2.01\ 10^{-8}$	$+1.51\ 10^{-8}$	$3.10\ 10^{-9}$
	15.5	$6.54\ 10^{-10}$	$7.33\ 10^{-9}$	$+5.60\ 10^{-9}$	$1.08\ 10^{-9}$
	18.6	$2.83\ 10^{-10}$	$3.24\ 10^{-9}$	$+2.50\ 10^{-9}$	$4.51\ 10^{-10}$
0.01	3.1	$2.30\ 10^{-7}$	$1.28\ 10^{-5}$	$+1.21\ 10^{-5}$	$4.12\ 10^{-7}$
	6.2	$1.07\ 10^{-8}$	$4.53\ 10^{-7}$	$+4.31\ 10^{-7}$	$1.18\ 10^{-8}$
	9.3	$1.42\ 10^{-9}$	$6.87\ 10^{-8}$	$+6.61\ 10^{-8}$	$1.25\ 10^{-9}$
	12.4	$3.43\ 10^{-10}$	$1.86\ 10^{-8}$	$+1.80\ 10^{-8}$	$1.81\ 10^{-10}$
	15.5	$1.15\ 10^{-10}$	$6.79\ 10^{-9}$	$+6.67\ 10^{-9}$	$7.90\ 10^{-11}$
	18.6	$4.77\ 10^{-11}$	$3.00\ 10^{-9}$	$+2.97\ 10^{-9}$	$2.16\ 10^{-11}$
0.001	3.1	$7.27\ 10^{-8}$	$1.25\ 10^{-5}$	$+1.24\ 10^{-5}$	$6.56\ 10^{-8}$
	6.2	$2.27\ 10^{-9}$	$4.46\ 10^{-7}$	$+4.42\ 10^{-7}$	$3.03\ 10^{-9}$
	9.3	$2.99\ 10^{-10}$	$6.76\ 10^{-8}$	$+6.76\ 10^{-8}$	$3.69\ 10^{-10}$
	12.4	$7.12\ 10^{-11}$	$1.83\ 10^{-8}$	$+1.85\ 10^{-8}$	$2.31\ 10^{-10}$
	15.5	$2.34\ 10^{-11}$	$6.70\ 10^{-9}$	$+6.82\ 10^{-9}$	$1.36\ 10^{-10}$
	18.6	$9.46\ 10^{-12}$	$2.96\ 10^{-9}$	$+3.03\ 10^{-9}$	$8.34\ 10^{-11}$
0	3.1	0	$1.25\ 10^{-5}$	$+1.25\ 10^{-5}$	$+1.59\ 10^{-8}$
	6.2	0	$4.42\ 10^{-7}$	$+4.45\ 10^{-7}$	$+2.31\ 10^{-9}$
	9.3	0	$6.73\ 10^{-8}$	$+6.80\ 10^{-8}$	$+7.24\ 10^{-10}$
	12.4	0	$1.82\ 10^{-8}$	$+1.85\ 10^{-8}$	$+3.18\ 10^{-10}$
	15.5	0	$6.68\ 10^{-9}$	$+6.84\ 10^{-9}$	$+1.66\ 10^{-10}$
	18.6	0	$2.95\ 10^{-9}$	$+3.05\ 10^{-9}$	$+9.55\ 10^{-11}$

*All forces are negative, indicating adsorption, EXCEPT those specifically written with positive (+) signs.

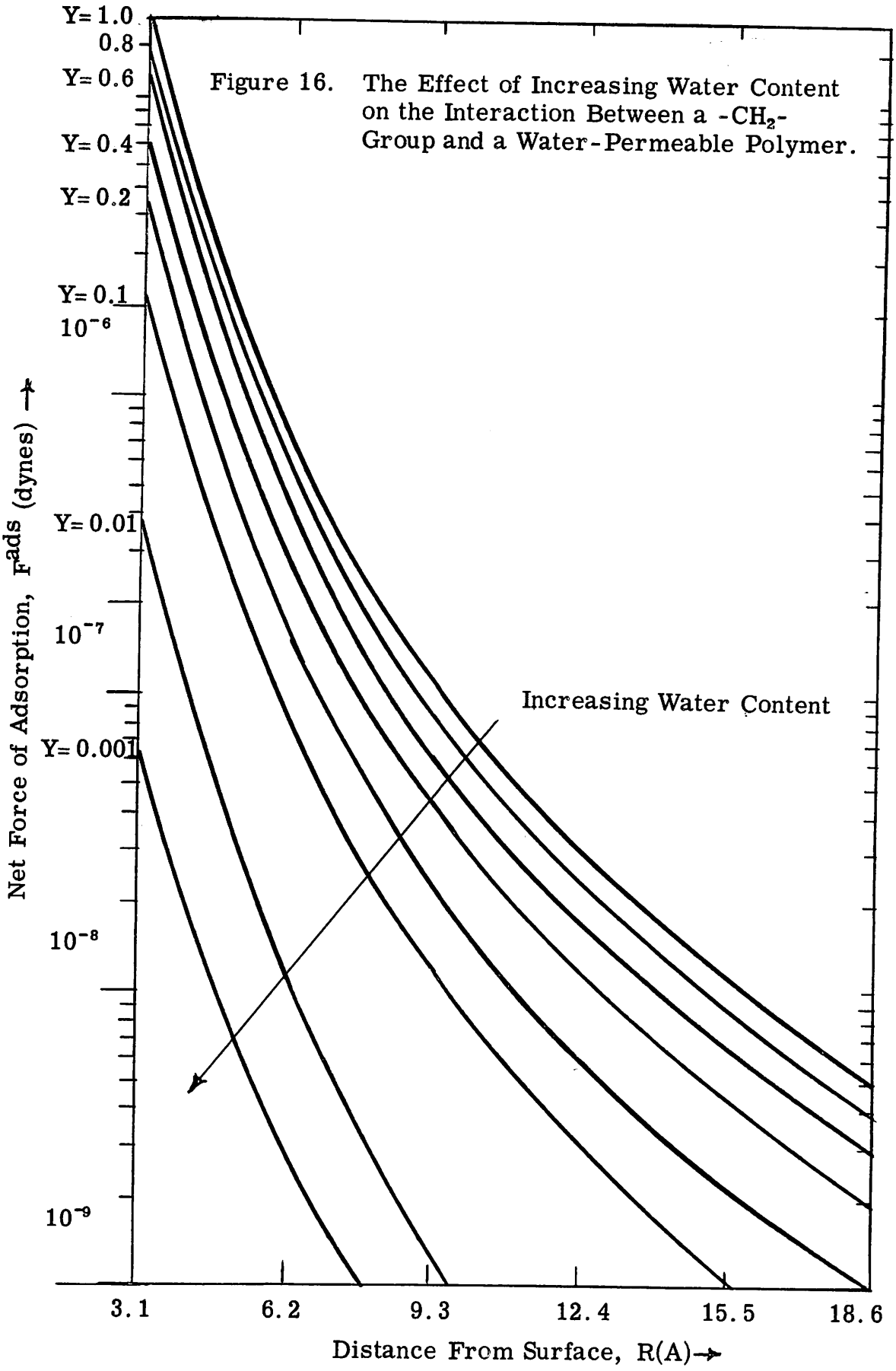


Figure 16. The Effect of Increasing Water Content on the Interaction Between a $-CH_2-$ Group and a Water-Permeable Polymer.

d. Interactions Between a Flat Surface and Large Particles

The expressions derived and used up to now have been for a single molecule or group interacting with a flat plate. It is clear from Figure 11a that the interactions between two flat plates can be calculated by performing another triple integration. Fortunately, the expressions for the interactions between simple shapes are available in the literature[127,132] and have been tabulated.[69] All of the expressions contain the quantity π^2N^2A, which is called the Hamaker constant,[69] where N is the number of molecules/cm^3 in the particles and A is the London constant (equation 10). The values of the ionization potentials for many molecules and groups are all roughly the same (within a factor of two or three). The average bond polarizability values do not vary by more than an order of magnitude. The molecular or group density of most organic materials is also within an order of magnitude of 10^{22} molecules/cm^2. The net result is that the Hamaker constant is of the order of 10^{-13} erg. Thus, for the interactions of large particles, the factors governing the interaction are primarily the distance of separation and the dimensions of the particle.

Vold[132] has shown that for particles of colloidal dimensions the total attractive energy is of the same order as kT energy when the mean diameter is roughly equal to the particle separation, independent of the shape of the particles. The mean diameter is defined as the cube root of the particle volume. At smaller separations the interaction is greatest for plates and decreases in the order plates, rods, cylinders, and spheres, as ex-

pected. Thus, the greater the axial ratio of a protein, the greater its dispersion interactions.

The above generalizations will be used to qualitatively discuss protein adsorption later.

4. The Effect of Orientation Dependence of the Polarizability:

a. Orientation of the Solute

The expressions derived up to now have assumed that the polarizability, and that the solute molecule can be considered as a point. The polarizability assumption is probably valid at large distances, but at small distances one might expect the solute to align itself with the surface in the position of maximum interaction.

Because of the large asymmetry in the polarizability of the C-C bond, one expects that such a bond (in ethane, for example) would orient perpendicular to a surface. The perpendicular orientation would interact nearly two orders of magnitude stronger than the parallel orientation. The situation changes, however, when a longer hydrocarbon is examined. Consider the extended chain in Figure 18. The problem is greatly simplified if the polarizabilities of the C-C bonds are resolved along the chain axis. The results are:

$$\alpha'_{\parallel} = \alpha_{\parallel} \cos 34 \simeq 15.6 \times 10^{-25} \text{ cm}^3$$

and

$$\alpha'_{\perp} = \alpha_{\parallel} \cos 56 \simeq 10.5 \times 10^{-25} \text{ cm}^3;$$

it is assumed that $\alpha_{\perp}$ is negligible with respect to $\alpha_{\parallel}$. The primes

denote the resolved quantities. It is clear that there is not a significant difference between $\alpha'_{\parallel}$ and $\alpha'_{\perp}$.

Consider a hydrocrabon chain, some distance away from the interface, rotating about its center of gravity (Figure 19a). If R is measured to the center of the molecule, then any rotation or movement which brings a portion of the molecule closer to the surface will be energetically favorable. The molecule will thus tend to approach the interface in a perpendicular orientation. As the separation distance decreases, then the molecule will tend to assume a parallel orientation, which minimizes R and optimizes the interaction; this case is shown in Figure 19b.

An extended chain solute molecule tends to approach an interface in a perpendicular orientation, though it will assume a parallel orientation at small separation distances. If the non-aqueous phase is a liquid, then the solute may continue to approach and even penetrate in the perpendicular orientation.

b. Orientation of the Polymer Surface

The directional dependence of the bonds in the polymer surface may also influence solute-surface interactions. In crystalline polymers the orientation of the chains is different on a fold surface than on a lateral surface. It was shown earlier that this results in significantly different energies for the lateral and fold surfaces. The

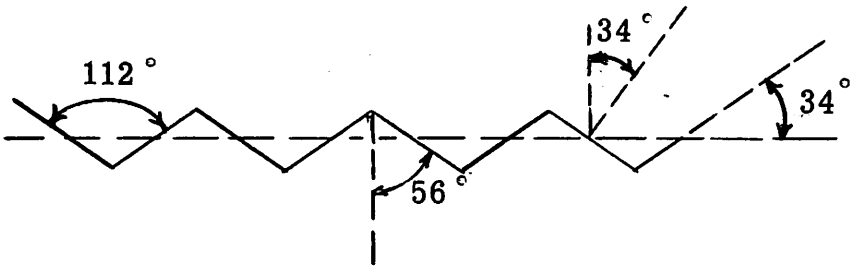


Figure 18. The Geometry of an Extended Hydrocarbon Chain.

a. At Large Separation Distances

b. At Small Separation Distances

Figure 19. Possible Orientations of a Solute Molecule Near an Interface.

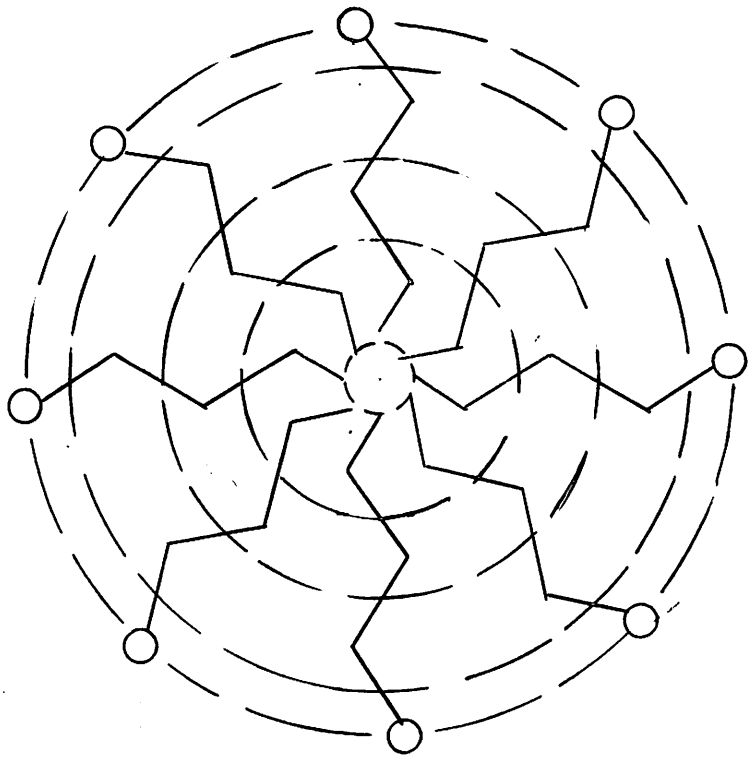
effect of the orientation is the same as that discussed for solute orientation. The chains making up a fold surface tend to be oriented perpendicular to the surface, while the lateral surfaces are composed of chains parallel to the surface. The bonds comprising a chain fold must be strained and distorted, so that conventional values of bond polarizabilities are probably not valid. Nevertheless, it is likely that the fold surfaces of polymer crystallites will interact more strongly than the lateral surfaces.

5. Complex Models:

a. Hypothetical Micelles

A very simple first approximation to the structure of a protein is a hypothetical micelle. A micelle is an aggregation of polar or charged molecules with long hydrocarbon chains. The intermolecular attraction leads to strong hydrophobic interactions among the apolar portions and strong dipole- or charge-solvent interactions with the polar portions. The result is often a structure of nearly spherical symmetry composed of an apolar interior and a polar exterior.

Consider the two-dimensional micelle of Figure 20a. This very idealized picture shows a symmetrical micelle composed of relatively small molecules and serves to illustrate micelle-surface interactions. The concentric circles in Figure 20a represent the areas occupied by the $-CH_2-$ groups as a function of radial distance. It is

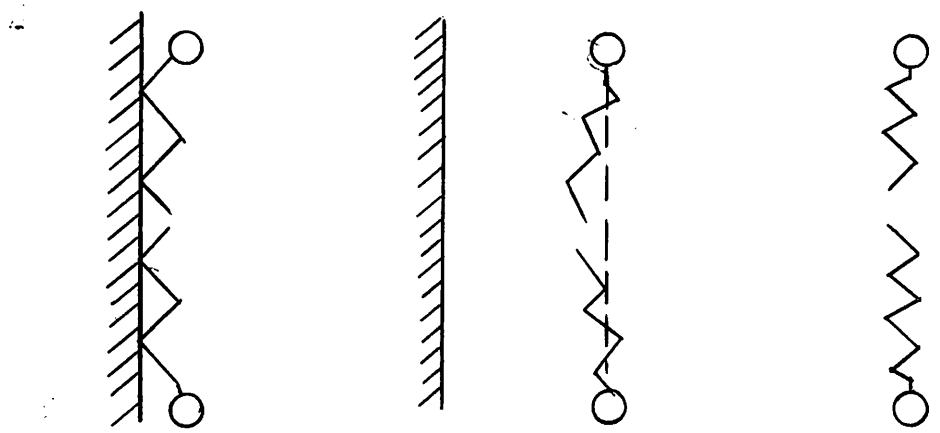


a.

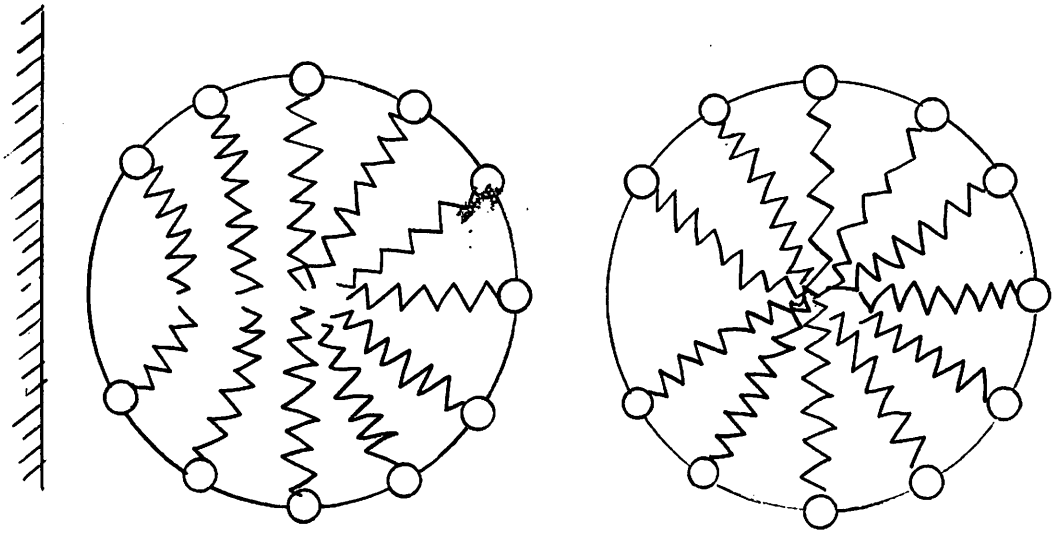
Figure 20. Views of Idealized Non-Ionic Micelles.

a. (Above) The Number of $-CH_2-$ Groups per Unit Area is Greater for the Inner Rings Than for the Outer Ones.

(See 20b and 20c next page.)



b.



c.

Figure 20. Views of Idealized Non-Ionic Micelles.

b. The Sketch on the Right Shows a Flat Micelle in Bulk Solution. The Center Sketch Shows it Undergoing a "Puckering" Distortion. The Left-most Sketch Shows its Probable Orientation on the Surface.

c. An "Unshielded" Nearly Spherical Micelle is Shown on the Right; a Possible Surface-Micelle Interaction Resulting in Distortion of the Micelle is Sketched on the Left.

assumed that because of steric hindrances, the center of the molecule is essentially empty. Each concentric ring contains the same number of $-CH_2-$ groups, but the area of each ring increases linearly with the radius. Thus, there would be a greater interaction force per unit area on the inner rings than on the outer rings if the micelle were oriented parallel to a surface. In such an orientation, each chain would be roughly parallel to the surface, which has already been shown to be an unfavorable orientation except at close separations. The net result would probably be a "puckering" of the micelle, as illustrated in Figure 20b, a side view of the two-dimensional micelle parallel to a surface. The puckering effect would produce an even greater interaction, as now the puckered groups would not only be closer to the surface, but would be more favorably oriented as well. The net result would tend to favor adsorption in the parallel configuration, but with the polar heads in the solvent and capable of extensive solvent-solute interactions.

Two-dimensional micelles probably do not exist. A three-dimensional micelle with perfect spherical symmetry would not tend to adsorb, as there would be extensive solvent-micelle interactions in all orientations. Thus, a spherical micelle would most likely never get close enough to a solid surface (10 to 20 A) for the interactions to be significant. If, however, one could have a micelle which is not truly spherical or well-shielded by the polar head groups, then certain orientations of the micelle might be favorable for interaction and even adsorption.

Such a micelle is sketched in Figure 20c, showing a slight puckering due to surface-micelle interactions. The micelle in Figure 20c is a reasonable approximation to the structure of some simple proteins; these will be discussed later.

Surface-solute interactions can be significant in distorting and reorienting structures to optimize the interactions and the consequent adsorption of solute. Such interaction and distortion can occur in spite of apparent shielding of the solute by polar-solvent interactions.

If the micelle is charged, then there would be even stronger interactions with the solvent, along with the increased complexity of counter ions. It is doubtful that a charged or even strongly polar micelle would adsorb from water solutions, as electrostatic interactions with the solvent would be much greater than the dispersion and induced-dipole interactions with the surface. For a very large micelle, e.g., a protein, the charge or dipole to surface area ratio would be much less, and the force of adsorption could be very significant.

b. Randomly Coiled Polymers

An apolar polymer probably could not exist in a random configuration in aqueous solution. Hydrophobic bonding would no doubt produce a relatively globular polymer configuration. This case is really no different from those treated earlier. As the polymer comes under the influence of the interface, certain "faces" of the globule could be

more energetically favorable for interaction than others; this will be evident later when ribonuclease is examined in detail. Rotations and distortions would occur to optimize the interaction. Once the polymer is at the interface, one might expect that the more mobile portions of the chain would tend to lie flat to further optimize the interaction. Certain bonds would no doubt be oriented for maximum interaction, perhaps on fold surfaces of crystallites, where both the C-C bonds of the surface and a C-C bond of the solute would be a parallel configuration, thus leading to extremely strong interactions. Such interactions may be irreversible and could be considered pinning points. An adsorbed polymer can, therefore, be visualized as a relatively globular (in aqueous solution) mass on the surface, with some portions of the chain oriented parallel to the surface, and with various groups optimally oriented, probably acting as pinning points.

Consider the effect after a monolayer of adsorbed polymer has formed. The structure of the monolayer is sketched in Figure 21 (see Ref. 89). Except possibly for extremely dilute solutions, one cannot expect the molecule to lie completely flat on the surface, though such a configuration might be the most energetically favorable. Thus the monolayer will have a thickness, d, probably related to the dimensions of the globule in solution. Because of the large number of fairly direct interactions, one would expect such an adsorbed layer to be irreversible, though the statistical fluctuations of flickering clusters and thermal

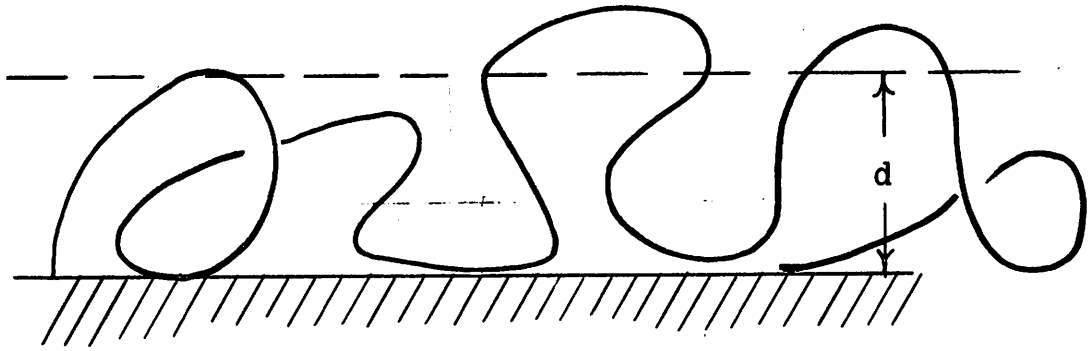


Figure 21. A Possible Structure for a Monolayer of Adsorbed Polymer (see Ref. 89).

energy would occasionally cause an adsorbed polymer to desorb; another one would most likely take its place. Eventually, the adsorbed polymers would be relatively fixed and permanent. The result of a fairly rigidly held and immobile monolayer is that a new surface is formed, roughly at a distance d from the original surface. The polymer density of the monolayer would be much lower than that of the polymer substrate, as it would contain trapped solvent molecules. It is reasonable to expect that d would be at least 10-15 A and probably greater. Thus, the substrate polymer would exert a negligible effect on additional solute interactions. The result is that the new surface exposed to the solution is composed of a low density polymer permeated by the solvent; this is the situation of Figure 16. The monolayer-solute interactions must be of smaller magnitude than the original substrate-solute interactions (Figure 16), due to the decreased density of the monolayer and its water content. Also, optimum binding sites, which

could have existed on the original polymer surface, are now absent. The net result is that adsorption on the monolayer must be relatively weak, though adsorption of the monolayer itself is quite strong. If adsorption on the monolayer does occur, it is probably reversible, as charge fluctuations would have a large effect on any species in the multilayers. The result of this discussion is that one must expect polymer adsorption to follow a monolayer isotherm, particularly in non-aqueous solutions, where the $F_n^S - F_n^W$ force could not be very significant. In aqueous solutions this force will be more important (because of the 3.3 term for water) and might lead to multilayer adsorption.

c. Amino Acids and Peptides

The adsorption of amino acids must be expected to be quite different from any solute considered so far. At neutral pH the carboxyl group is ionized, and the amino group may also be charged. Even though the net charge is zero at the isoelectric point, the dipole-water interractions would be very significant. In addition, the peptide bond is capable of strong dipole-dipole interactions. If these were the things to be considered, one would expect that amino acids could not be adsorbed from aqueous solution except possibly by highly charged surfaces. However, the effect of the highly variable side group leads to many possible surface interactions. It is at least conceptually pos-

sible that amino acids with long apolar side chains might interact with an apolar surface strongly enough to adsorb. Such adsorption would be transient, however, as cluster flickerings and thermal excitement could produce desorption very readily.

It is, therefore, possible that amino acids with strongly apolar side chains might adsorb from aqueous solutions, though such adsorption on apolar surfaces would probably be weak and hardly extensive. Amino acids without apolar side chains cannot be expected to adsorb from aqueous solutions onto apolar surfaces.

A peptide is a polymerized string of amino acids. Its adsorption properties should be similar to those of its constituent amino acids, though the charge-water and dipole-water interactions would not be as extensive as for an amino acid. A peptide will adsorb much more readily than its constituent amino acids, but again adsorption will be negligible unless it contains apolar side shains. A very long chain peptide can be considered a simple protein, if it is made up of many different kinds of amino acids.

d. Proteins

Detailed calculations of the dispersion interactions between proteins and a polymer surface will not be attempted. Such calculations are theoretically possible for those proteins whose structure is completely known, but the task would be immense. The polarizabilities

of C-N and N-H bonds are about the same as for C-C and C-H bonds, respectively. The polarizability of the carbonyl bond is also quite high ($\alpha_{\parallel}$ is about 20, $\alpha_{\perp}$ about 10). One must, therefore, expect significant dispersion contributions from the peptide linkages as well as from the apolar side chains. Order of magnitude estimates of the interaction energy can be made using the generalizations deduced by Vold [132] and previously discussed. Before doing this, it will be useful to closely examine a protein whose structure is completely known. Such information is only available for quite small proteins.

The volume and mean diameter of some proteins is given in Table XIII along with data on surface area and surface charge density (in square angstroms per net charge). Values of the net charges and polar-apolar ratios (P/AP) were given in Table II.

The properties of ribonuclease were given in Table II; its amino acid sequence is given in Figure 22, in which the disulfide bridges are shown and each cysteine is given a number (I to VIII). The numbers in Figure 22 are keyed to Figures 23a and 23b, which show Scheraga's schematic model of the structure of ribonuclease. The enzyme is composed of six helical sections, one helix at right angles to the other five. The detailed molecular model has been constructed and photographs of it are available.[156-7] Table XIV gives the amino acid sequence, net charge, and polar-apolar nature of the

TABLE XIII

PHYSICAL AND STRUCTURAL DATA FOR SOME PROTEINS

Protein	M	Semi-Major Axis, a, A	Semi-Minor Area, b, A	Surface Area, A^2 *	Volume A^3 **	Mean Diameter ***	Surface Area / Net Charge ****
Ribonuclease	14,000	25	12	3,160	15,000	11	790
Albumin	69,000	80	20	16,200	134,000	51	300
Gamma Globulin	156,000	120	25	30,600	315,000	68	≠
Fibrinogen: Ellipsoidal	340,000 to 400,000	325	32	102,000	1,400,000	110	340
Nodular	"	see Fig. 24	see Fig. 24	56,000	410,000	74	190

* The surface area of a prolate spheroid (formed by the rotation of an ellipse about its major axis, 2a) is:

Surface Area = $2\pi(b^2 + (ab/\epsilon)\sin^{-1}\epsilon)$, where ϵ = eccentricity of the ellipse = $\sqrt{a^2 - b^2}/a$ and $0<\epsilon<1$.

** The volume of a prolate spheroid is $V = 4\pi a b^2/3$.

*** The mean diameter is given by: $d = V^{0.33} = [4\pi ab^2/3]^{1/3}$.

****See Table II.

≠ If one assumes all side chains have access to the solvent, the value is about 260. However, this is a very poor assumption, as the pI data of Table II indicate. The variable structure of the gamma globulins prevents one from deducing a value for the surface charge density.

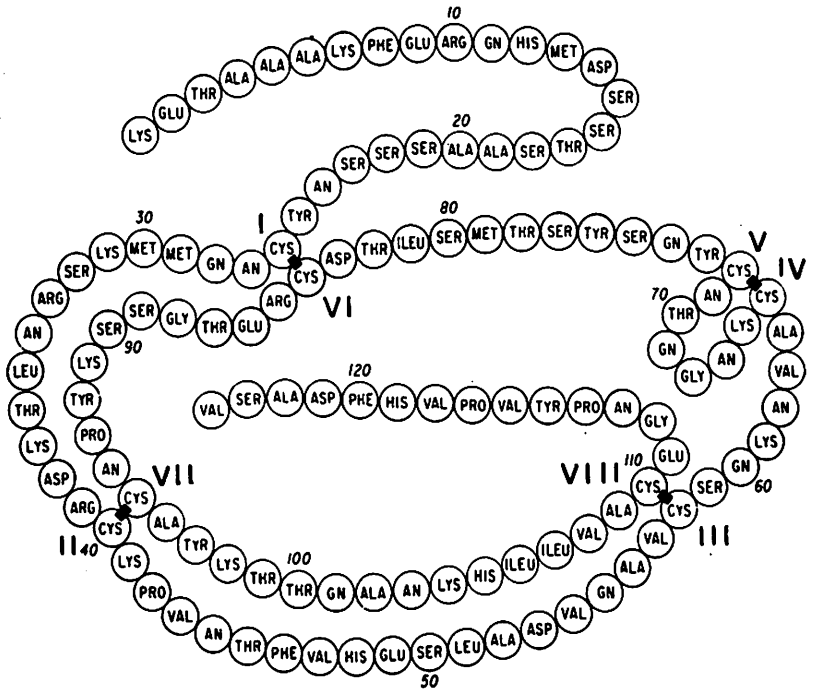
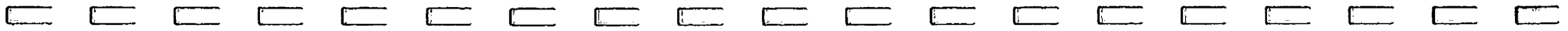


Figure 22. The Amino Acid Sequence of Ribonuclease. The Roman numerals Refer to the Disulfide Bridges in Figure 23. (After Ref. 36, p. 11.)



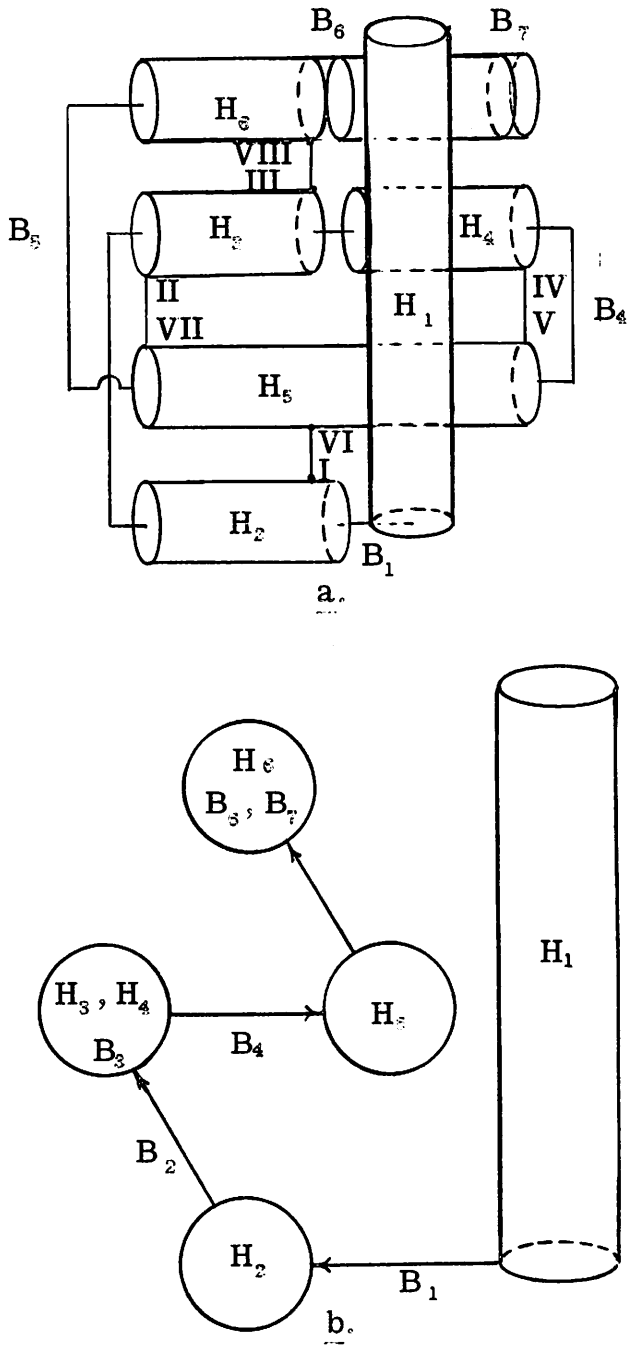


Figure 23. A Schematic Representation of the Structure of Ribonuclease (From Refs. 156, 157); a. Top View; b. Side View.

TABLE XIV

THE AMINO ACID CONTENT, NET CHARGE, AND P/AP RATIOS FOR THE HELICES OF RIBONUCLEASE*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
$NH_2 \oplus \rightarrow$	+	-	P	AP	AP	AP	+	AP	-	+	P	P	AP	-	P	P	P	P	AP	AP	P

Helix = H_1; P/AP = 2.0; Net Charge = 0

22																					37
P	P	P	P	P	P	P	AP	AP	+	P	+	P	AP	P	+						

Helix = H_2; P/AP = 4.3; Net Charge = +3

40																									65
P	+	AP	AP	P	P	AP	AP	P	-	P	AP	AP	-	AP	AP	P	P	P	+	P	+	P	AP	AP	P

Helix = H_3 - H_4; P/AP = 1.4; Net Charge = 0

72																									94
P	P	P	P	P	P	P	AP	P	AP	P	-	P	+	-	P	?	P	P	+	P	AP	P			

Helix = H_5; P/AP = 6.3; Net Charge = 0

98																												124	
+	P	P	P	AP	P	+	P	AP	AP	AP	AP	P	-	?	P	AP	P	AP	AP	AP	P	AP	-	AP	P	AP	⊖		

Helix = H_6; P/AP = 1.2; Net Charge = 0 COOH

*See also Figures 22 and 23. The N- and C- terminal groups were not counted in the P/AP ratio and net charge determinations.

different helices (helices 3 and 4 are combined in Table XIV). The non-helical portions of the molecule are not considered. It is obvious from Table XIV that the different helices have strikingly different polar-apolar natures. The short helix, H_2, is strongly polar and is the only helix with a net charge. Helix H_5, is very highly polar, but its net charge is zero. Thus, the characteristics and properties of ribonuclease certainly cannot be expected to be symmetrical, even though the shape of the molecule is roughly spherical. The interactions of ribonuclease with a surface will thus be strongly dependent on the orientation of the protein with respect to the interface. Helices H_1, H_3-H_4, and H_6 should be able to approach quite closely to a surface and interact strongly by dispersion forces; charge-induced dipole interactions would tend to cancel out, as the net charge on these helices is zero. The presence of charge- and dipole-bound water would hinder the approach to the surface, however. If the adsorbing surface is negatively charged, one might expect H_2 to be more energetically favorable, because of its net positive charge. For an apolar surface, only helices H_6 and H_3-H_4 can be expected to interact strongly, due to their greater apolar nature. The other helices would interact with water much more strongly, thus their approach to the surface would be hindered.

The consideration of the individual helices serves to indicate that the P/AP ratio of Table II may be highly misleading, at least

for the consideration of surface interactions. According to the ratio, ribonuclease is very polar, but from Table XIV it is clear that much of that polarity resides in helix H_5. Helix H_5 cannot be expected to interact directly with a surface, as it is essentially shielded or at least hindered by all of the other helices.

The interaction of H_3 -H_4 and H_8 may be aided by the puckering mechanism of Figure 20. The non-helical and non-hydrogen bonded regions, B_5 and B_8 (see Figure 23a), may be easily deformed, particularly at B_5, as it is somewhat apolar. Adsorption could, therefore, occur first at certain preferentially deformable sites; once these sites have tentatively affixed the molecule to the surface, other interactions would become important, as the separation distance would then be relatively small.

If one ignores the detailed structure of ribonuclease and simply considers it as an ellipsoidal organic particle, the interaction energy would only become significant at a distance of the order of 10-11 A from the surface (the value of the mean diameter, Table XIII).

The above discussion shows that one must expect a protein to be a very heterogeneous structure—capable of adsorbing by different mechanisms in different orientations on different surfaces. The heterogeneity of ribonuclease may be greater than in non-enzymatic proteins, as an enxymatically active site may require significant property differences among the participating helices. Ribonuclease is also peculiar

in that it is positively charged at neutral pH, while most plasma proteins are negatively charged. Also, the effect of non-helical regions has been ignored. These could be very important for both their own particular interacting behavior and the steric influences they might exert on the helix-surface interactions.

Albumin has been discussed and some of its properties were given in Tables II and XIII. As it is composed of a single polypeptide chain, the hypothetical protein of Figure 9 may be a reasonable approximation for the structure of albumin. Though the P/AP ratio for albumin is not particularly high (Table II), it is a very polar protein. Its polarity is indicated by its solubility properties, as well as by the relatively high surface charge density (about one net negative charge for every 300 A^2 of surface; Table XIII). If the protein is hollow[64] and the inner core contains water, then the charge density would be much lower.

As shown earlier, charge-induced dipole interactions are negligible until the molecule is very close to the surface; thus albumin must interact primarily by the adsorption forces discussed earlier. There could easily be relatively apolar portions of the molecule, as observed for ribonuclease. Again a puckering or distortion mechanism of initial adsorption could be acting.

Vold's mean diameter (Table XIII) criteria[132] indicate that the surface-albumin interaction becomes quite significant at a separation distance of about 50 A. Thus, albumin must be brought to the interface

by dispersion interactions; the interactions are optimized if the long axis of the molecule is parallel to the surface. Maximum interaction occurs when the long axis is flat on the surface, but the actual thickness of a monolayer must be somewhat greater than the minor axis, due to the non-efficient packing of an adsorbed layer. It is possible that short-range charge-induced dipole interactions would modify the orientation, but this is doubtful as the charges would be interacting strongly with the solvent and most of them would probably never come close enough to the surface to have a very significant effect.

It is unlikely that the adsorption process could produce a layer of efficient packing, thus the density of the adsorbed layer would probably be less than that of the substrate. Also, the adsorbed proteins would contain some bound and trapped water. This means that adsorption is expected to stop after the formation of a monolayer (see Figure 11). In addition to the density effect, the monolayer would now exhibit the net charge of the proteins within it. Thus there would be a tendency for the monolayer to repel similarly charged proteins. These two reasons for the absence of multilayer formation are not absolute, however. If the charge distributions are strongly localized, as discussed earlier, then multilayer formation could still occur.

The gamma globulins are much larger than albumin, though the overall shape seems to be about the same. Their mean diameter is 68 A, thus one would expect strong dispersion interactions out to

about 70 A. It is clear from the earlier discussion and the data of Table II that it is not possible to draw conclusions about the surface charge or structure of the gamma globulins. One might expect that their weak structure and resultant ability to respond structurally to subtle influences would make them easily denaturable. This same tendency would make them more susceptible to puckering distortions. As the isoelectric point of the gamma globulins can vary between 5.8 and 7.3 (Table II), depending on the fraction, some fractions can be uncharged at neutral pH, thus the argument of electrical repulsions between adsorbed solute molecules would not hold. Monolayer formation is still expected, however, due to packing, density, and permeability considerations.

It is reasonable to assume that the density of gamma globulin must be less than albumin, because the gamma globulins have proportionally a much lower alpha-helix content and fewer disulfide bridges than albumin. This assumption is not supported by the data of Table XIII, but this could be due to the ellipsoidal shapes of the molecules. The overall ellipsoidal shape of the molecule also includes its hydration layer, which can be a sizeable contribution to the total volume (Figure 9).

If gamma globulins are much less polar than albumin, as has been discussed, they would tend to have a much smaller hydration layer. Thus, the volumes given in Table XIII may be deceptive, as the volume

given for albumin may be significantly larger than the true volume. If this is the case, gamma globulin would indeed have a lower density than albumin, which would mean its dispersion interactions would not be as strong (per unit volume). One must not, therefore, expect gamma globulin to adsorb more strongly than albumin. A monolayer of gamma globulin would still be thicker than a monolayer of albumin because of the greater size of the molecule.

Fibrinogen cannot be considered as a globular or even ellipsoidal molecule. The data of Table XIII for the ellipsoidal model indicate that the density of fibrinogen (molecular weight per unit volume) is half that of albumin or gamma globulin, which is not reasonable. The dumbell-shaped structure sketched in Figure 24 will, therefore, be used for fibrinogen. This results in a surface area about twice that of gamma globulin but a volume not much greater than that for gamma globulin. The surface charge density is quite high, higher even than albumin. Part of this difference is because the dumbbell-shape dimensions are for the dry molecule and do not consider the volume of hydration. Thus the true area and volume in solution is probably a compromise between the two structures given in Table XIII. In any event, the net charge density will still be relatively high. The previous discussions indicated a lack of charge asymmetry along the long axis. The charge must, therefore, be evenly distributed.

Fibrinogen-surface dispersion interactions are probably significant out to between 70 and 100 A. The greatest interaction would be in the parallel orientation. One must expect fibrinogen to be a rather clumsy, unpredictable protein for adsorption considerations. As it contains large nodules, its orientation for adsorption is not necessarily as straight-forward as for albumin and gamma globulins. If a fibrinogen molecule "stumbles" into the vicinity of an interface in the perpendicular orientation of Figure 24, it will most likely continue to be adsorbed in that orientation if it is within 50 A of the surface (the mean diameter for a sphere 65 A in diameter is about 50 A*). It is probably not reasonable, therefore, to assume adsorbed fibrinogen to have a particular orientation. The majority of the molecules should be oriented parallel while many may have the perpendicular orientation, at least initially; after initial adsorption, close range interactions could pull a molecule from the perpendicular orientation into a more parallel position. This is not unreasonable, and is somewhat compatible with the structural properties of fibrinogen, i.e., that the nodules are connected by "...loose, sponge-like segments..." (see Ref 64).

Fibrinogen adsorption should result in a loosely packed layer of flat molecules entangled with bent or distorted molecules which were initially adsorbed in the perpendicular orientation. The thickness of such a layer should be somewhere between 70 and perhaps several

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*This curious result is due merely to the definition of mean diameter. The mean diameter is defined as the cube root of the volume.[132]

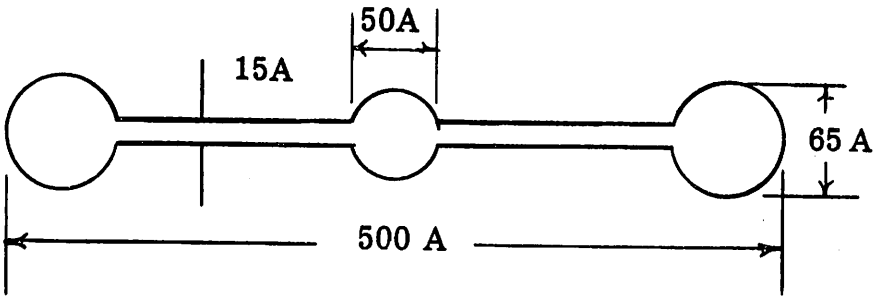

Figure 24. The Nodular Structure of Fibrinogen

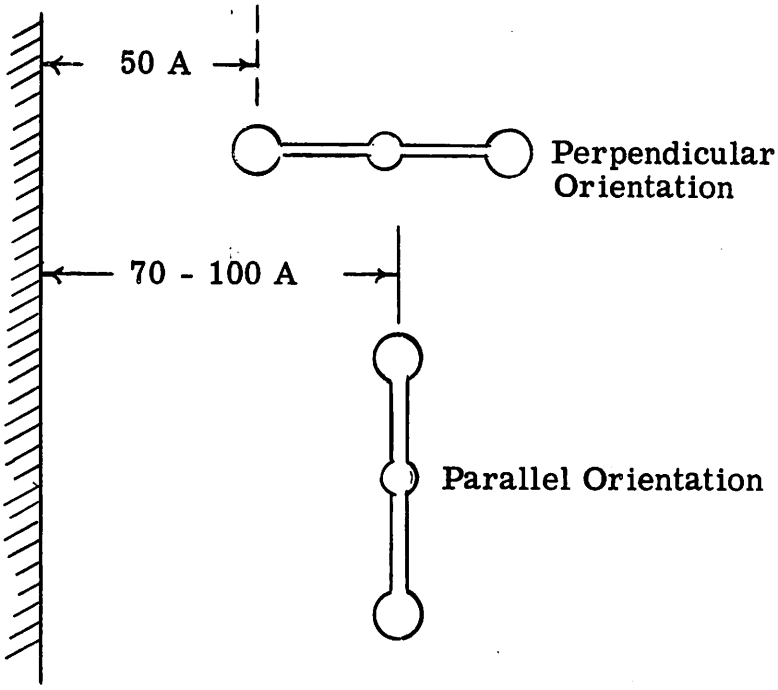

Figure 25. Two Limiting Orientations for a Fibrinogen Molecule in the Vicinity of an Interface.

hundred angstroms. Multilayer formation is not expected.

Fibrinogen will interact significantly with a surface by dispersion forces at greater distances than the other proteins considered. Thus, adsorption of fibrinogen can be expected to be more rapid than for the other proteins discussed. It is also possible that, because of its higher molecular weight and rod-like structure, it will interact more strongly with a surface than albumin or gamma globulin. This could result in competitive adsorption, where fibrinogen may successfully compete for occupied regions of the surface.

C. Comparison of the Model with Experiment

1. Simple Compounds:

The model predicts that apolar compounds should have relatively large adsorption forces acting on them, as the 3.3 term will dominate the other terms in equation (24); this will be the case at air/water and apolar polymer/water interfaces. The data of Tables IX to XI show that the forces and energies of adsorption must be significant at distances of 10 to 15 A, depending on the size of the molecule considered. Figure 14 clearly shows that the adsorption of large molecules is favored over smaller ones. It was demonstrated in Figure 15 that the differences between solute molecules result in significantly greater adsorption forces than differences among the apolar polymer surfaces. The solute molecule is also expected to approach the surface in a more or less perpendicular orientation, though it will tend to assume a parallel orientation if space and time are available for it to do so. Because of the relatively low polarizability of water (Table IV), F_p^s is usually greater than F_p^w, thus there is a slightly greater adsorption tendency at polymer/water than at air/water interfaces. This conclusion is evident from Figure 17, where the Y=0 curve is equivalent to that for an air/water interface, while the Y=1 curve represents the adsorption force at the polymer/water interface.

The model also predicts multilayer and monolayer adsorption, depending on the structure and nature of the first adsorbed layer. The

data of Table XII and Figure 16 clearly demonstrate that the adsorption forces decrease rapidly as the water content of the surface increases. An adsorbed layer which contains trapped or bound water molecules will, therefore, interact more weakly than the original surface. This has been discussed and was sketched in Figure 21 for random coil polymers, but the analysis is just as valid in the general case. If the adsorbed layer does not contain water, as might be expected of an adsorbed hydrocarbon, then the situation is given by Figure 17. Though the monolayer must have a lower density than the original surface, the force of adsorption is only slightly decreased. If the monolayer was of zero density and contained no water, adsorption would still occur, just as it does at the air/water interface.

The above predictions are in good agreement with experiment. Most of the generalizations and conclusions in Section A. 3. b (Adsorption of Simple Compounds) are in agreement with the predictions of the model. It was noted in that section that adsorption tends to be preferential for that component which most reduces the surface or interfacial tension; this conclusion is evident from Figure 7 and equation (24).

Perhaps the most complete study of adsorption of simple compounds at a polymer/water interface is that by Schneider et al.[158] They studied the adsorption of several hydrocarbons, alcohols, and organic acids from aqueous solution onto polystyrene beads. Their isotherms for ethane, propane, and n-butane are replotted in Figure 26; the

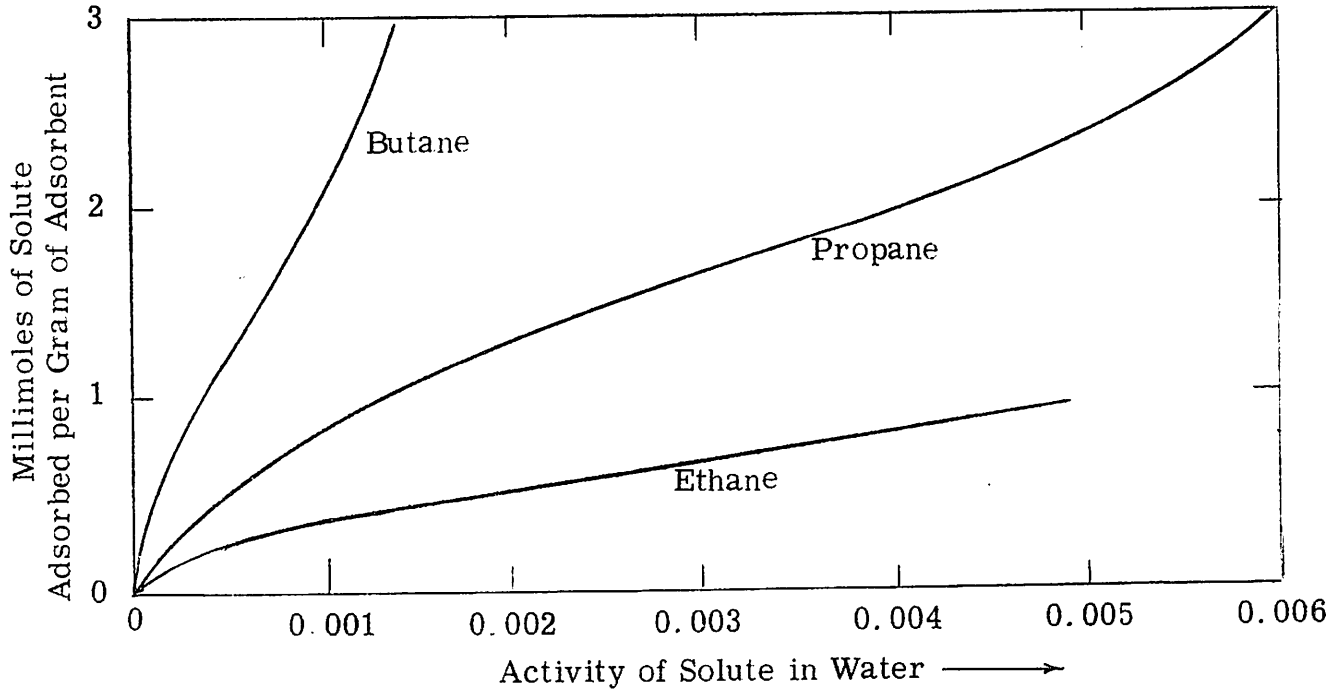

Figure 26. Adsorption Isotherms for the Adsorption of Simple n-Alkanes onto Polystyrene Beads (After Ref. 158).

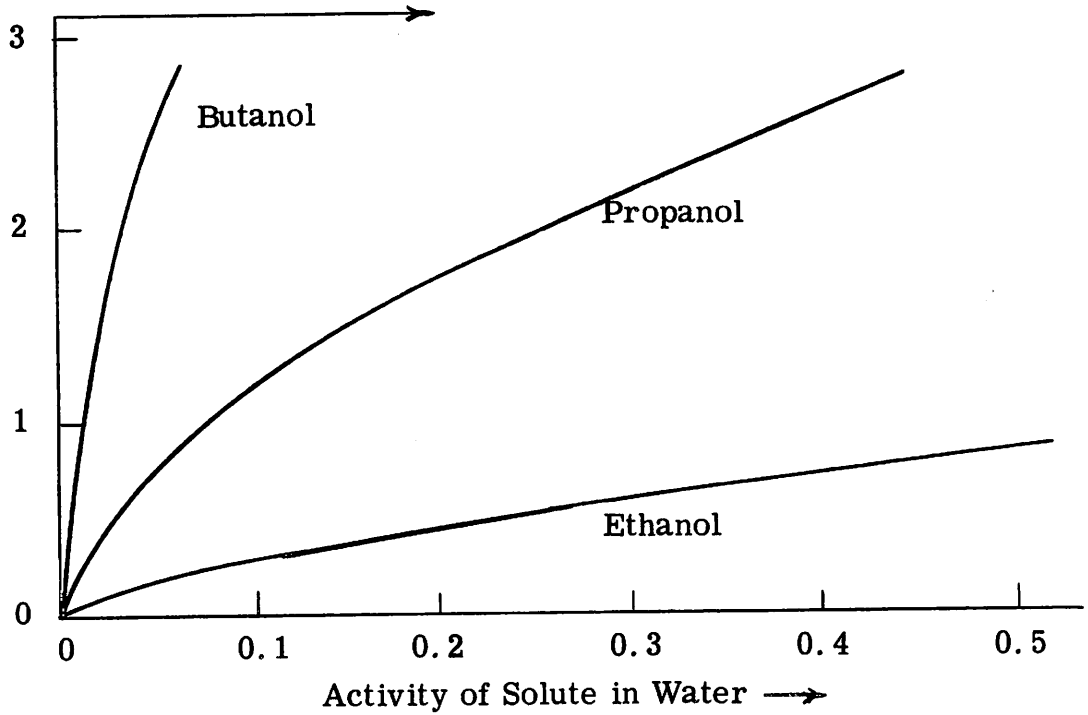

Figure 27. Adsorption Isotherms for the Adsorption of Simple n-Alkanols onto Polystyrene Beads. (After Rev. 158.)

isotherms for ethanol, propanol, and n-butanol are given in Figure 27. These isotherms (and most adsorption isotherms) are for equilibrium adsorption, thus one cannot obtain kinetic information from them. It is clear from the isotherms presented and from most isotherms in the literature that the degree of surface coverage or amount adsorbed is not merely a simple function of the size of the molecule. Propane would be expected to occupy roughly 50% more surface area than ethane, and butane twice as much area, yet the isotherms certainly do not show any such relationship. If adsorption is due to active sites, one would expect the number of moles of solute adsorbed per unit area of surface coverage to be independent of molecular size, as there is little reason to expect an active adsorption site for propane to differ from one for butane.

The extent of the isotherms presented is of course limited by the solubility of the solute in water. Ethanol is highly soluble, while butanol is much less soluble. There is some question about the hydrocarbon isotherms. In the case of butane and propane the isotherm indicates multilayer formation. It is possible, as pointed out by the authors,[158] that the adsorbed alkanes may diffuse into the polystyrene.

It is difficult to quantitatively examine Figures 26 and 27, as the surface area of the swollen polystyrene adsorbent is not known. It is clear, however, that the alkanes adsorb very readily from very dilute solutions; the alkanols require a hundred-fold greater concentration to produce the same amount of adsorption. The alkanols appear

to follow a monolayer (Langmuir) isotherm, while the alkanes seem to form multilayers.

The model predicts that alkanes should tend to produce multilayer isotherms, as discussed above. Small alkanes should not trap any water as they are adsorbed; in fact, the movement of the water between the solute and the interface is one of the major driving forces for adsorption (Figure 7). Therefore, one would expect a layer of adsorbed hydrocarbon to be relatively water free and probably less dense than the substrate. Thus adsorption of multilayers is expected to occur, though probably to a lesser extent than the original adsorption. These results are in good agreement with the isotherms of Figure 26.

The adsorption of small alkanols is expected to be relatively weak. The force of adsorption is now greatly decreased as compared to alkanes because of the extensive dipole-dipole interactions which must take place between the water solvent and the -OH group of the alcohols. Any adsorption which does occur would probably be quite reversible, as desorption must occur relatively easily whenever a water cluster forms nearby in an optimum interaction orientation. In order to obtain significant adsorption, Schneider et al.[158] had to use relatively high alcohol concentrations; this no doubt acted to eliminate some of the reversibility of adsorption.

Figures 26 and 27 are also in agreement with the conclusion

that large molecules are expected to interact more effectively than smaller ones. This conclusion is due to two effects; the greater force on a large molecule gives it an advantage in coming to the interface; once it is at the interface, it can interact strongly with each of its polarizable regions, which will tend to hinder desorption (the greater the number of strongly interacting sites, the less chance that desorption will occur). The net result is that larger molecules probably are adsorbed more rapidly than smaller molecules and, once adsorbed, they tend to stay there longer. The isotherms of Figures 26 and 27 are thus in qualitative agreement with the model.

The adsorption of polar compounds has been discussed qualitatively and some comparisons can be made. Data are available on the adsorption of some amino acids and peptides,[158] as well as on the alkanols just discussed. The following amino acids were studied[158] in solution at their isoelectric point: glycine (AP), alanine (AP), proline (AP), serine (P), glutamic acid (P), aspartic acid (P), tyrosine (p), and phenylalanine (AP). Only the latter two aromatic amino acids were adsorbed, but not to a significant degree. The adsorbent was polystyrene beads, thus the aromatic acids probably interacted directly with the pi-orbitals of the polystyrene. It is clear, however, that amino acids do not tend to adsorb. It would have been interesting if the study[158] had included leucine or isoleucine as well, as the long apolar side chain might have been more favorable for adsorption.

The same study did consider two peptides: gly-gly-gly-gly and leu-gly-gly. The former did not adsorb, supporting the contention made at the bottom of Table II, i.e., that though glycine can be considered to have an apolar side chain, steric effects must prevent it from interacting significantly. The leu-gly-gly peptide did adsorb (on polystyrene), showing that the leucine side chain can exert a considerable influence on the adsorption properties of a peptide or amino acid. Thus the results cited for amino acids and peptides are in agreement with the qualitative predictions made earlier.

2. Polymer Adsorption:

Polymer adsorption has not been widely studied and few generalizations are available. The model predicts that polymers adsorbed from solution onto polymer surfaces should form a relatively loosely packed and solvent permeated monolayer. Multilayer adsorption is not expected; the argument is the same as given above. Even though the molecule remains relatively globular and loosely packed on the surface, its very nature and size provides a large number of close-range interactions with the surface. As noted earlier, it is statistically improbable that all of these interactions could be disrupted simultaneously, thus polymer adsorption tends to be irreversible. Also, as noted above, the larger the molecule, the more stable it must be on the surface. Thus polymers of higher molecular weight must have

a longer surface lifetime than those of lower molecular weight; this is the basis of competitive adsorption. Equilibrium is thus difficult to achieve and requires a long time. The initial adsorption is expected to be very rapid, however, following the trend discussed above (the larger the molecule, the greater its adsorption tendency).

3. Protein Adsorption.

The detailed discussion of ribonuclease showed that protein adsorption must be extremely complex, as different portions of the same molecule may interact by very different mechanisms. Until the detailed structure of many more proteins is available, one must resort to qualitative discussions and extremely rough calculations. Using Vold's criteria[182] and applying it to protein-solid dispersion interactions, it was concluded that protein adsorption (to a first approximation) will be a function of the size and density of the molecule. Thus, it is expected that adsorption would increase in the order albumin, gamma globulin, and fibrinogen. Only monolayer adsorption should occur, with the monolayer thicknesses somewhat greater than the minor axis but significantly less than the major axis. The rod-like nature of fibrinogen will probably allow it to interact more strongly with a surface than the other proteins, thus, as discussed above, competitive adsorption should be expected.

Lyman et al.[19] have succeeded in determining protein adsorp-

sorption isotherms on plane polymer surfaces by means of total reflection infra-red spectroscopy. Their isotherm for gamma-globulin adsorption on polystyrene (at 37 C for two hours from distilled water) is given as Figure 28. They studied the adsorption of albumin, gamma globulin, and fibrinogen on commercial polystyrene, polyethylenes (low density), polydimethyl siloxane, and on a fluorinated ethylene-propylene copolymer (Teflon FEP). Except for gamma globulin on Teflon FEP, the behavior was analogous to Figure 28 for all combinations. Their results are given in Table XV; film thicknesses varied from 44 to 138 A (assuming a protein density of 1.3) but there was no adsorption of gamma globulin on the fluorocarbon. It is thus clear that the surface free energy concept[39] does not hold for protein adsorption since there is no trend of absorbed thickness with surface energy, though Lyman et al.[19] showed that such a trend does hold for platelet adsorption. The adsorbed proteins could not be desorbed over a wide pH range, thus Lyman et al.[19] concluded that "...adsorption in these systems is not reversible."

The above results are in qualitative agreement with the earlier discussion, though the values for polystyrene are lower than expected. The earlier calculations show polystyrene interacting more strongly than the other polymers. Lyman has also found[15 9] that adsorption increases in the order albumin, gamma globulin, fibrinogen. (See also Table XV).

TABLE XV

EQUILIBRIUM LAYER THICKNESSES FOR THE ADSORPTION OF PLASMA PROTEINS ON SEVERAL POLYMER SURFACES*

(After Ref. 19, p. 252)

Polymer	Layer Thickness, Angstroms		
	Albumin	Gamma Globulin	Fibrinogen
Polystyrene	44	54	130
Polyethylene (Low Density)	62	77	96
Polydimethyl siloxane	120	138	120
Fluorinated Ethylene-Propylene Copolymer (Teflon FEP)	62	0	108

*Adsorbed from distilled water at 37 C for 2 hours.

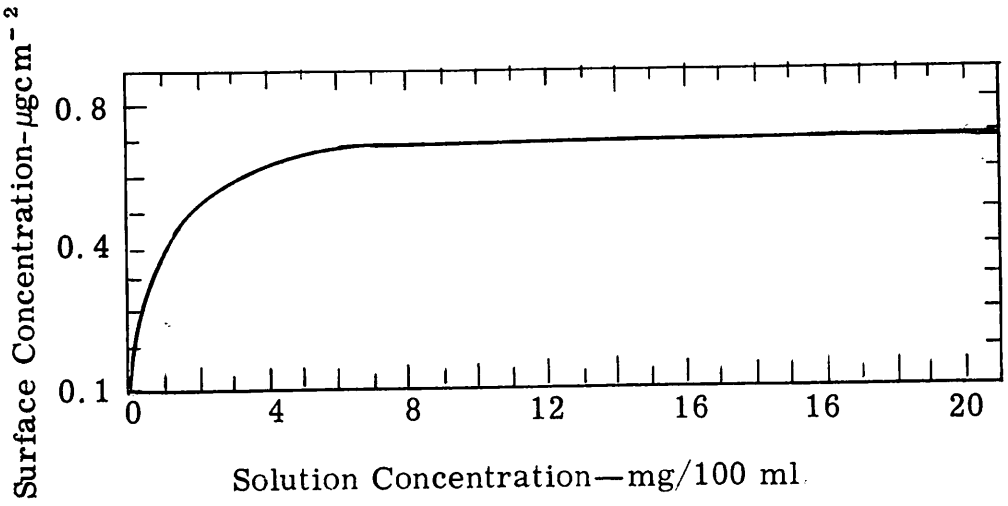

Figure 28. Adsorption of Gamma Globulin onto Polystyrene at 37 C. (From Lyman et al., Ref. 19, p. 251).

The layer thicknesses are quite large. They were interpreted as due to [19] "...the formation of a monolayer with the dimensionally intact globular molecules in closely packed array more or less end-on to the surface." This interpretation is compatible with the discussion of layer thicknesses given earlier.

Figure 13 showed that polyethylene and PTFE tend to interact to the same degree with solute molecules. This result is confirmed in Table XV by the data for polyethylene and Teflon FEP for albumin and fibrinogen. The result for gamma globulin is not explained; it is very interesting, but unpredictable by the criteria given here.

Vroman has studied the interaction of blood and blood proteins with surfaces using an ellipsometer. Much of his work and views on the role of surfaces in blood coagulation and protein adsorption are summarized in two recent reviews.[2,160] He has exhaustively surveyed and studied the role of hydrophobic surfaces in coagulation in an earlier paper.[18] The nature of the ellipsometric technique produces limitations on the selection of suitable substrates. Vroman usually uses tantalum oxide or silicon surfaces which are either wettable or non-wettable, depending on the surface treatments used (see Ref. 18). Thus the surfaces studied are certainly not polymeric but can be considered to consist of relatively loosely packed hydrocarbon chains for the non-wettable surfaces, as it is often prepared by rubbing on a monolayer of ferric stearate.

Ellipsometric studies are the only current source of information on adsorption dynamics. Vroman has found that "...all protein-containing solutes showed adsorption onto all four types of surfaces tested, at an initial rate of about 10-20 A per minute." (Ref. 2. p. 299). There was no difference in adsorption rates at room temperature on wettable or non-wettable surfaces,[18] which tends to indicate that the solute-solvent interactions dominate solute-surface interactions. After a few minutes the rate decreased abruptly for purified protein solutions and somewhat gradually for mixtures of proteins. This result tends to indicate monolayer formation of the purified proteins and continuing competitive adsorption of the protein mixture. Vroman has shown that multiple protein layers can be adsorbed under appropriate conditions.[160] Adsorption studies on hydrophobic powders have shown that many proteins (particularly certain coagulation factors) are preferentially adsorbed, including fibrinogen.[18,160] In the case of fibrinogen the preferential adsorption may be due to its great size and geometry; the coagulation factors which favor non-wettable surfaces may have relatively apolar "faces" as observed for ribonuclease. The film thicknesses Vroman gets are usually 30 to 40 A for most proteins and up to 80 A for fibrinogen.[18] These results are more in agreement with the discussion given earlier than those of Lyman.[19]

The conclusion that adsorption will not tend to occur on a surface containing a high water content (Figure 16) leads to some possibilities

for the preparation of adsorption-resistant surfaces. Such a surface probably results when a protein is chemically bonded to a polymer surface. The proteinated surface is probably similar to that of an adsorbed layer of protein, except that chemically bonded protein could not be desorbed; competitive adsorption thus could not occur. Such a surface has been prepared by binding albumin to chloromethylated polystyrene using an aqueous Friedel-Crafts alkylation reaction (See Chapter II). Attenuated total reflection infra-red data showed significant amounts of bound protein (Figures 1 and 2). Vena cava rings of this material were implanted in dogs by Dr. Vincent Gott of the Johns Hopkins University School of Medicine; initial results for chronic (two hour) and acute (two week) tests were very good, as no clots were found in the rings. The preliminary results of this severe test indicate that a proteinated surface may be a good coagulation-resistant material. Long-term tests, particularly on the stability and life-time of such a surface, are needed before definite conclusions can be made. The behavior of the surface is compatible with the model, if blood coagulation is truly a protein adsorption-dependent process.

Protein denaturation was not specifically discussed. It was noted that orientations of maximum interaction are favored. It is, therefore, reasonable to suspect that the forces of adsorption will continue to operate until the solute is flattened down on the surface and cannot be "pushed" farther. If the bonds responsible for the tertiary

structure in a protein are weaker than the adsorption force, those bonds may be disrupted, and denaturation will occur.

The discussions on protein adsorption given in Section B are thus in reasonable agreement with experiment. Much more adsorption data and further development of the model will be required, however, before a mechanistic model of protein adsorption can be produced.

solutions, especially prepared surfaces, which can significantly intersect with water, may show promise as adsorption-resistant materials.

2. The Role of the Solute in Adsorption from Solution

In general, the larger the solute molecule, the greater its tendency to adsorb by dispersion interactions. This trend is clearly demonstrated in Figures 14 and 15, as well as by Vold's conclusions.[132] If the solute can compete for solvent interactions, then the force of adsorption will be decreased; this is evident in Figure 7. In some cases solute-solvent interactions may be greater than solvent-solvent interactions; the result in this case would be negative adsorption (Figures 4 and 7). Adsorption tends to become irreversible when the solute becomes quite large, as for a polymer or a protein.

A complex solute, such as a protein, probably adsorbs by different mechanisms, depending on the surface and on the orientation of approach of the solute. Certain regions of the molecule may interact in a particularly strong manner; if such regions are not tightly bound to other portions of the solute, distortion (puckering) effects may occur, which optimize the interactions.

3. The Role of the Solvent in Adsorption from Solution

Adsorption from aqueous solution is primarily dependent on the cohesiveness of water. The primary adsorption force is due to solvent-solvent interactions, influenced by solute-solvent effects.

Adsorption from other, less cohesive, solvents is expected to be much different, as then the B and C curves in Figure 7 would be much closer together; solvent competition effects would then be more important.

4. Critique and Limitations of the Model

The model is limited in that it considers only non-ionic aqueous solutions and apolar polymer surfaces. It is further limited in that only dispersion interactions are computed and that solute structuring effects are essentially ignored. Such structuring can be treated as an adsorbed layer; treatments of this type have been given by Vold.[163] The treatment of proteins has been very qualitative and there was no attempt to discuss the competitive adsorption of a number of different proteins. The greatest limitation, however, is the failure to specifically consider dipole-dipole and charge-dipole interactions between the solvent and the charged and polar groups on a protein molecule.

The model does provide a satisfying mechanistic picture of adsorption on the molecular level. It explains in a fairly satisfactory manner a phenomenon which was previously not explainable: monolayer adsorption of polymers and proteins. Perhaps its greatest contribution, however, will be that it can be used to design experiments which will lead to a better understanding of the mechanisms of adsorption.

5. Future Work

The model raises many questions and focuses attention on several areas of inquiry. The main conclusion is that the solvent plays a fundumental and probably major role in adsorption processes, particularly for polar solvents. This result can be tested by studies of adsorption from a series of solvents of varying cohesiveness.

Another major focus is the structure of the adsorbed layer and its role as a "new" surface. Suitable model systems can be prepared by chemically binding molecules to a surface and then studying the adsorption properties of the new surface.

The great bulk of data available on adsorption apparently resulted from many isolated experiments (see Ref. 21 for a discussion). There have been few studies designed to truly pin down a fundamental variable or concept. The work of Zisman[48] is one of the rare cases where detailed studies have been performed leading to basic, general conclusions.

The author intends to study adsorption of simple compounds from aqueous solutions by radioisotope methods as a function of ion content, pH, solute, and nature of the surface. The hope is that eventually it may be possible to formulate some general, fundamental conclusions. He also intends to study the role of surface morphology and crystallinity in the adsorption process by microautoradiographic methods.

CHAPTER IV

SUMMARY

The blood/materials interface is a crucial factor in the successful use of solid materials for blood-contact applications. The interactions which occur at such interfaces will not be understood until one thoroughly understands the mechanism of adsorption from aqueous solution, particularly onto polymer surfaces.

A mechanistic model of adsorption of apolar molecules from aqueous solution onto apolar polymer surfaces is presented. The nature of adsorption, the structure of water, and the forces which exist between molecules are all considered. The model shows that adsorption is a natural consequence of the asymmetric force field which exists in the vicinity of an interface. It shows that solvent-solvent and solvent-solute interactions are of particular importance, especially in aqueous systems. The solvent content of the adsorbate is considered, resulting in the conclusion that adsorption will not tend to occur on a solvent-loaded surface. The model predicts and provides a mechanistic explanation for monolayer and multilayer adsorption; it also discusses and predicts the orientations of adsorbed species. The role of polymer crystallinity effects and "active sites" is briefly examined; however, the role of the solid surface is shown to be minor with respect to solvent-solvent and solvent-solute effects. Calculations are presented

for a $-CH_2-$ group, ethane, butane, and hexane. Qualitative discussions are given for the adsorption of polar molecules and macromolecules, notably proteins. The structure of ribonuclease is examined; it is shown that different areas on the protein will have significantly different intermolecular interactions with the surrounding solvent or with a near-by adsorbate. The conclusion is that a protein must be expected to adsorb by different mechanisms on different surfaces. The surface-protein interactions may be highly dependent on the orientation of the protein with respect to the solid surface. The adsorption of albumin, gamma globulins and fibrinogen is also discussed. The model and its predictions are compared with available experimental data.

The rationale for the preparation of the potentially enzyme-inhibitory and non-thrombogenic polymers, the polyorganofluorophosphates, is briefly discussed. The rationale for preparing proteinated surfaces is also analyzed; the preparation of albuminated polystyrene is treated in detail and its non-thrombogenic behavior is discussed. A brief mention is given to the potential use of fluoroescence microscopy as a tool for studying protein adsorption on the microscopic level.

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ABSTRACT

The blood/materials interface is a crucial factor in the successful use of solid materials for blood-contact applications. The interactions which occur at such interfaces will not be understood until one thoroughly understands the mechanism of adsorption from aqueous solution, particularly onto polymer surfaces.

A mechanistic model of adsorption of apolar molecules from aqueous solution onto apolar polymer surfaces is presented. The nature of adsorption, the structure of water, and the forces which exist between molecules are all considered. The model shows that adsorption is a natural consequence of the asymmetric force field which exists in the vicinity of an interface. It shows that solvent-solvent and solvent-solute interactions are of particular importance, especially in aqueous systems. The solvent content of the adsorbate is considered, resulting in the conclusion that adsorption will not tend to occur on a solvent-loaded surface. The model predicts and provides a mechanistic explanation for monolayer and multilayer adsorption; it also discusses and predicts the orientations of adsorbed species. The role of polymer crystallinity effects and "active sites" is briefly examined; however, the role of the solid surface is shown to be minor with respect to solvent-solvent and solvent-solute effects. Calculations are presented for a $-CH_2-$ group, ethane, butane, and hex-

ane. Qualitative discussions are given for the adsorption of polar molecules and macromolecules, notably proteins. The structure of ribonuclease is examined; it is shown that different areas on the protein will have significantly different intermolecular interactions with the surrounding solvent or with a nearby adsorbate. The conclusion is that a protein must be expected to adsorb by different mechanisms on different surfaces. The surface-protein interactions may be highly dependent on the orientation of the protein with respect to the solid surface. The adsorption of albumin, gamma globulins, and fibrinogen is also discussed. The model and its predictions are compared with available experimental data.

The rationale for the preparation of potentially enzyme inhibitory and non-thrombogenic/polymers, the polyorganofluorophosphates, is briefly discussed. The rationale for preparing proteinated surfaces is also analyzed; the preparation of albuminated polystyrene is treated in detail and its non-thrombogenic behavior is discussed. A brief mention is given to the potential use of fluorescence microscopy as a tool for studying protein adsorption on the microscopic level.

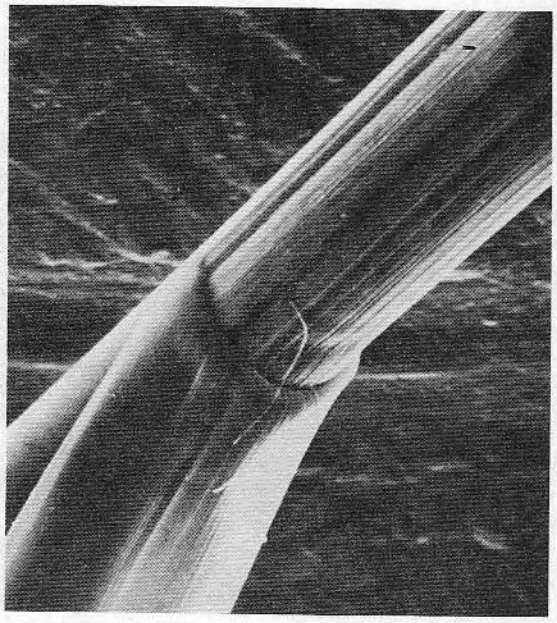
MATERIALS SCIENCE & ENGINEERING— A MODERN MULTIDISCIPLINE

JOSEPH ANDRADE
University of Utah,
Salt Lake City, Utah

Weave together much of modern solid-state chemistry and solid-state physics, include the principles of engineering design and mathematical analysis, and add some medical and biological principles and you have the modern discipline called materials science and engineering. What is it? It is, literally, the study and application of the engineering and scientific aspects of materials: metals and alloys, ceramics, polymers, and composites.

This field ranges from the most sophisticated theories and mathematical methods to the most practical everyday applications and covers everything in between. The scope and diversity are so great that it has been called a superdiscipline by some, a way of attacking problems or a state of mind by others. W. A. Tiller, chairman of the department of materials science at Stanford University, feels that ". . . materials science is not a discipline in its own right but is an area where disciplines converge to give balanced understanding about real problems."

Multidiscipline, superdiscipline, or interdiscipline, materials science and engineering is concerned with the fundamental phenomena of how atoms and molecules come together to form solids, the microscopic and macroscopic structure of these solids, their physical, chemical, and mechanical properties, and, perhaps most importantly, how these solids and their properties can be utilized for the benefit of man. As the structure–property relationships in solids become better understood,



Courtesy William O. Statton, University of Utah

Figure 1. A synthetic polymer being peeled in banana fashion, showing internal microfibrils, 500 Å in diameter.

the materials scientist becomes more concerned with modifying and optimizing the structure to further improve and even tailor-design the properties. This has been called molecular engineering.

Materials science and engineering is one of the oldest fields of inquiry and endeavor known to man. Man was forming and firing pottery long before recorded history. The history and evolution of civilization itself are recorded in terms of metallurgical advances—for example, "Bronze Age," and "Iron Age." Some of the first tools used by prehistoric man were composites (bones) and man became concerned with polymers when he first began to use wood and hides.

Metals, ceramics, and polymers constitute three fundamental classes of materials, each with its relatively unique elemental makeup, bonding type, and resultant characteristics and properties. Many materials cannot be so easily categorized; there are large areas of overlap between the three fundamental types.

Perhaps the best way to introduce one to the subject of materials science and engineering is to present an outline which will perhaps serve to whet your appetite and encourage you to do a little reading in those areas which intrigue you.

The subjects in materials science and engineering can be broadly and generally broken up into ten areas:

—*Bonding.* Metallic bonding in metals and alloys, ionic bonding between dissimilar atoms in ceramics, and covalent bonding along the chains in polymers with secondary bonding between the chains, as well as some covalent cross-links

—*Packing.* Directional bonding (ionic and some covalent), nondirectional bonding (metallic, van der Waals), and differences in atomic or ionic sizes

—*Structure.* Crystalline structures (metals, most ceramics), amorphous or noncrystalline materials (glass, many polymers), semi- or *para*-crystalline materials (many polymers), crystal structure types, ionic crystals, molecular crystals, polymeric crystals, and chain folding

—*Surfaces and defects.* Bonding discontinuities, surface energy, atomic or molecular vacancies, dislocations (a missing or dislocated plane of atoms), space charges, and catalysis

—*Thermodynamics and kinetics.* Entropy, free energy, laws of thermodynamics, activation energy, reaction rates, equilibrium, diffusion processes, and phase analysis

—*Degradation and corrosion.* Electromotive series and galvanic corrosion, oxidation, solubility, stability, chain scission, and enzymatic attack

—*Mechanical behavior.* Elastic properties, mechanical tests, stress, strain, fracture, creep, fatigue, plastic deformation, dislocation theory, strengthening mechanisms, and precipitation hardening

—*Physical properties.* Electrical conduction, electron emission, semiconductors, thermoelectricity, magnetic properties, superconductivity, dielectric and optical properties

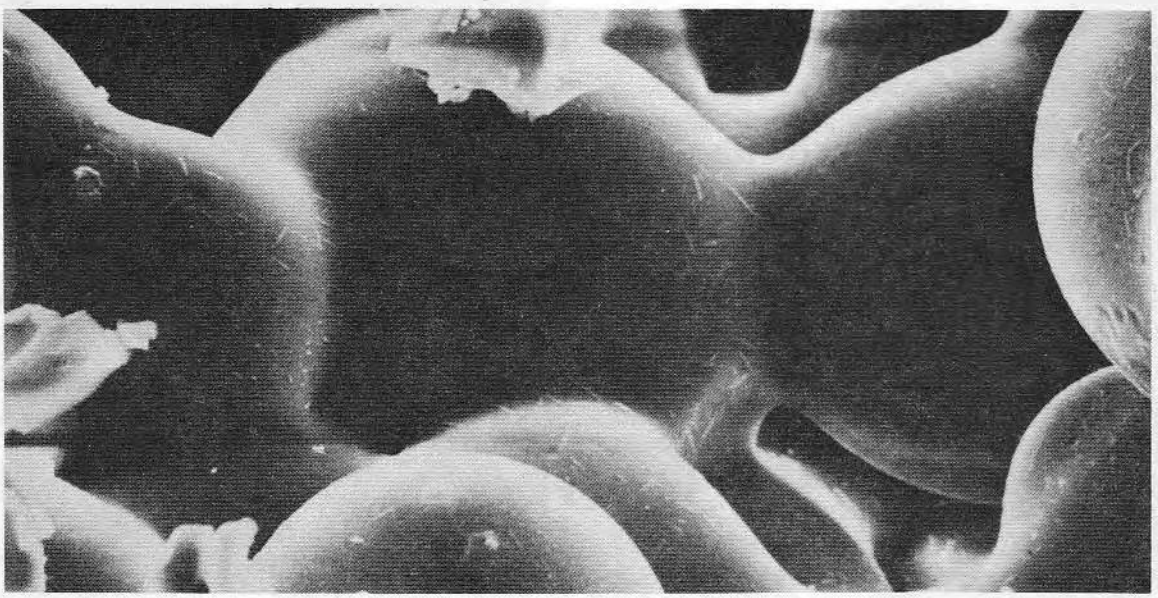


Figure 2. Glass spheres are often used in studying fabrication of materials by sintering. This scanning electron micrograph shows the necking of glass spheres (0.001 inch diameter) after heating to the temperature where 3.5% shrinkage has occurred *Courtesy Ivan B. Cutler, University of Utah*

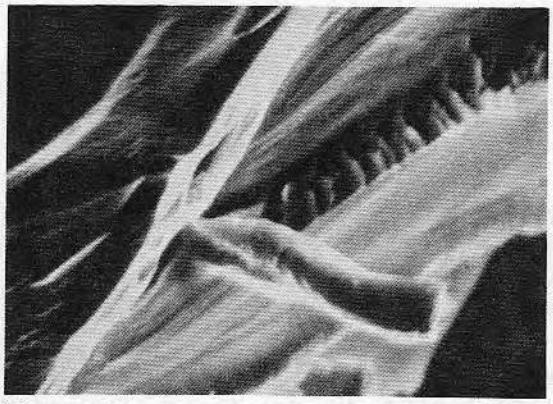


Figure 3. Fractograph of chemically vapor-deposited tungsten unannealed (× 11,000) *Courtesy John S. Chun, University of Utah and Bob Jernigan, Engis Equipment Co.*

—*Biomedical behavior.* Blood coagulation, hemolysis (red cell destruction), foreign body reaction, corrosion and degradation in biological environments, cell/material interactions, and biochemical material effects

—*Molecular engineering.* Superalloys, high temperature materials, biologically compatible materials, polymer synthesis, composite materials and structure modifications

Nearly all of these areas have direct, practical applications in engineering and technology. The development of superalloys for jet engines, of high temperature-resistant materials, of ceramic body armor, of semiconductor materials and devices (responsible for the diode, transistor, and electronics revolution), of materials for use in artificial internal organs, of the plastics now in common use (Styrofoam, Dacron, Plexiglas, and polyethylene) of lasers, and of the Apollo spacecraft and Lunar Module—all of these developments and applications of materials depend on the principles and techniques of materials science and engineering.

In most colleges and universities, materials science is in the engineering school or college and one must satisfy the basic core of engineering courses as well as the particular requirements of the materials program. In some cases the materials curriculum is an option or division within another department.

The prospective materials major should select a curriculum which is balanced in all areas of materials science. Many departments carry the name but are merely metallurgy or ceramics departments which have changed their name without changing their emphasis or curriculum. The requirements of modern engineering make it imperative that all materials be considered in any application, as each class of materials has relatively unique properties which make it suitable for diverse applications.

There is no materials science society to which you can write for information but there are societies in the respective materials areas which do have information available for distribution. Each one is, however, biased towards its own particular interests and you must weigh them all together and in perspective. Some of these groups are: American Ceramic Society, 4055 North High Street, Columbus, Ohio 43214; American Institute for Metallurgical Engineers (AIME), 345 East 47th Street, New York, N.Y. 10017; American Society for Metals (ASM), Metals Park, Ohio 44073; Society of Plastics Engineers, Inc., 65 Prospect Street, Stamford, Conn. 06902.

Many excellent and readable books are also available. The Doubleday Science Series is particularly relevant and inexpensive. Some are: "Ceramics in the Modern World," M. Chandler, 1967; "Giant Molecules: the Technology of Plastics, Fibers, and Rubber," M. Kaufman, 1968; "Glass in the Modern World," F. J. T. Maloney, 1967; "Metals in the Modern World," E. Slade, 1967; "Spare-Part Surgery," D. Longmore, 1968. The September, 1967, issue of *Scientific American* is also an excellent introduction to materials.

The student particularly interested in biomedical materials will find information and programs more difficult to come by. Several graduate-level programs exist, but undergraduate courses are virtually nonexistent. However, the University of Utah's division of materials science and engineering is presently offering biomaterials course work on the undergraduate level. There are about 100 colleges and universities offering programs in materials, and among them you should find a faculty and curriculum suited to your interests, goals, and ideals. For further information on college study, consult your counselor, the societies mentioned above, or the author at 2006 Merrill Engineering Building University of Utah, Salt Lake City, Utah 84112. Ω

JOSEPH ANDRADE received his B.S. from San Jose State College in California in materials science and his Ph.D. in 1969 from the University of Denver. Presently he is an assistant professor in the division of materials science and engineering at the University of Utah and also holds an appointment as research instructor of surgery in the division of artificial organs of the College of Medicine. His major interests are the interactions which occur at the interface between nonliving materials and living systems.

COATED ADSORBENTS FOR DIRECT BLOOD PERFUSION: HEMA/ACTIVATED CARBON

J. D. Andrade, K. Kunitomo, R. Van Wagenen, B. Kastigir, D. Gough, and W. J. Kolff

BACKGROUND AND RATIONALE

Activated carbon. There has been an interest in the direct perfusion of blood over adsorbents, notably activated charcoal[1,2]. The rapid adsorption of creatinine and uric acid as well as other complex and colored molecules makes charcoal an attractive adsorbent for use in the treatment of uremia and acute intoxication.

Data on the mechanism and kinetics of solute adsorption on charcoal are available. Dedrick, et al have done work in this area with compounds of interest to toxicology and uremia[4]. Other kinetic studies are also available[3]. A voluminous literature is available on activated carbon, which has been a significant purifier in the chemical industries for over a century. This information has been critically reviewed in a recent monograph[5].

Direct blood perfusion. Yatzidis[1], in 1964, demonstrated that a charcoal cartridge could be used clinically in chronic uremia. He did analyses for a wide variety of chemical species and showed significant adsorption of creatinine, uric acid, indican, phenols, guanidine bases, and organic acids. He estimated that a 60 min hemoperfusion over 2-3 charcoal columns has about the same efficiency as a hemodialysis of 3-6 hr duration. He also noted, however, that charcoal does not remove cholesterol, urea, water, electrolytes, or bilirubin, but it does remove urochromes and some poisons, notably the barbiturates, salicylates, and glutethimide. These promising results led Dunea and Kolff[2] to study and apply charcoal clinically as an artificial kidney. They perfused heparinized blood directly over 12/28 mesh activated charcoal for varying periods at various flow rates. They found a high initial creatinine clearance which decreased with time as the charcoal became saturated. The clearance increased with flow rate. Serum creatinine and uric acid levels were cut approximately in half by a 90 min perfusion and a rebound was observed the next day. Platelet levels also dropped by half. Platelet counts dropped more rapidly as the flow rates increased. Gross hemolysis was not observed. A relatively large blood loss occurred (100-150 ml) due to charcoal caking.

The promising results with uric acid and creatinine adsorption in uremia led a number of investigators to study direct blood perfusion over charcoal for the treatment of poisoning. The effectiveness of activated charcoal for the adsorption of barbiturates, salicylates, and glutethimide had already been demonstrated by Yatzidis[1]. He later used the procedure for the clinical treatment of severe barbiturate intoxication[6]. DeMyttenaere, et al[7], in a series of dog and clinical studies, demonstrated that a simple charcoal hemoperfusion can dramatically alter the prognosis for "lethal" doses of glutethimide. Other investigators have also demonstrated the efficiency of hemoperfusion over charcoal for uric acid, creatinine, barbiturates, salicylates, and glutethimide removal[1,2,6-8].

The general procedure in all of these studies is to place 100-200 Gm of a relatively large granular carbon (1/2-2 mm dia) in a cylinder. Filters are placed on the inflow and outflow ends. Arterial blood is generally admitted at the bottom of the vertical cylinder. The animal or patient is well-heparinized (100-300 mg heparin). Flow rates are usually 100-200 ml/min. Perfusions run from 30 min to 2 hr and longer, though nearly all of the solute removal is observed in the first 30 min.

The advantages of such a treatment method are: 1) rapid removal of selected toxins directly from the blood, 2) simplicity; the equipment involved is minimal and inexpensive, as contrasted with hemodialysis, 3) versatility; other adsorbers can be used to develop a gross selectivity for other classes of compounds. Ion-exchange resins have been used. Lipophilic resins have also been used[9].

The main problem appears to be the decreased platelet and white blood cell levels. In all the reported cases the platelet count is cut at least in half, after a relatively short perfusion. There are also problems with charcoal particles getting into the circulation and lodging in the lungs, spleen, and kidneys[10]. This can probably be corrected by thorough removal of the fines and the use of fine mesh filters. The use of a larger particle size has also been shown to reduce the incidence[8]. Charcoal caking is also a problem, decreasing the flow path and reducing the efficiency. The loss of red blood cells in the column is of concern. A thorough study of intermittent charcoal hemoperfusion over long periods is not available. It is conceivable, and perhaps probable, that long-term blood problems may result, particularly decreased cell lifetimes, hemolysis, and protein changes.

Gel surfaces. Hydrophilic surfaces are often more blood compatible than hydrophobic surfaces. This is reasonable, as the vascular linings are themselves hydrophilic. Hydrophilic surfaces may have higher surface free energies than the hydrophobic surfaces, but their interfacial free energies in aqueous solutions are much lower. Indeed the interfacial energy between an aqueous gel surface and an aqueous solution approaches zero.

From the Division of Artificial Organs, University of Utah, Salt Lake City, Utah.
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Thus, gel-like or solvent-loaded surfaces minimize interaction potential discontinuities, thus minimizing the interactions themselves[11]. A gel surface should, therefore, minimize the adsorption and denaturation of proteins and should have minimal interactions with cellular elements. Lyman and Andrade[12] have shown that a layer of protein on a hydrophobic surface does succeed in producing a hydrophilic interface which has low platelet adhesion and is non-thrombogenic.

The acrylic hydrogels, in which water content can be varied from 20-97%, have been known for over a decade[13]. The acrylic gels are compatible with many types of biological tissue, including blood[14,15]. These materials are quite stable in aqueous solutions.

The common Hydron materials are based on the esters of methacrylic acid. Polyhydroxyethyl methacrylate (polyHEMA), also called Hydron - S[14], is the material most commonly used. Commercial Hydron is cross-linked with ethylene dimethacrylate to give a gel containing 40% water and able to transmit molecules up to about 8,000 MW[14].

Encapsulated adsorbents. A significant amount of the blood trauma associated with direct blood perfusion over carbon can be eliminated if the charcoal is coated or encapsulated with a blood compatible material. Yatzidis has claimed that coating the charcoal with a solution of cellulose acetate solves the platelet adsorption problem[7]. Chang[16-18] has obtained good results by encapsulating the charcoal in collodion and nylon membranes. We already know that an intact protein layer on a surface reduces platelet adsorption to virtually zero[12]. Indeed, charcoal would probably do much more blood damage were it not that plasma proteins rapidly adsorb on its outer surfaces and, in effect, encapsulate it[19]. Chang's results on the coating of collodion with albumin further substantiate the improved blood compatibility of albumin surfaces[16].

Our experience with polyhydroxyethylmethacrylate and albumin surfaces[20] suggested their use as coating agents for activated carbon. This paper will concentrate on the polyHEMA coatings. The albumin coatings will be discussed in detail in a later paper.

METHODS

Activated carbon and coating procedures. Columbia Activated Carbon granules, 12/28 mesh, Type LCK were used. This material, donated by the Union Carbide Corporation, is a liquid treating grade designed for adsorption of relatively small molecules. All the work reported here has been with this carbon.

The carbon is prepared before using by sizing through a 20 mesh screen (0.833 mm) to remove the small granules and fines. It is then washed in ethanol for 10 min and dried to constant weight before using. This is the "uncoated" charcoal referred to here.

The charcoal is coated by placing it in a pre-dried, pre-weighed mesh sack (such as a woman's nylon stocking). The charcoal and mesh are vacuum dried overnight, weighed, and placed in the coating solution for the appropriate time. The sack is removed from the solution, placed and shaken on absorbent paper, and polymerized in vacuo at 80°C for 2 hr; extraction in ethanol or saline is usually performed. The sack, charcoal, and mesh are weighed. The weight increase of the charcoal due to the coating is thus known.

The coating solution is hydroxyethyl methacrylate (HEMA) in 95% ethanol containing 0.4% t-butyl peroctoate initiator. The albumin coating solution is 5 mg/ml bovine serum albumin (BSA) in 0.0175 M acetate buffer (pH 4.9) containing 0.25% glutaraldehyde as the cross-linking agent.

Attempts were made to detect the coatings optically and via scanning electron microscopy.

In vitro adsorption studies. A stirred-batch procedure utilizing radiolabeled solutes (C^{14} and I^{125}) was used and the analysis made by liquid scintillation counting. The studies reported here were done at room temperature (about 20°C). About 5.0 Gm (uncoated weight) of the charcoal were dried to constant weight in a vacuum oven and quickly weighed accurately. The dry charcoal was placed in a beaker on a magnetic stirrer. 200 ml of the radiotagged solution (1 to 2 μc) was added with stirring. In some runs the charcoal was equilibrated in water before the solute was added. For the protein runs, monolayer complications were avoided by first covering the charcoal with buffer, then adding the radioiodinated human serum albumin (RIHSA) solution. The final solution concentration was 2 mg/100 ml. Phosphate buffered saline was used for the albumin and most of the creatinine and uric acid studies. One cc samples were taken at various times. The samples were counted by liquid scintillation using 10 ml of Insta-Gel (Packard Instrument Co.) as the scintillation and solubilization medium. Appropriate blanks, standards, and calibrations were performed.

Circuit design. In our initial studies we found that the typical arrangement used by other investigators was relatively inefficient. Dye flow studies showed that a 200 ml/min flow into a right cylinder of carbon granules produced the flow pattern of Figure 1. Thus, about half of the charcoal volume was unperfused. In addition, activated carbon in blood and other fluids tends to pack and sludge unless kept well agitated. This effect has been noted previously[2]. The packing often leads to a decreased flow and the development of channels in the carbon, further reducing its effectiveness. One of us (RVW) designed and constructed a number of containers which would optimize particle agitation and eliminate all unneeded volume. These are illustrated in Figure 2.

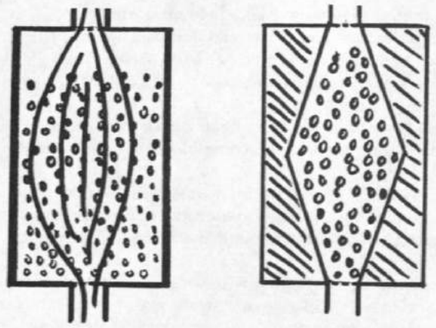


Figure 1. Schematic representation of the flow pattern in a right cylinder (left) and in a design which minimizes stagnant regions (right).

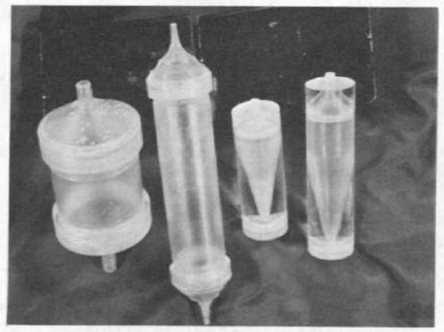


Figure 2. a. (far left) A typical right cylinder cartridge; b. (mid left) A more effective design; c. (mid right) The first, and very effective conical design. d. (right) The current design, with a capacity of up to 20 Gm of 12/28 mesh charcoal. Arterial flow is from the bottom.

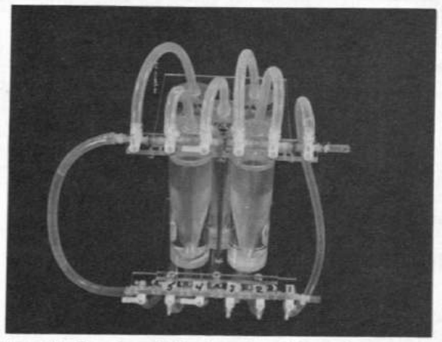


Figure 3. The charcoal circuit consists of 5 cartridges in parallel with a suitable valving arrangement to permit flow through only one cartridge (or the A-V shunt) at a time. Arterial inflow is at the bottom right, venous outflow at the top right.

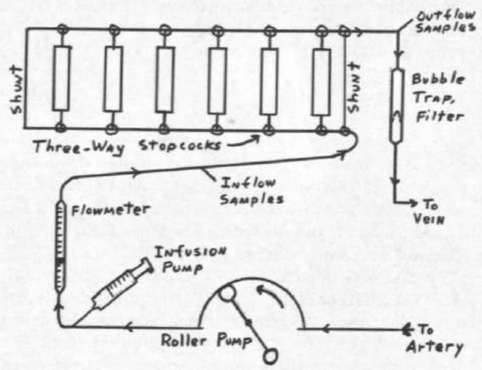
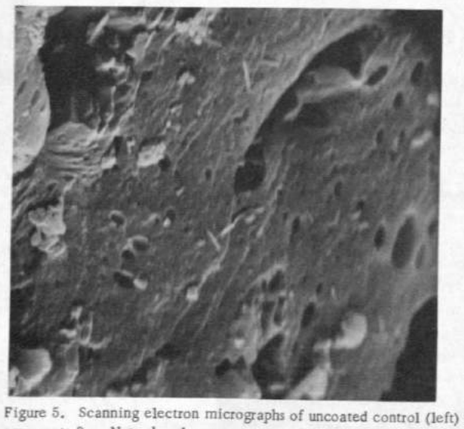


Figure 4. Schematic representation of the ex vivo perfusion circuit for determining clearances.



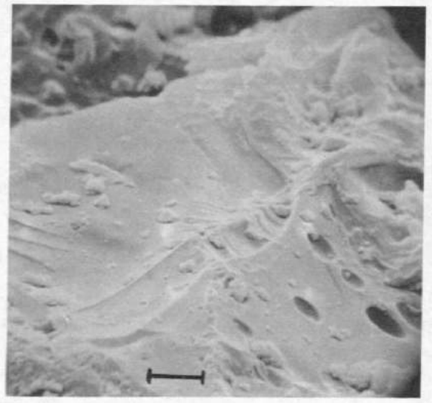


Figure 5. Scanning electron micrographs of uncoated control (left) and BSA coated, (right) carbon. The mark on the bottom of the right photo represents 2μ. Note the clean open pores of the control (left), as contrasted with the pores of the coated sample, which appear to be covered with a thin, concave coating, perhaps a film of cross-linked albumin.

Arterial flow from the bottom in Figure 2d leads to excellent particle agitation and flow lines throughout the container volume. The charcoal particles are constantly in motion and are in contact with the fluid stream as optimally as possible. However, only about 20 Gm of the charcoal could be kept completely fluidized. Thus, a circuit was designed in which up to 5 20 Gm cartridges could be connected in parallel with a suitable valving arrangement to permit one at a time to be perfused (Figure 3).

In vivo studies. Early in vivo studies utilized 1 or 2 cartridges of the type in Figure 2. These studies were mainly for platelet compatibility.

The circuit was connected as a carotid-jugular shunt to 70-80 Kg sheep. The fasted animal was brought in, given light Brevane (sodium methohexital) or Halothane anesthesia, intubated, respirated, and placed on the table. Carotid-jugular cutdown was performed and conventional artificial kidney shunts inserted. The animal was given 100-150 mg heparin I.V. The circuit (previously primed with saline containing 100-150 mg heparin) was connected and flow initiated and monitored. Samples were taken from inflow and outflow sides of the cartridge at 2, 5, 10, 20, 30, and 60 min. Platelet counts and hematocrit were determined.

In vivo clearance studies for uric acid and creatinine were performed using the arrangement in Figure 4. The circuit of Figure 3 was connected and the blood shunted. Creatinine and uric acid infusion was begun on the arterial side using an infusion pump. The infusion rate was 1.0 cc min of a solution containing 15 mg% creatinine, 15 mg% uric acid, and 25 mg% piperazine hydrate[21]. Analyses were performed for platelets, Ca, PO_4, Glucose, BUN, uric acid, creatinine, cholesterol, alkaline phosphatase, LDH, SGOT, total protein, albumin, bilirubin, and hematocrit. Most of the determinations were performed by a local laboratory using an SMA 12/60 Auto Analyzer. Uric acid and creatinine concentrations were determined with a commercial clinical test kit.

RESULTS AND DISCUSSION

Charcoal coating. The results of the polyHEMA coating of activated carbon granules are summarized in Table I. Various solvents and coating conditions were evaluated. The 20% HEMA/ethanol solution and a 10 min

TABLE I

HEMA COATING OF ACTIVATED CARBON - CONDITIONS AND RESULTS

Sample	wt % HEMA in ethanol*	Coating Time	Conditions (0.4% initiator)	Weight Increase %
A	50	3 days	aspirated	50
B	50	10 min	aspirated	30
C	10	1, 10, 100 min	unaspirated	∿ 10
D	1	1, 10 min	unaspirated	∿ 10
1	20	10 min	aspirated	15
2, 10	20	10 min	unaspirated	11
7	20	10 min	coating solution prepolymerized for 45 min at 70°C	14
8	20 in xylene	10 min	unaspirated	13
13	20 in ether	10 min	unaspirated	29
9	20	10 min	carbon presoaked in sucrose solution for 1 hr, dried	24
11, 12	20 in ether	10 min	carbon presoaked in sucrose solution for 15 min with aspiration, dried	19

*unless otherwise noted

soak (sample no. 10) was chosen as the best coating to date for reasons which will become evident. Attempts were made to "prefill" the pores with a poorly adsorbed, highly water soluble compound, such as sucrose, to prevent the HEMA from penetrating into the interior of the particles. The rationale was that after the HEMA coating was produced, the sucrose could be removed. The results with such coatings (9, 11, and 12 in Table I) have not been satisfactory to date. Attempts to pre-polymerize the HEMA (7 in Table I) before the coating procedure fared somewhat better; this approach is being continued.

The albumin (BSA) coatings produced a 9-10% weight increase. The albumin-coated sample is designated as No. 5.

If the weight due to the coating is all on the surface of the carbon granules, one should be able to detect a capsule or membrane. Optical microscopy studies could not detect such a membrane. Scanning electron microscopy (SEM) indicated that the gross morphology and heterogeneous structure of active carbon was unchanged by the various coatings. However, the macropores clearly evident in uncoated material were much less evident in coated material.

The left portion of Figure 5 is an uncoated, control carbon surface. Note the relatively large, open, clean macropores. The right photo is that of a BSA-coated carbon. The pores appear to be covered with a thin film, somewhat concave over the pore itself. The pores are closed. This photograph is the first hint of a membrane coating that we have obtained by direct optical observation.

In vitro adsorption studies. Stirred batch adsorption studies were performed using creatinine, uric acid, and albumin as solutes in distilled water, saline, and PBS at solute concentrations ranging from 10 to 400 mg%. The data confirmed that charcoal rapidly adsorbs uric acid and creatinine from solution. Ten Gm of carbon removes 360 mg of uric acid from 100 ml of a 400 mg% solution in 30 min, for a solution depletion of 90%. HEMA coated carbon, 15% weight increase, produces a solution depletion of over 60% in 30 min. Similar results were obtained for creatinine. Adsorption of albumin was 100 to 200 μg/Gm of charcoal in 1 hr, which is almost negligible compared to the quantities of uric acid and creatinine adsorbed.

Figure 6 gives the solution depletion curves for 5 Gm of carbon and a 68 mg% creatinine solution in PBS at 20 C. The solution depletion tends to increase with decreased coating weight. Most of the curves begin to approach a plateau value at 20 to 30 min. It is therefore clear that HEMA and albumin-coated activated carbon can retain their high adsorptive capacity for uric acid and creatinine as long as the coating is not too "heavy."

Studying the capacities of such coated carbons, it was evident that under ideal conditions the concentration of 5 L of a 10 mg% creatinine or uric acid solution could be reduced to nearly zero by 50 Gm of carbon.

In vivo studies. Our use of uncoated charcoal confirmed the results of other investigators in that even with high levels of heparinization (300 mg) packing occurred and clumping developed in the charcoal. Flow was thus sporadic and difficult to control. Platelet levels were cut in half in one pass through the uncoated charcoal. In 25 min of perfusion, the platelet count dropped from nearly 300,000 to around 80,000/mm^3.

The use of coated charcoal completely changes these results. The use of a HEMA-coated charcoal (50% weight increase) virtually eliminated the platelet drop. The values remained scattered around 200,000 platelets/mm^3 with no depletion trend. Packing, clumping, and channeling were eliminated. Good particle agitation and good flow were obtained.

Certain combinations of coating and heparin are necessary to prevent coagulation and charcoal caking. It was found that coated charcoal alone will not prevent clotting, but that a certain amount of HEMA on the surface of the charcoal does prevent caking. By beginning at the extremes of coating and noting the resulting adsorption effects, we began working toward an acceptable combination. It has been determined that a 20% HEMA/ethanol solution (10 to 15% weight increase) and 3 mg/Kg heparin dose gives the best results with efficient adsorption for a sheep. With less heparinization the charcoal in the cartridge may clump and the blood flow is then channeled between the charcoal clumps and the inside surface of the cartridge. The caking may eventually occlude the outflow (venous side) of the cartridge. Proper doses of heparin and HEMA coating prevent a fibrin network from forming on the charcoal and subsequent clot formation, allowing for adequate charcoal perfusion for at least 1 hr. We have investigated the effect of HEMA coating on platelets. With coatings from HEMA solution concentrations of 20% by weight or greater, platelet levels were only slightly altered. No gross hemolysis was observed during any of the experiments, and there were no fluctuations in the hematocrit.

The results of the in vivo clearance studies are presented in Figure 7. The flow rate was 150 ml/min, and the experimental set up is illustrated in Figure 4. The change in uric acid and creatinine levels is evident. There was no change in Ca, PO_4, BUN, cholesterol, total protein, albumin, and bilirubin. Both the uric acid and creatinine levels increased during the experiment, indicating that the animal was not completely clearing these compounds. Nevertheless, there was a significant clearance through the cartridges. The uric acid clearances were about 40, 30, and 40 ml/min for cartridges 1, 2, and 3, respectively. The creatinine clearances were slightly lower. It is clear that unlike the in vitro studies, the charcoal was not saturated after 20 min of perfusion. These results were obtained with a constant creatinine, uric acid (and piperazine) infusion, producing a steadily increasing concentration. In spite of this aggravated situation, a single 20 Gm activated carbon cartridge can maintain a significant clearance in excess of 20 min of perfusion - with minimal blood damage, clumping, or packing due to the polyHEMA coating.

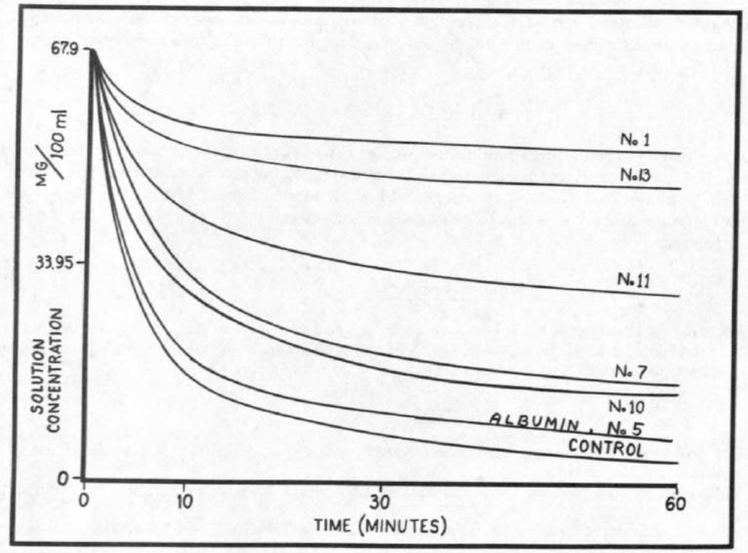


Figure 6. Representative solution depletion curves for 5 Gm of various coated and control carbon in a 68 mg% creatinine solution in PBS. The sample numbers correspond to those in Table I. The #10 (HEMA) and #5 (Albumin) are fairly close in adsorption behavior to the uncoated carbon.

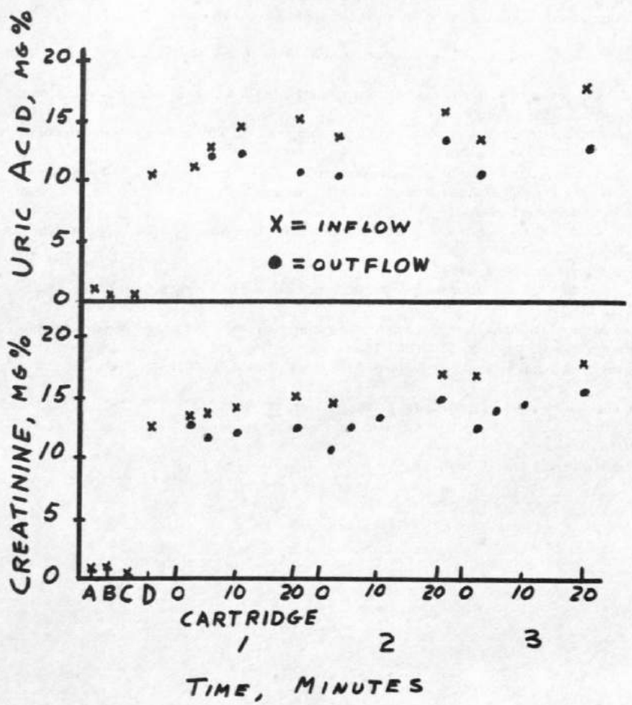


Figure 7. Uric acid and creatinine concentrations for a typical in vivo perfusion run (150 ml/min). A, B, and C are control samples. D was taken shortly after the infusion pump was started. The cartridges each contain 20 Gm of #10 HEMA-coated carbon. Note the good clearances obtained, from 30-40 ml/min for uric acid.

We are confident that by optimizing the carbon type, the coating, and the engineering design, a small, safe, inexpensive, highly efficient adsorption device can be developed as an aid to the treatment of chronic uremia and acute intoxication.

SUMMARY

PolyHEMA coated activated carbon is highly effective for the removal of creatinine and uric acid in vitro and in vivo. A carbon container and associated hardware has been designed, constructed, and tested to permit maximum effectiveness with minimum quantities of charcoal and low priming volume. The coated carbon does not produce gross platelet depletion and is resistant to caking and channeling. In vitro and in vivo capacities and clearances have been determined.

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IUPAC Preprint

INTERFACE CONVERSION FOR THE PREPARATION OF HYDROPHILIC GEL INTERFACES

~1970

J. D. Andrade, D. Gough, R. Van Wagenon, D. Hill
Institute for Biomedical Engineering
University of Utah
Salt Lake City, Utah 84112 USA

Introduction and Background

The acrylic hydrogels, in which water content can be varied from 20% to 97%, have been known for over a decade(1). A substantial amount of information is available on their biological properties(1-3). The acrylic gels are compatible with many types of biological tissue, including blood(3). Refojo(4) has done extensive studies on the hydroxethyl and glyceryl methacrylate gels. Wichterle and Lim have proposed many medical applications for such gels(1) including their use as plasma expanders(5). These materials are quite stable in aqueous solutions and do not suffer from hydrolysis, as do many other gel systems.

Protein gels are also biologically compatible under certain conditions. Fibrinogen and fibrin have been used in some cases(6). Lightly cross-linked gelatin(7) and suitably modified collagen materials(8) have been shown to be blood compatible. However, the biological response of these materials is highly dependent on their molecular configuration, and as a result adverse blood interactions can also be initiated by these same generic materials. Protein gels may also be susceptible to enzymatic attack.

The problem of poor mechanical strength of gels may be circumvented by utilizing the unique biological compatibility of a gel only at the surface of the device, perhaps as a simple coating. Theoretical evidence(9) would indicate that a neutral interface of the order of a hundred Angstroms thick and having a high water content will not adsorb compounds from an aqueous solution. The interfacial tension of a gel interface approaches zero and thus there is little free energy advantage in adsorption.

A gel surface should neither adsorb or denature proteins and should have a minimal interaction with cellular elements. Lyman(10,11) and Andrade(9, 11) have shown that a layer of protein on a hydrophobic surface does succeed in producing a hydrophilic interface which has virtually no platelet adhesion and is non-thrombogenic. Hydrogel coated sutures and tubes also show no tissue reaction(12). Albumin coatings tend to reduce blood cell interactions or destruction(13). Lyman(14) has also shown that hydrophilic monomers, such as N-vinyl pyrrolidone, radiation grafted to hydrophobic polymers, show increased blood compatibility.

It is thus evident that one may obtain the biocompatibility characteristics of a gel with the desired physical and mechanical properties of a substrate material by surface grafting or coating. The interface between the gel surface and the solution is not a true interface at all. Lightly cross-linked gel systems are not "inert, passive" barriers or discontinuities in a physiological environment. Ionic transport, molecular migration and water transport can occur.

There are two basic approaches to a surface coating: 1) a chemical attachment to the underlying substrate, and 2) a physical attachment to the underlying substrate.

We are studying the Co^{60} radiation-induced grafting of hydroxyethyl methacrylate (HEMA) and similar monomers to a variety of polymer substrates. The result is a polyHEMA surface in which some of the gel molecules are

chemically bonded to the polymer. The other approach is to polymerize the HEMA on a rough or porous surface to permit good physical attachment of the polyHEMA to the substrate. This can be aided by first swelling the material to be coated in a solvent and letting the monomer diffuse in before polymerization is permitted to occur.

The rationale and background for protein coatings has already been discussed. Our initial efforts in this area are in press(11). There are two different approaches to protein bonding - direct chemical attachment of protein to the base material or a physical attachment of an insolubilized, cross-linked protein gel. A simple procedure is to use glutaraldehyde as a coupling agent between two amino groups.

An easy and effective way of placing amino groups on polymer surfaces is via the aminoalkoxysilane coupling agents or adhesion promoters. 3-amino propyltriethoxysilane (APTES) is the most effective of these compounds(15,16) for a number of applications. It is used as a coupling agent between silastic and heparin(15). It bonds to -SiOH groups and other hydroxyl-containing surfaces. The silica filler in Silastic is readily bonded to APTES(15,16). The resulting amino-containing surface can then be coupled to protein via the glutaraldehyde coupling agent.

An approach of interest is the in situ cross-linking of protein to form a protein gel capsule around the sample. As glutaraldehyde is a coupling agent between two $-NH_2$ groups, it is clear that it will cross-link proteins to form insoluble protein gels. If the surface is rough or irregular, the protein coating is usually well adhered and stable.

Experiments and Results

The experimental procedures for the proteination of silicone rubber have previously been reported(17). Surface concentrations were determined by the use of radioiodinated human serum albumin (RIHSA). After extensive washings, the minimum surface concentration was about 5 micrograms/cm^2 as opposed to 0.7 to 1.0 micrograms/cm^2 for the controls which are typical "monolayer" values. The surface concentrations could be varied up to 15 micrograms/cm^2 by additional reactions. The surfaces were stable for at least several months in phosphate buffered saline and for at least 8 hours in pumping blood - long term stability tests are in progress. Soda-lime glass and tantalum oxide surfaces have also been proteinated by the same procedures. The APTES-glutaraldehyde - albuminated surfaces retain their immuno-chemical specificity for anti-albumin(18). Blood clotting tests were carried out using the Maloney method(7) for eliminating air interface complications. Silastic tubing proteinated by the above method was less thrombogenic than untreated silastic controls.

Hydroxyethyl methacrylate (HEMA) has been bonded to a variety of hydrophobic polymer surfaces by Co^{60} radiation-induced grafting. Dosages have ranged up to 0.7 Megarad at a dose rate of about 500 rads/minute. Irradiation and grafting was performed with the polymer substrate immersed in a 5% HEMA solution in ethanol. This procedure lead to significant grafting ($\sim$ 700 A HEMA layer) on polydimethyl siloxane and polyethylene substrates. Stress-strain curves were obtained and a deterioration of mechanical properties was usually observed for the 0.7 Mrad treated samples. The results of cell culture studies on such surfaces are quite revealing. A stable subline of baby hamster kidney cells (BHK-21) was cultured on the treated surfaces (19). All the HEMA-grafted surfaces did not exhibit cell adhesion; cells do not culture on such surfaces. This includes the surface which showed no weight increase, or "insignificant' grafting. The cells can detect what is significant much more effectively than can an analytical balance. One might attribute this effect to free radicals remaining on the surface, but the irradiated controls do not show the same behavior. Certainly other phenomena may be occurring, but this is at least encouraging evidence indicating the ability of such surfaces to reduce or eliminate cell adhesion.

HEMA coatings on activated carbon granules have also been prepared(17, 20). The purpose of this procedure was to prepare an adsorbent for the direct removal of toxins from blood with minimal blood damage. The important results, for the purposes of this paper, was the in vivo blood compatibility of the HEMA coated granules. They were placed in a plexiglas container, designed for optimal granule agitation, and connected to 70 - 80 kg sheep as a carotid-jugular shunt. The circuit was primed with saline containing 150 mg heparin. The sheep were also given 150 mg heparin i-v before perfusion began. Uncoated granules clumped readily and channeling developed. Coated granules permitted perfusions of one hour duration with no clumping and excellent particle agitation; platelet depletion with the coated granules was minimal. Platelet counts with the uncoated granules dropped from 230,000/mm^3 to 60,000/mm^3 after 25 minutes. These results have been confirmed in six separate in vivo experiments.(21)

These experiments indicate that polyHEMA coated activated carbon may be suitable for the atraumatic removal of adsorbable compounds directly from blood. Studies are presently in progress on the properties of albumin-coated activated carbon and other sorbents.(21)

Discussion and Conclusions

These studies and results indicate that hydroxyethyl methacrylate and albumin coatings can be prepared on a variety of medical polymers in such a manner that the mechanical and physical properties of the substrate polymer are not adversely compromised. The coatings can be prepared by cross-linking in place, in effect encapsulating the substrate, or by direct chemical attachment to the substrate. The coatings are relatively stable and non-toxic. The coating thickness can be varied. The hydrophilicity of the surface may also be varied.

Such surfaces are biocompatible in the sense that they neither promote nor adversely affect cell cultures. The protein surface is biocompatible in the sense that its antigenicity has not been compromised. The surfaces are blood compatible in the sense that they produce much less platelet depletion than control surfaces. They are partially thromboresistant in the sense that they exhibit longer whole blood clotting times (by the method of Ref. 7) than controls.

The behavior of these hydrophilic surfaces is in accordance with the mechanistic model of adsorption previously mentioned(9).

The design and fabrication of artificial organ devices is an extremely complex task. The designer should be able to select and use materials with the physical and mechanical characteristics desired. Ideally, the completed, sterile device could then be subjected to one or more interface conversion processes to render the entire system biocompatible.

We hope that the treatment processes outlined may help serve such needs.

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Activated Carbon and Blood Perfusion: A Critical Review

J D ANDRADE, K KOPP, R Van WAGENEN, C CHEN, W J KOLFF
Colleges of Medicine and Engineering,
University of Utah, Salt Lake City, Utah, USA

Activated carbon is a very high surface area adsorbent with the property of adsorbing relatively non-polar molecules, even from aqueous solution (Bock, 1920; Anderson, 1946). It adsorbs creatinine, uric acid, salicylates, barbiturates and a variety of other important compounds (Blaney et al, 1966; Sparks et al, 1966; Dedrick & Beckmann, 1967). It is often used as an oral antidote for ingested poisons (Holt & Holz, 1963; Fiser et al, 1971). Activated carbon has been studied for dialysate optimisation (Kolobow & Dedrick, 1965) and is now in commercial use in a recirculating dialysate system (Gordon et al, 1969). Yatzidis (1964) pioneered the use of activated carbon in direct contact with blood. A large number of investigators have followed Yatzidis' lead in studying and applying charcoal haemoadsorption.

This paper is a brief critical review of the literature experience with activated carbon haemoadsorption, with emphasis on the characteristics, properties and cleanliness of the activated carbons employed.

ACTIVATED CARBON

Adsorbent haemoperfusion requires the use of particles which can be contained without difficulty in an extracorporeal circuit. This largely eliminates carbons of very small particle size, unless such carbons are encapsulated or otherwise contained. Granular and pelletised carbons are available in size ranges suitable for blood perfusion (about 0.5mm in diameter or larger).

The properties of activated carbon are a function of the raw material from which it is prepared (coconut shells, bone, coal, peat, wood, petroleum, etc), the method of activation (see Smisek & Cerny, 1970), the surface area (expressed as square m/g), the pore volume (expressed as ml/g), the ash content, and the pore size distribution (pores in the 100 to 300 nm diameter range are the most useful for compounds of interest in uraemia and acute intoxication). In addition, one should know the mechanical properties of the carbon, ie its tendency to fragment or fracture. The presence of fine-

ultra-fine particles attached to the carbon surface, which are potential charcoal emboli, must also be determined and characterised. Unfortunately, such data are rarely, if ever, available from industrial activated carbon suppliers. Most of the activated carbons we have considered are inadequately characterised. Andrade et al (1972) have presented a table listing the USA granular and pelletised carbons and their properties. The authors would appreciate similar data for European and other activated carbons.

NON-COATED ACTIVATED CARBON

Yatzidis (1964) packed about 200 g of a granular (0.50-0.75mm diameter) Merck carbon into a 6 x 20cm siliconised glass cylinder filled with 100 mesh filters. He states that the system is "... well washed with deionised tap water" and then sterilised. A flow of 140 ml/min can be maintained through the low resistance device by arterial pressure. The device was evaluated on 5 dogs and a number of patients (20 perfusions). Temperature elevations and blood pressure drops were noted initially in a few of the cases. Extensive chemical determinations were made. Haematological studies were also performed — platelet and fibrinogen drops were noted. There was no mention of fine particles, possible fine carbon emboli generation or pathological examinations. There was no mention of other carbon types or why that particular carbon was selected. Yatzidis (1965) did mention that a number of side effects of the perfusion might be "attributable to sulphur compounds which are liberated from the charcoal. By careful washing of the charcoal with ammonia before use, these reactions can be partly avoided".

Dunea and Kolff (1965) utilised a system very similar to that of Yatzidis (1964). Eighteen haemoadsorptions were performed on three patients. They utilised a Union Carbide Corp. carbon, coconut base, 12/28 mesh, acid washed, and of 0.045% ash content. The carbon was gas sterilised with ethylene oxide in dichlorodifluoromethane. One must expect some adsorption of these compounds on the carbon — desorption could be quite slow. The device was assembled and 4 to 8 l of saline "... were run through the cylinder to remove fine dust". This description of carbon properties and washing procedure is one of the best to be found in the haemoadsorption literature, yet it is very inadequate. One must question the suitability of gas sterilisation and the very inadequate washing procedure (see Andrade et al, 1972). Dunea and Kolff noted caking of the carbon and significant platelet drops. Clearance data for creatinine, uric acid, and salicylate were very good initially but dropped rapidly during perfusion. They concluded: "Haemoperfusion appeared to be a safe procedure. Side effects were few. Nausea and vomiting occurred several times. No pyrogenic reactions were noted". No mention was made of fine particles or pathological studies.

The first histopathological study of activated carbon haemoperfusion was

reported by Hagstam et al (1966a, b). They used a 1.9 x 25cm plastic tube containing 23 g of activated carbon. They used 0.3 to 0.5mm Merck, 0.5 to 0.75mm Merck, and a 0.5 to 1.0mm Graves carbon. The apparatus was extensively rinsed until the "... rinsing fluid was free from macroscopically observable charcoal particles. Centrifugation of the rinsing fluid revealed, however, continual occurrence of microscopic charcoal particles". (Hagstam et al, 1966a). They thus noted the inadequacy of simple washing procedures for the removal of small particles. They did note the effect of particle size on column resistance. Extensive physiological monitoring was performed during the in vivo rabbit haemoperfusions. Carbon particles (5-35μm size) were noted in outflow blood smears. Eleven rabbits were treated with the haemoadsorption system for 30-210 minutes using one to three carbon columns. The animals' condition remained stable. No significant changes were noted in the parameters monitored. Chemical changes confirmed the results previously reported. The animals were killed two days to nine months after perfusion. No macroscopic abnormalities were noted. Additional studies were performed on phenobarbital-intoxicated rabbits and rabbits rendered acutely uraemic. In all, 34 rabbits received haemoadsorption treatment. Six rabbits which had not undergone charcoal perfusion were examined histologically. "In none of these six were any charcoal particles found in the lungs, liver, spleen or kidneys. All the 34 perfused animals had charcoal particles in their lungs. In 28 of them charcoal particles were also found in the spleen. In 9 of the animals particles were seen in the kidneys; two animals may have had carbon in the brain; no carbon could be found in the heart".

Hagstam et al (1966a) further showed that the amount of carbon observed was correlated with the rate and length of perfusion and the number of columns; no difference was noted between the two kinds of carbon used. A histopathological discussion was included in their paper. Hardly any tissue reaction to the particles was noted. The work by Hagstam et al (1966a, b) clearly demonstrates, both in vitro and in vivo, the inadequacy of simple washing procedures for removal of fine carbon particles. They further noted that mutual agitation and rubbing of carbon particles may lead to fine particle generation during perfusion. They did not notice any physiological changes which could be attributable to carbon deposition. Nevertheless, they recommended that "until more is known about this phenomenon (carbon deposition in the organs), haemoperfusion through activated charcoal should be done only in restricted cases", and finally concluded that "the pathogenetic significance of this charcoal deposition cannot yet be evaluated". Even in this excellent study, however, the carbon used was not adequately identified nor was the role of carbon type or washing conditions on fine particle generation discussed.

DeMyttenaere et al (1967a, b) used activated carbon haemoperfusion for

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DeMyttenaere et al (1967a, b) used activated carbon haemoperfusion for

the treatment of acute glutethimide poisoning. A 10/18 mesh Fisher carbo (coconut base) was used, "... previously washed with water". In vitro and in vivo (dog) studies were performed on glutethimide removal. The carbon for the in vivo studies was "... rinsed with a large volume of tap water... and autoclaved. Before actual perfusion, 4 to 6 litres of saline were pumpe "... through the circuit and the charcoal column at a high flow rate to remo the fine dust". This washing procedure is probably inadequate (see Andrad et al, 1972). Platelet counts were generally reduced by over 50% after cha coal perfusion. Histopathological data were not reported.

Rosenbaum et al (1968) also evaluated activated carbon haemoperfusion as a treatment for acute intoxication. They used a 350 g column of Union Carbide Corp. NXAC 20/48 mesh activated carbon on five dogs. No washi procedure was described, though "... the column was initially irrigated wi 6 l of isotonic saline and sodium bicarbonate in a 2:1 ratio. It was then ste lised with ethylene oxide and again irrigated with 4 l ..." of solution. The was a very dramatic decrease in barbiturate levels during perfusion. Plate let levels dropped 86% and leucocyte count dropped 85%. Rosenbaum et al (1968) noted marked hypotension by the third hour of perfusion. "Autopsies revealed emboli of charcoal particles....", primarily located in the pulmo nary arterioles "and to a lesser extent in other viscera including the heart, spleen and liver". They concluded that "In spite of the efficiency of carbon haemoperfusion, human application is not justified due to toxic effects".

Dutton et al (1969) studied charcoal haemoperfusion in cats as a techni for the production of acute thrombocytopenia. They used 33 "ml" of 1.2 to 1.4mm diameter carbon granules (NXAC C-66-202 14/16 mesh, National Carbon Co.). Perfusion of blood at 16 ml/min resulted in an average plate let drop of 78% after one hour of perfusion. There was no discussion of washing, fine particles, or histopathological analysis.

Barakat and MacPhee (1970) studied carbon haemoperfusion using a dif ferent cartridge design. Most other investigators have preferred to flow in an upward direction, thereby partially fluidising the particle bed. Baraka and MacPhee essentially have a downward flow device. They used about 40 "ml" of particles. (Their carbon is identified as 2080, 5/10 mesh, B.S.S. Sutcliffe, Speakman & Co., Leigh, Lanes, Great Britain.) The system wa sterilised by dry heat and "...perfused with normal saline to wash the carb and to displace air". They apparently had a system with a high resistance and low flows, thereby running into packing and clotting problems. The nature of the system or the carbon used led to haemolysis problems: "... haemolysis was regularly found, as is so commonly the case in extracorporeal circulations". The latter statement should not be taken as gospel, printed as such. Their chemistry determinations confirmed the results of previous investigators for barbiturate and creatinine removal. Some urea

removal was demonstrated, and they state that "... the clearance of urea ... by this method has already been proved". This is misleading. Some urea is removed, but not nearly enough to make carbon perfusion attractive for urea removal. Histopathological studies were performed. Carbon deposits were noted in the lungs, liver and kidney when they used 15/30 mesh carbon, though they claimed that when 5/15 mesh carbon was used "... no further carbon emboli were encountered". Platelet decreases were also noted.

Barakat and MacPhee (1971) have used charcoal perfusion for the removal of bilirubin and alkaline phosphatase. These results are intriguing, as they are quite different from those of other investigators. Yatzidis (1964) and Andrade et al (1971) found negligible removal of bilirubin. The pH changes encountered in Barakat and MacPhee's (1971) in vitro experiments perhaps indicate that an acid-washed carbon was used. One of the final statements in their paper deserves mention. They state_ "we were disappointed to observe that ammonia was not adsorbed by the wet carbon column".

Activated carbon is not a magical sponge which will adsorb and thereby remove anything we desire. It can exhibit some ion exchange behaviour depending on the surface treatments to which it has been exposed. Adsorption of gases on activated carbons can be rather non-specific. Adsorption from aqueous solution is another and much more complex matter. Grades of activated carbon used for treating acqueous solutions and often called decolourising carbons tend to adsorb apolar, relatively insoluble compounds. Different carbons may have substantially different adsorption properties, particularly for creatinine (see Andrade et al, 1972).

It is of interest to note that we have reviewed the work of seven groups (11 papers) and in all cases the properties of the carbon were inadequately known or at least inadequately discussed. In all cases the washing procedure was inadequate or inadequately discussed. In all the studies cited in this section there was no mention of evaluating different carbon types.

Merrill, in a recent editorial (Merrill, 1971), indicated substantial concern over the blood damage and emboli (fine particle) problems associated with activated carbon haemoperfusion. Andrade et al (1972) have shown that there are substantial differences in the cleanliness and, more importantly, the "washability" of various USA carbons. One might expect that the same would be true of European carbons.

COATED ACTIVATED CARBON

Properly coated or encapsulated activated carbon may minimise the blood damage and fine particle emboli problems associated with carbon haemodialysis.

Yatzidis (1966) used cellulose acetate-coated carbon, claiming that such coated carbon almost completely eliminated the undesirable ill effects noted

with uncoated carbon. He could not detect any fine carbon particles in the rinsing fluid from a 200 g column of coated carbon. He further claimed that the coating did not significantly affect the adsorptive power of the carbon. Six patients with acute barbiturate poisoning and 11 chronic renal failure patients (29 perfusions) were treated. The results were quite dramatic and similar to those reported earlier (Yatzidis, 1964; Yatzidis et al, 1965). In vitro adsorption data were also reported. Yatzidis (1966) also discussed the adsorption of "non-dialysable toxic factors" on activated carbon.

Rosenbaum et al (1968) also evaluated cellulose acetate-coated carbon (a different coating procedure to that used by Yatzidis, 1966) using the apparatus and carbon previously described. The coated carbon was apparently ethylene oxide sterilised and washed in the same manner as the uncoated carbon previously discussed. Though the coating technique was well described, little description of washing procedure was presented.

Rosenbaum et al (1968) were much less enthusiastic about carbon or coated carbon perfusion than Yatzidis (1964). Severe drops in platelet and in leucocyte counts were noted. Charcoal emboli were readily observable in the pulmonary arterioles, just as with uncoated carbon. They conclude that "the emboli are not prevented by cellulose coating or the use of blood filters". Rosenbaum et al (1968) may have had a carbon with low washability (see Andrade et al, 1972) which was then inadequately washed, or the coating method which they utilised may not have produced a good, strong encapsulation.

Neither Yatzidis (1966) nor Rosenbaum et al (1968) discussed the properties of their coating, the extent of encapsulation, or the cleaning processes they may have used before and after coating.

The most extensive, complete and well characterised study of coated activated carbon haemoperfusion is that by Chang et al (1966-1972). Chang (1966) has pioneered the field of microencapsulation (artificial cells) for medical applications. Chang et al (1968) evaluated three different encapsulation materials (nylon, collodion and heparin-complexed collodion) for blood compatibility, concluding that the nylon material showed the greatest platelet and WBC decrease. The heparin-complexed collodion surface showed no effect on platelet or WBC levels. Chang noted early in his work (1966) that deformable capsules were not suitable for use in a large column, as they would pack closely, producing a very high resistance to flow. Chang et al (1968) thus coated activated carbon in a manner similar to that of Yatzidis (1966). Heparin-complexed collodion was used, and the coating procedure is well-documented (Chang et al, 1968). Dog experiments showed that platelet levels remained approximately normal during and after perfusion over coated carbon, while uncoated carbon produced a 50% drop in arterial platelet levels. A relatively small amount of carbon (40 g) was used and the flow

rate was 100 ml/min. These results clearly indicated that activated carbon could be coated in such a manner as to greatly minimise blood damage with haemoadsorption. In vitro adsorption data, clearance data and fine carbon particles were not evaluated. The carbon was not identified.

Chang (1969) used 300 g of 6/14 mesh Fisher scientific carbon in a study of toxin removal. Collodion, heparin-collodion and collodion-adsorbed albumin coatings were studied. The coating procedures were well described. Carbon was washed before and after coating with 6 l of saline per 300 g of carbon. In vivo (dog) adsorption and platelet data were presented for all three coatings. The collodion-albumin system proved to be the best compromise for creatinine removal and platelet compatibility. He did note that fine carbon particles were observed in the effluent blood from the shunt when uncoated carbon was used "... but none was found in that of the microencapsulated activated charcoal shunts". Excellent removals of pentabarbital were also obtained. No rationale was given for the selection of the Fisher carbon nor for the washing conditions used. No histopathological analyses were reported.

Chang and Malave (1971) have reported on the first clinical use of their system. They again used the Fisher Scientific carbon – 6/14 mesh, coconut base, encapsulated with collodion-albumin. The coated carbon was ".... washed repeatedly with distilled water through a sieve (40 mesh) until all free particles which have escaped microencapsulation are removed". In vitro adsorption and clearance studies were reported. Twenty bilaterally nephrectomised dogs were perfused with 300 g of encapsulated carbon. The 10-30 mg/100ml initial creatinine level was decreased by more than 35 % after 2 hours of perfusion at a flow rate of 120ml/min. Uncoated, collodion-coated and collodion-albumin coated carbon all showed roughly the same creatinine removal. Uric acid, urea, and other molecules were also studied. Haematological findings were comparable to those previously reported by Chang (1969). Chang and Malave (1971) studied a number of sterilisation methods for activated carbon in preparation for clinical trials, finally settling on autoclaving.

Histological studies of organs of dogs perfused with collodion-albumin coated carbon which had been carefully washed following autoclaving showed no evidence of charcoal particles in the lungs, liver, spleen, or kidney. Following these promising results, an initial clinical trial was conducted utilising 300 g of collodion-albumin activated carbon, and a 90 min perfusion. The perfusion was successful. After 60 min of perfusion the creatinine level had only dropped from 16.5 to 14.8 mg/100 ml – uric acid went from 12.9 to 9 mg/100 ml. No significant decrease in formed elements was noted.

Chang et al reported their clinical trials in 1971 and 1972. A detailed description of the washing and coating procedure is available (1971). Creatinine

and uric acid clearances were found to be linear with flow rate. Data were presented for nembutal, salicylate, glutethimide and guanidine. Chang et al (1971) have managed to design and construct a system of very low resistance, which can function adequately from arterial inflow without a blood pump. Resistances and clearances of various dialysers were compared with carbon perfusion.

Further clinical experience with coated activated carbon haemoadsorption was reported by Chang et al (1972a, b). Chang and his group have therefore evaluated various coatings and cartridge designs in the development of a system for haemoadsorption. They have extensively studied the system in vitro and with experimental animals. Finally, they have documented the safety and efficiency of coated activated carbon haemoperfusion in a series of continuing and expanding clinical trials.

There are still many unanswered questions, however. Our group (Andrade et al, 1971, 1972) has studied other coating systems, carbon types and cartridge designs. An extensive study of granular, decolourising USA-activated carbons was carried out (Andrade et al, 1972). In vitro adsorption data for

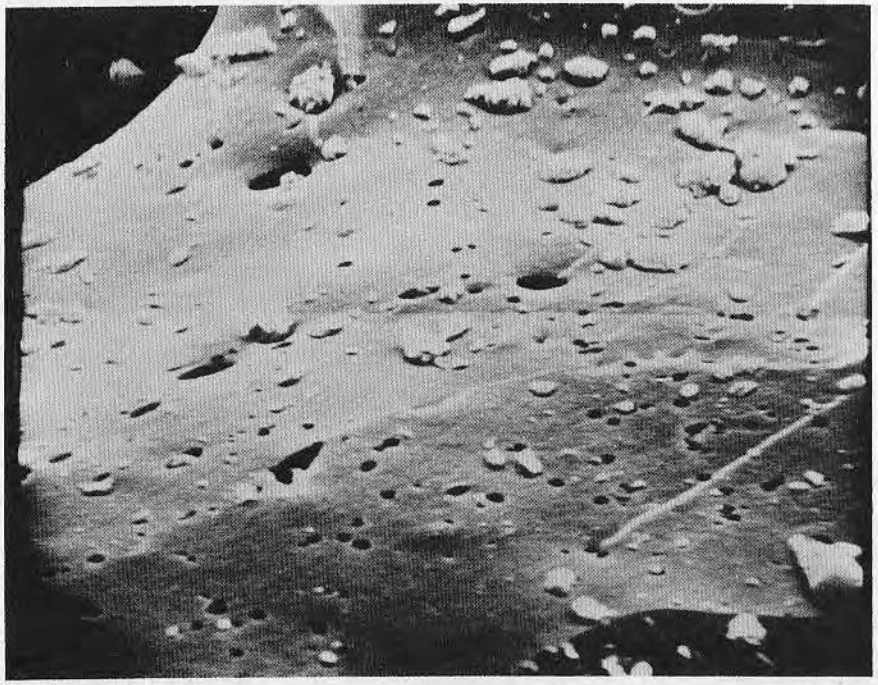

Figure 1. Witco Chemical Company Activated Carbon, as received. Note the surface pores and the fine particles. Scanning Electron Micrograph, 5300 x original magnification

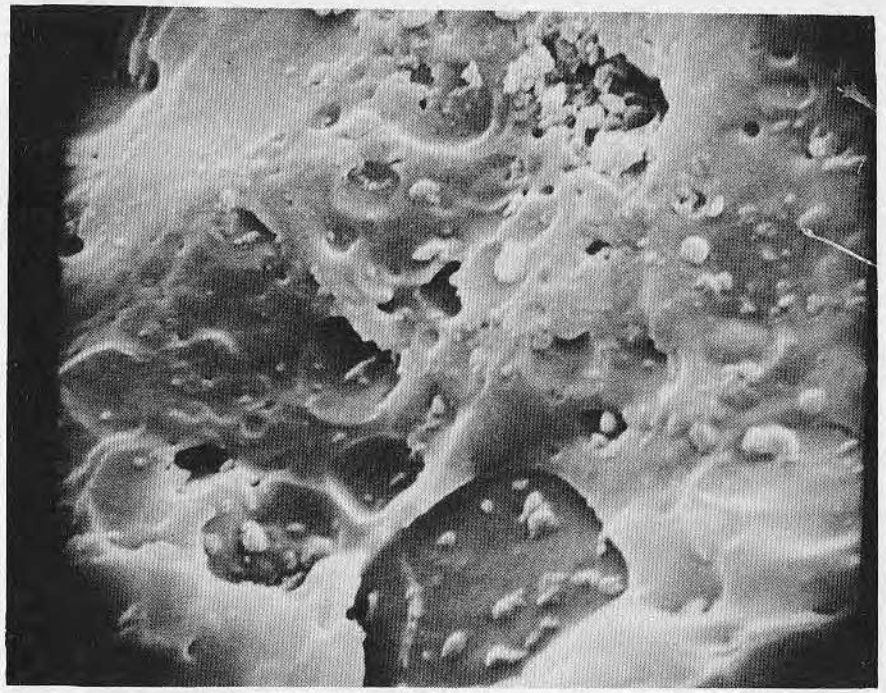

Figure 2. Witco Chemical Company Activated Carbon coated with Hydron Biomedical Polymer. This particular coating is very blood tolerable and not a significant hindrance to adsorption. Scanning Electron Micrograph, 5300 x original magnification

creatinine and other solutes were determined. Extensive washing and washability studies were also conducted, concluding that Witco 517 activated carbon (Figure 1) was perhaps the best. A detailed washing protocol is available. We evaluated three different coatings – glutaraldehyde cross-linked albumin; polyhydroxyethyl methacrylate (polyHEMA) prepared in place by direct monomer polymerisation; and Hydron Biomedical Polymer (trademark of HydroMed Sciences, Inc., New Brunswick, New Jersey, USA), prepared by cross-linking polyhydroxyethyl metacrylate (Figure 2). The latter two coatings are representative of the hydroxy alkyl methacrylate hydrogels, materials now well known for their biological compatibility (Wichterle & Lim, 1960). All exhibited good platelet compatibility and excellent in vitro and in vivo adsorption. The advantage of the polyHEMA and Hydron Biomedical Polymer coatings is that it is quite simple to prepare and is autoclavable. The coated carbon is thus relatively easy to prepare and sterilise.

Andrade et al (1972) concluded that:

1 Perfusion of blood over about 200 g of coated activated carbon can reduce blood creatinine values in 70-80 kg sheep from 15 mg/100 ml to 4 mg/

100 ml in less than three hours of perfusion. Clearances for creatinine can be maintained at **over** 100 ml/min for three hours for a 15 mg/100ml inflow concentration. Salicylate clearances can be maintained at **over** 60 ml/min for two hours for a 100 mg/100 ml inflow concentration.

2 Albumin and PolyHEMA coated carbon is relatively resistant to packing and sludging, albumin being better than the PolyHEMA. Hydron Biomedical Polymer coated activated carbon is also resistant to packing and sludging.

3 Albumin coated carbon produces a platelet drop of 20-50% in the first hour of perfusion.

4 PolyHEMA and Hydron Biomedical Polymer-coated carbon produce a platelet drop of the order of 20% or less in one hour of perfusion.

5 There is no optimum particle size in the 12-24 mesh range.

6 A single compartment cartridge is just as effective as a multi-compartment-fluidised one.

7 Charcoal which is **properly** and **thoroughly** washed does not produce readily detectable charcoal emboli — particularly when such charcoal is encapsulated. This is a tentative conclusion.

8 High resolution electrophoresis patterns of perfused blood reveal no protein changes. Inflow and outflow samples show no differences. Serum amino acid chromatograms also show no differences.

9 Activated carbon which is encapsulated may be even more effective than uncoated carbon in removing material from blood due to the lack of sludging and packing.

10 Witco 517 activated carbon appears to be superior to all others tested with respect to cleanliness and washability. It is comparable to many others for the in vitro adsorption of creatinine, salicylic acid and Pentobarbital.

11 Hydron Biomedical Polymer-coated activated carbon (Witco) may be suitable for use in clinical adsorbent haemoperfusion applications.

The work discussed on coated carbon, particularly the work of Chang et al (1966-1972) and Andrade et al (1971-1972) clearly demonstrates that a number of the problems cited in the editorial by Merrill (1971) have been successfully overcome.

DISCUSSION AND CONCLUSIONS

There is no doubt that activated carbon is an extremely effective adsorbent for creatinine, uric acid, guanidine, salicylates, barbiturates, glutethimide,

amphetamine, and a number of other compounds of interest in chronic uraemia and acute drug intoxication.

There is no doubt that perfusion over columns of most commercial activated carbons will produce readily detectable fine-particle emboli in the organs and in effluent blood smears, **unless** the carbon is extensively and thoroughly washed. Well-washed, properly washed carbon, particularly one of the more easily "washable" types, does not appear to produce fine carbon emboli. There is ample evidence that properly washed **and** properly coated carbon exhibits no signs of carbon emboli.

Activated carbon haemoperfusion cannot be a substitute for haemodialysis, but it can be an effective supplement to dialysis. Activated carbon removes best what haemodialysis removes the most poorly — creatinine and uric acid. Two to three hours of activated carbon perfusion can remove quantities of creatinine and uric acid equivalent to a full term haemodialysis. It is thus conceivable that activated carbon perfusion may permit haemodialysis treatment times to be cut in half (Scribner, 1971).

The usefulness of activated carbon haemoperfusion in the treatment of acute drug intoxication is well documented. Such work should be encouraged and expanded. Haemoadsorption devices suitable for pediatric use are presently under development and study. Commercialisation of coated activated carbon perfusion systems is undergoing extensive study.

Unless activated carbons (granules, pellets or fibres) can be produced to the exacting specifications required for medical applications, particularly freedom from fine particles, all investigators should consider the use of properly coated or encapsulated carbons. Coating systems are presently under development, which should permit haemoperfusion with minimal amounts of anticoagulation.

Other adsorbents, ion-exchange materials, and biochemically-specific perfusion systems have not been discussed. These areas are being studied by a number of groups and merit attention.

This review has emphasized the most ignored parameter in carbon haemoperfusion — the carbon itself. We hope that future workers will realize that all activated carbons are not equal — and that all coating systems are not equivalent.

ACKNOWLEDGMENT

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E DENTI (Saluggia): I want to ask Mr Kopp two questions. First, in the experimental work carried on jointly by Sorin Research Centre, Saluggie and by Medical Semeiotics Institute of the University of Pisa, we found that the activated carbons commercially produced always contain a more or less significant amount of various impurities that are slowly released into body fluids put into contact with such activated carbons. The main impurities are sodium, calcium, potassium, magnesium, copper, iron; and in some cases also zinc, manganese, aluminium and boron. The amount of impurities is such that the granulated carbons must be purified by complicated and time-consuming washing processes, in order to avoid troubles in the application of absorption techniques on the animals and the humans. I would like, therefore, to ask the author about his own experience of similar problems.

The second question is about the formation of dust from the carbon granules. We have remarked that the transport conditions are the most significant factor, the quantity of dust being of course a function of vibration duration and intensity, with the accompanying attrition between granules. It is difficult, therefore, to define the dustiness of a carbon in comparison with another carbon, without taking into account these modifications taking place during the transport. The same remark applies also, by our own experience, to the cellulose acetate coated granules. To avoid this, only coatings of relatively much greater thickness are useful, as we have found recently in Saluggia and Pisa.

KOPP: Well I am very happy; Dr Denti fully confirms what I said, that not all carbons are equal, and not all washing procedures are equivalent. Thank you, Dr Denti.

T M S CHANG (Montreal): I just want to make the point that the microencapsulation of activated charcoal is just one example of the possible use of the microcapsule artificial kidney. If you do some calculations you will find that 10 ml of microcapsules has the same total surface area as the whole artificial kidney, and thus if anybody comes up with a better absorbent than activated charcoal, then it is possible to make an even smaller 10 ml microcapsule artificial kidney.

KOPP: I think I pointed out that Dr Chang has done probably the very best work on encapsulated carbon. I encourage you very much to see his demonstration downstairs (see page 568, Eds).

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COATED ADSORBENTS FOR DIRECT BLOOD PERFUSION II

J. D. Andrade, R. Van Wagenen, C. Chen, M. Ghavamian, J. Volder,
R. Kirkham, and W. J. Kolff

Other investigators. Activated carbon (AC) is well known for its ability to adsorb a variety of poisons, drugs, and metabolites. It is often used as an oral antidote for many orally ingested toxins[1]. In vitro adsorption and kinetic studies have been carried out[2, 3]. Decolorizing and liquid treating grades of activated carbon readily adsorb a variety of compounds, particularly relatively nonpolar, poorly water soluble compounds. Creatinine, uric acid, and other compounds of interest are readily adsorbed[4].

There has been some interest in the use of AC in the dialyzing fluid to optimize the concentration gradient for the removal of creatinine and uric acid[5, 6].

There has been interest in the direct perfusion of blood over activated carbon granules or pellets for the partial treatment of uremia or acute poisoning. Yatzidis in 1964[7] demonstrated the efficacy of removing creatinine, uric acid, phenols, guanidines, salicylates, barbiturates, and glutethimides from blood by directly passing blood through a cartridge containing about 200 Gm of well-washed carbon granules. A number of investigators followed Yatzidis' lead in applying hemo adsorption over activated carbon for the treatment of uremia and acute drug intoxication[7-16, 21].

The general results are very similar. Clearly the method is very effective for the removal of a variety of toxins of clinical interest. One problem is acute thrombocytopenic effects. Platelet levels generally drop at least 50% in the first hour of perfusion. In fact, perfusion over an activated carbon column has been proposed as a method of inducing experimental thrombocytopenia[12]. The data on WBC and protein changes are inconclusive. The data on removal of urea and other compounds, particularly bilirubin, are contradictory[7, 13, 14].

A more severe problem is fine particle release or granule fragmentation. All of the studies cited except reference 10 failed to adequately describe the carbon used and the washing and cleaning procedures. There were some general notes as to nausea, vomiting, and temperature increase. There was little effort to isolate the cause - fine charcoal emboli, pyrogens, or other. Though most of the authors cited mentioned washing the carbon, few details were given. Two of the studies documented that extensive charcoal fines are readily observed in histological sections of the organs of perfused animals[10, 13]. One study[13] claimed that these were not observed when larger size carbon granules were used. The studies cited did not indicate any knowledge or even significant concern for the mechanical strength of the carbons, fines content, processing conditions which might affect fines, washing by the manufacturer, or even washing by the user. There was no mention or comparison of different carbon types.

A number of studies have shown that properly coated or encapsulated activated carbon minimizes the blood incompatibility problems noted with uncoated carbon and may even decrease charcoal emboli. Yatzidis[15] has coated carbon with cellulose acetate; greatly improved blood compatibility was noted.

Yatzidis' coating method consists of placing the charcoal for 30 min in a cellulose acetate solution (0.5 Gm cellulose acetate/L of one part alcohol, 9 parts chloroform). The carbon is dried at room temperature. "We verified by high centrifugation that the rinsing fluid from a 200 Gm column of treated charcoal in a closed circulation for 30 min with a flow rate of 200-300 ml/min remains free of microscopic charcoal particles"[15]. He further claimed that this treatment results in a 0 to 6% drop in adsorptive capacity. No data were given for blood compatibility of the cellulose acetate coated carbon.

Rosenbaum also tried the cellulose acetate coating[21], though his coating method was quite different from Yatzidis'. Though his coated carbon was less damaging than the uncoated, the platelet drop was 70% after 6 hr of perfusion.

Chang's studies of encapsulated activated charcoal are the most extensive available to date[16-19]. He has evaluated a number of coating systems and appears to have settled on collodion coating which is then coated with adsorbed albumin[19]. He has utilized Fisher carbon exclusively. His cartridge is 10 cm diameter by 8 cm high, containing about 300 Gm of carbon, with a pressure drop of 25-45 mm Hg; thus no blood pump is needed. Clearances for uric acid and creatinine are quite high - usually in excess of 100 ml/min; platelet depletion is minimal - about 10% average drop. Chang has stated that fairly extensive histological studies of dogs perfused with washed and coated activated carbon show no charcoal in the organs[17]. Chang's successful clinical application of activated carbon hemoadsorption[17, 18, 24] clearly indicate that it is a most promising method, deserving of further development and application.

We have encapsulated activated carbon granules with glutaraldehyde cross-linked bovine albumin and with polyhydroxyethylmethacrylate (poly HEMA), a synthetic hydrogel[20]. Properly coated carbon is not significantly different from uncoated carbon with respect to in vitro and in vivo removal of creatinine and uric acid[20]. Both coating materials significantly reduce the platelet depletion effect observed with uncoated carbon. Both

From the Division of Artificial Organs, and the Division of Materials Science and Engineering, University of Utah, Salt Lake City, Utah
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coatings reduce and virtually eliminate the channeling and sludging commonly observed when uncoated carbon is perfused with blood[20].

Hydrophilic surfaces. Our rationale for the use of hydrophilic gel coatings and surfaces to induce biocompatibility has been documented[20, 25]. The primary physical parameter of importance in blood compatibility are the flow conditions in the vicinity of the interface, surface roughness, and interfacial free energy - all, of course, being interrelated. The interfacial energy between a non-ionic aqueous gel surface and an aqueous solution must be very low, thus largely minimizing interfacial phenomena. One can naively, but correctly, say that there can be no interfacial phenomena when there is no interface. There is no interface when the interfacial free energy is zero. This situation is approached with non-ionic gel surfaces of high water content[25].

Activated carbon. Adsorbent hemoperfusion requires the use of particles which can be contained without difficulty within an extracorporeal circuit. This largely eliminates carbons of very fine particle sizes. Granular carbons and pelletized carbons are available in size ranges suitable for blood perfusion. The granular and pelletized decolorizing domestic activated carbons which are readily available are listed in Table I, along with some of their physical properties. Commercially available carbons are often not well characterized, particularly with respect to washing conditions, mechanical behavior, and fines content.

TABLE I

ACTIVATED CARBON DATA

Name/Brand*	Mesh	Raw Material	Max. % Ash	Surface Area m^2/gram	Pore Volume cc/gram	Approx. Density gram/cc	Pore Fraction Over 15 A°	Evaluated?
Darco	4/12 12/20	Lignite	---	700	1.00	0.39	"Mean radius" 29 A°	Yes
Norit - Granular	4/14 8/20	Wood Charcoal, peat, "etc."	5-10	665	0.9-1.10	0.26-0.30	0.55 (over 12 A°)	Yes
Norit - extruded	4/6 6/8 8/12 16/20	---	---	900-1000	---	0.42-0.45	---	No
Pittsburgh - CAL	10/40	Bituminous coal	8.5	1000-1100	0.94	0.44	0.93	Yes
SGL	8/30	"	8	950-1050	0.85	0.48	0.86	Yes
CPG	14/40	"	6	1000-1100	0.90	0.48	0.95	No
Columbia LCK	12/28	---	---	1200	---	0.46	0.80	Yes
JXC	4/10	---	2-3	---	---	0.48	0.80	No
3LXC	4/10	---	2	---	---	0.50	---	No
SXWC	4/10	---	3	---	---	0.45	---	No
NXC	4/6	---	2	---	---	0.50	---	No
ACC	6/14	---	2	---	---	0.51	---	No
MBV	4/6-6/8	---	12	---	---	0.43	---	No
Witco 517	12/30	Petroleum	1	1050	0.60	0.48	---	Yes
256	4/10	Petroleum	--	---	---	--	---	Yes
Fisher	6/14	Coconut	--	---	---	--	---	Yes

*Witco: Witco Chemical Co.,
277 Park Avenue
New York, New York

Norit: American Norit Co., Inc.
6301 Glidden Way
Jacksonville, Florida

Darco: Atlas Chemical Inc.
Chemicals Division
Wilmington, Delaware

Pittsburgh: Pittsburgh Activated Carbon Division
Calgon Corporation
Calgon Center
Pittsburgh, Pennsylvania

Columbia: Carbon Products Division
Union Carbide Corporation
120 South Riverside Plaza
Chicago, Illinois

Fisher: Fisher Scientific Company
Fairlawn, New Jersey

METHODS

Cleaning and washing. The as-received granular activated carbon is contacted with a polypropylene surface, which removes a large number of the fines, probably by a static charge interaction. Fifty Gm portions are then placed loosely in a nylon mesh sack and subjected to a 60-80 L/min air stream for about 5 min. The carbon is then sieved on conventional mesh screens and the 12/24 mesh fraction is retained. The material is then placed in saline (200 Gm carbon in 400 ml of saline) and deaerated in a vacuum dessicator at room temperature for 2-3 hr. As the air is removed from the smaller pores of the carbon, the fines evidently come with it, rising to the surface of the solution. The surface debris is aspirated off and the carbon transferred to a polypropylene washing

cylinder fitted with a support screen (50 mesh) on the lower end. The cylinder is then moved up and down in a piston-like fashion into saline solution. This motion produces a gentle agitation of the carbon; the fines removed remain suspended in the washing medium. The wash water is discarded and replenished every 10 min for up to an hour. After 4 to 5 changes, the solution is visibly free of fines for some carbon types, though microscopic fines usually remain. These are detected by taking a 20 cc sample of the wash solution and passing it through a 0.22 μ, 25 mm diameter Millipore filter. The carbon is finally rinsed in distilled water, packaged moist in plastic bags, and stored under refrigeration. Prior to use in a perfusion circuit, the carbon is again gently washed in distilled water and then soaked in a heparinized saline solution overnight.

The washing procedure described has evolved empirically in our Laboratory. It is not necessarily an optimum procedure.

Coating. The coating procedures utilizing hydroxyethylmethacrylate and albumin have been previously described[20]. One might expect that a coating medium containing molecules of fairly high molecular weight would be much more suitable for our purposes than monomer or low molecular weight polymer (such as albumin). Thus we arranged for Hydro Med Sciences, New Brunswick, New Jersey to coat our carbon with Type NL Hydron Biomedical Polymer, a well-characterized polyHEMA. The carbon is carefully coated in the polymer solution and dried; multiple coating and drying steps are involved, depending on the coating properties desired. Cross-linking is accomplished with a derivative of the monomer, HEMA. The coating is carried out under conditions which minimize any mechanical abrasion of the carbon granules.

In vitro adsorption. The carbons noted in Table I were evaluated in stirred batch adsorption studies for in vitro rate and capacity (5.0 Gm of carbon were placed in 200 ml of phosphate - buffered saline (PBS), pH 7.4, at 37° C). The appropriate radiolabeled solute was added rapidly and samples taken as a function of time. Samples were placed in scintillation vials containing 10 ml of Aquasol (New England Nuclear Company), a commercial liquid scintillation cocktail solubilizer, and counted to $\pm 1\%$ at a 95% confidence level.

Cartridge. The 3 cartridge designs we have used are illustrated in Figure 1. The rationale behind the multi-compartment cartridge was that we wanted to minimize packing in the column and maximize fluidization of the particles. Each compartment has a priming volume of about 20 cc when filled with 20 Gm of carbon granules. The large single compartment cartridge is 6.3 cm in diameter and 12.6 cm long (ignoring the cone end caps); it can contain 200 Gm of carbon with a screen-to-screen priming volume of 110 cc. The small cartridge in Figure 1 is designed for small animal studies and possibly for eventual pediatric applications[26]. It is 2.5 cm I.D. and 18.5 cm long (screen-to-screen); it can contain 40 Gm of carbon with a 40 cc priming volume. It is important to realize that activated charcoal has a substantial internal pore volume (0.6 cc to 1.0 cc/Gm, see Table I), thus carbon may displace relatively little water.

In vivo studies. The most common experimental set-up utilizes a sheep of approximately 70 Kg. The animal is anesthetized with halothane and respirated. A carotid-jugular cutdown is performed, conventional silicone rubber dialysis shunts inserted (Extracorporeal Saf-T-Shunt), and the circuit connected. The circuit is illustrated in Figure 2. Heparin dosage was usually 20,000 units initially (about 3 mg/Kg), followed by 3-5,000 units/hr thereafter. Total dose per experiment ranged from 30,000 to 50,000 units, depending on the duration. Blood samples were taken at various times. Rectal temperatures were monitored in a number of experiments. A series of animals was acutely bilaterally nephrectomized, a series was unilaterally nephrectomized, and another series was not nephrectomized. Clearance studies were performed on the non- and unilaterally nephrectomized animals using a constant infusion (before the roller pump in Figure 2). A number of preliminary experiments were done using a single-needle access systems[27] for arterial access and also for venous access.

Blood analysis. Creatinine and salicylate determinations were made utilizing standard Medi-Chem Laboratory test kits. WBC counts were made on a Model ZB1 Coulter Counter. Platelets were also counted with the Coulter Counter as well as manually. Electrophoresis patterns were made according to the Ortec, Inc., Oak Ridge, Tennessee, method, utilizing a polyacrylamide gel slab with 4 different pore size zones and a pulsed power system[28]. Amino acid scans were made using the Kodak Chromato/0/screen screening chromatography kit (Eastman Kodak Co., Rochester, New York). Selected other data were obtained with an autoanalyzer.

Multiple section pathological examination of liver, spleen, kidney, lung, and myocardium was conducted by Dr. Kent van Kampen, Veterinary Pathology Labs, Murray, Utah.

RESULTS AND DISCUSSION

Cleaning and washing. The cleanliness of activated carbon is of great importance for any in vivo application, due to the potential generation of charcoal emboli. Figure 3a is a scanning electron micrograph (SEM) of as-received Witco 517 (See Table I). The material clearly has a large amount of fines in its surface, in the cracks, and within the pores. After treating the material with a flow of air (see METHODS), one observes a very great decrease in the number of fines on the carbon (Figure 3b). After saline washing, the fines are further reduced, as evident in Figure 3c. All of the granular carbons we have evaluated (Table I) exhibit a surface with a high proportion of fines.

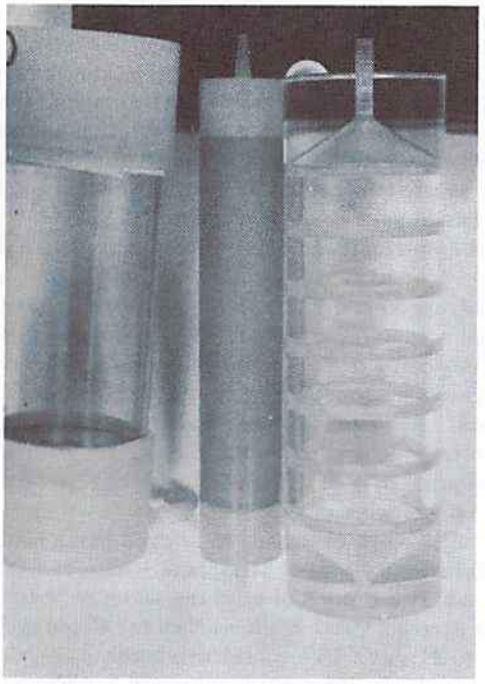

Figure 1. The 3 different cartridge designs referred to in the text. On the right is the multi-compartment device designed for maximal particle agitation. On the left is the present 200 Gm single compartment cartridge. The smaller unit has a 40 Gm capacity for small animal studies.

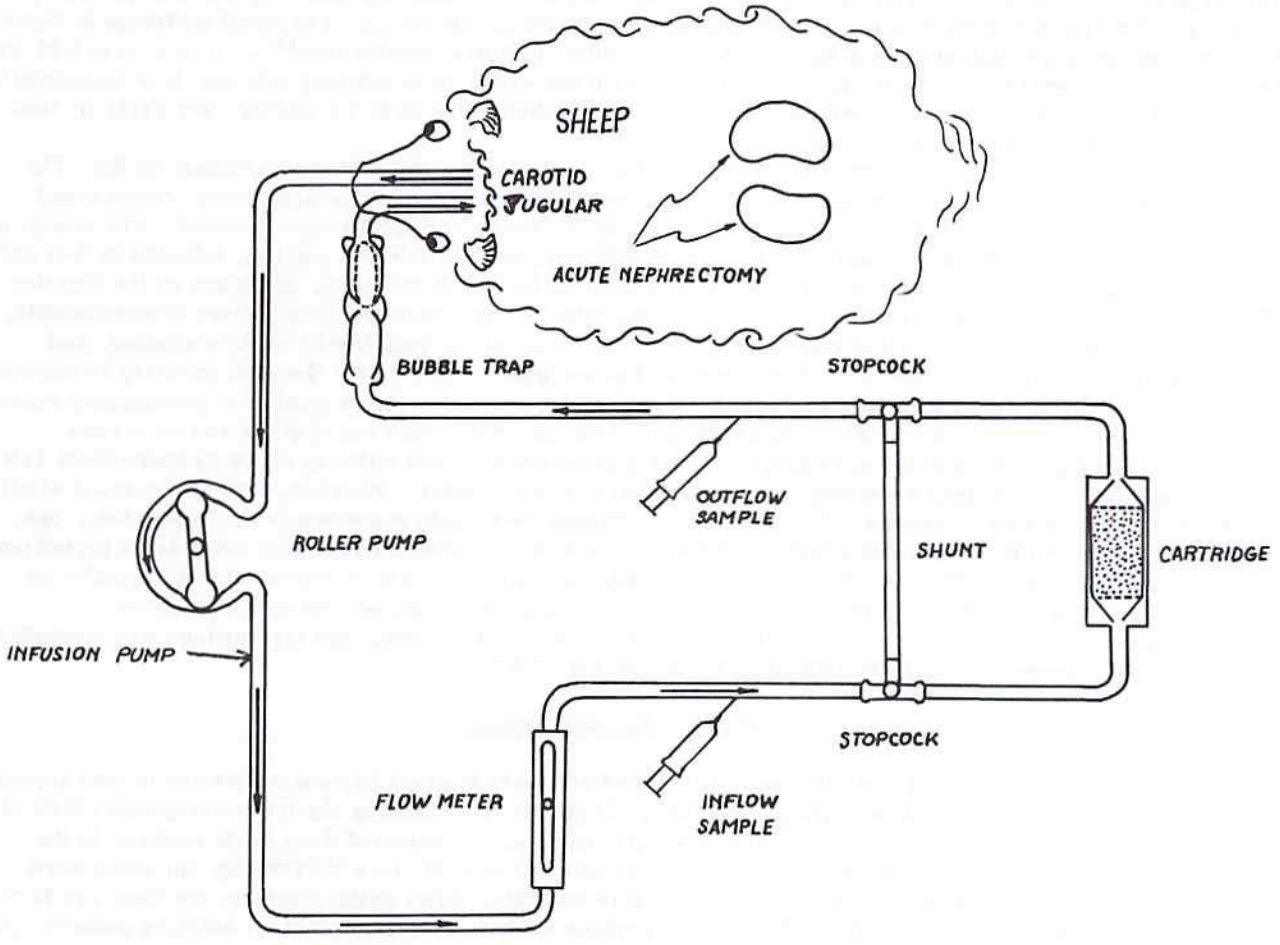

Figure 2. The experimental set-up for most of the acute in vivo experiments.

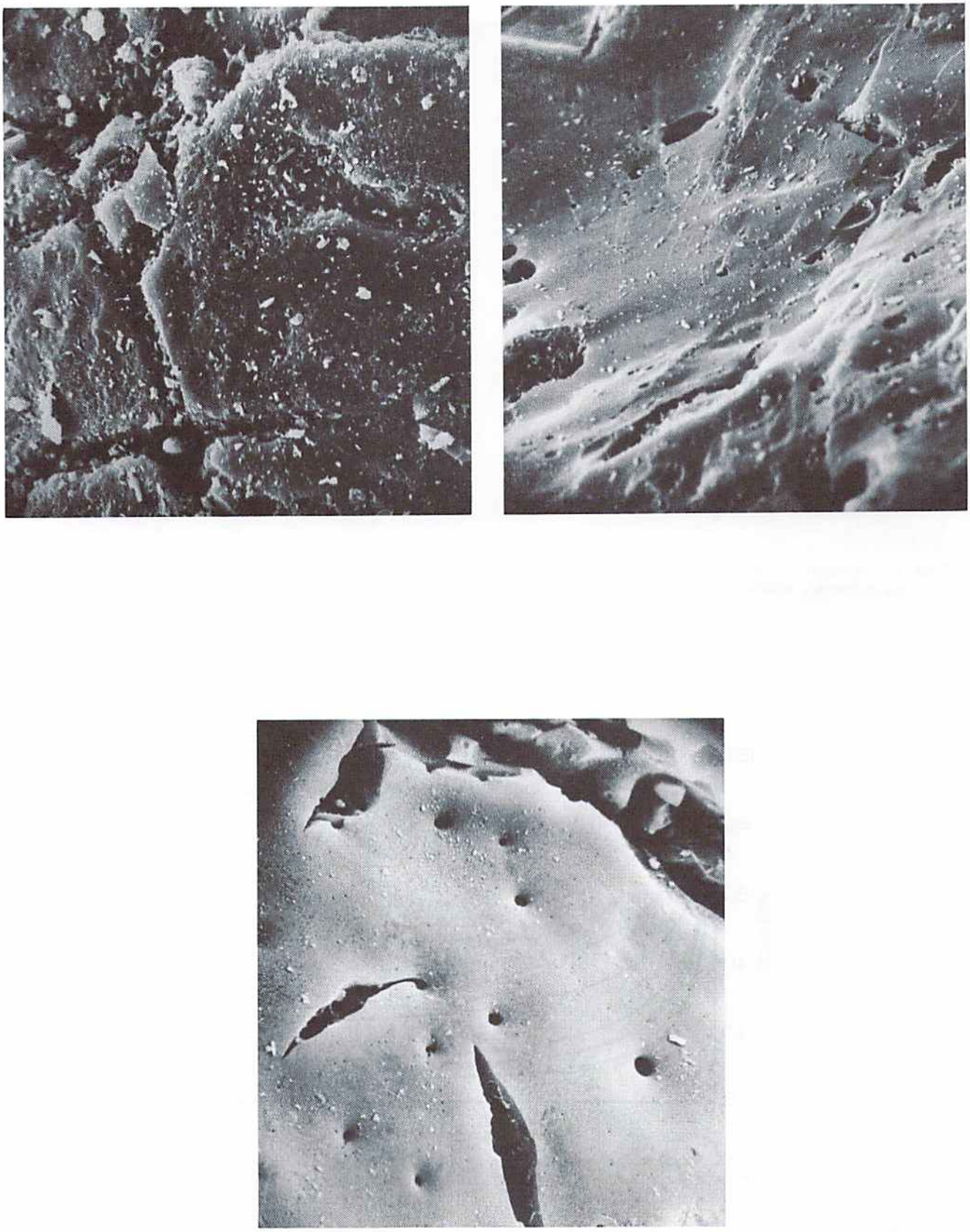

Figure 3. Scanning Electron Micrographs of Witco 517 12 x 24 mesh activated carbon. All samples are gold-coated and observed at 20, 000 volts. (a) As-received - 1000 x original magnification, (b) Air washed - 1150 x original magnification, (c) Air and saline washed - 1150 x original magnification.

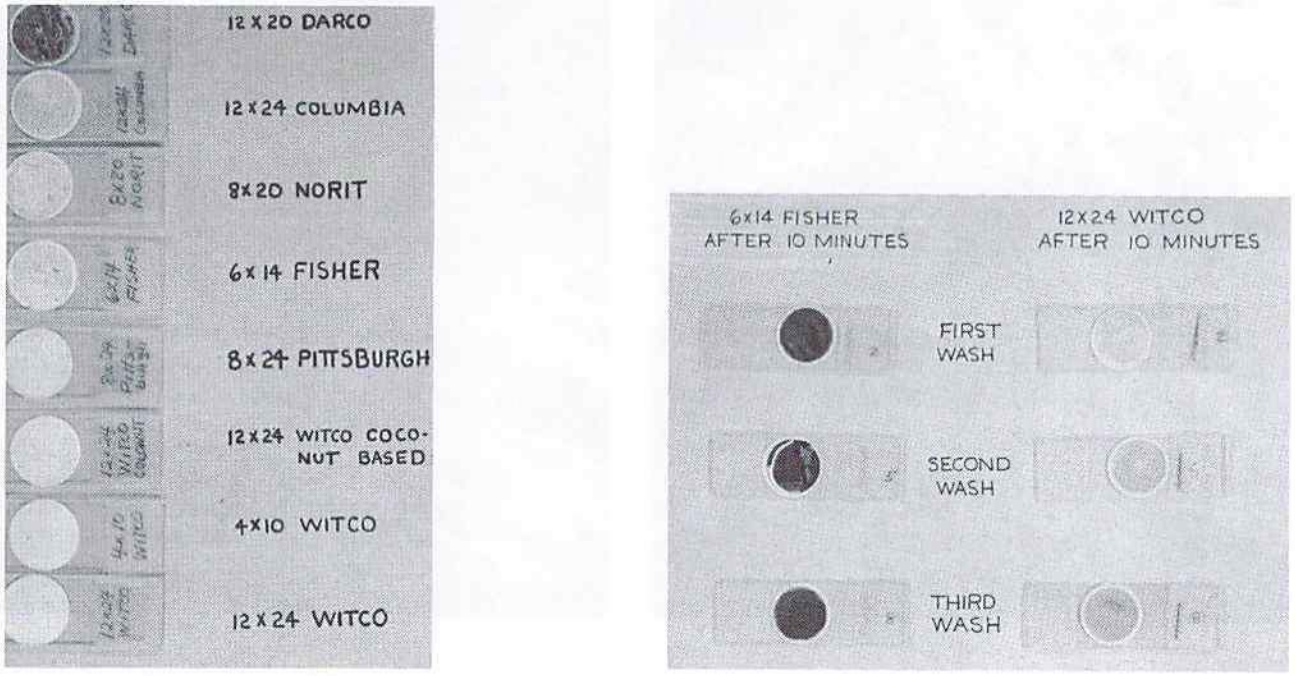

Figure 4. Comparative washing studies - See text for explanation. (a) Comparative study of 8 different carbons - all saline washed 4 times, (b) Comparative study of Fisher and Witco carbons - each washed 15 times.

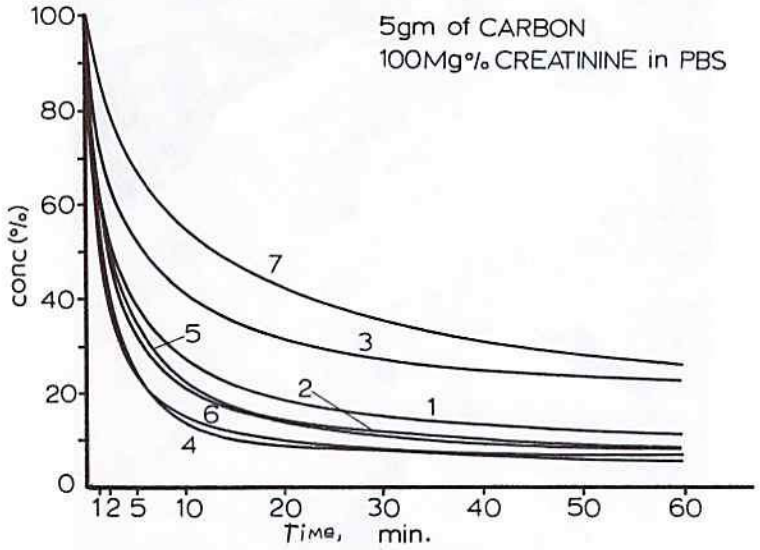

Figure 5. Stirred batch adsorption data for the adsorption of creatinine from 200 ml of a 100 mg% solution in PBS, pH 7.4, 37° C, onto 5 Gm of activated carbon granules:

1. Norit (8 x 20)
2. Witco (12 x 30)
3. Darco (12 x 30)
4. Columbia (12 x 28)
5. Pittsburgh (8 x 30)
6. Pittsburgh (12 x 40)
7. Fisher

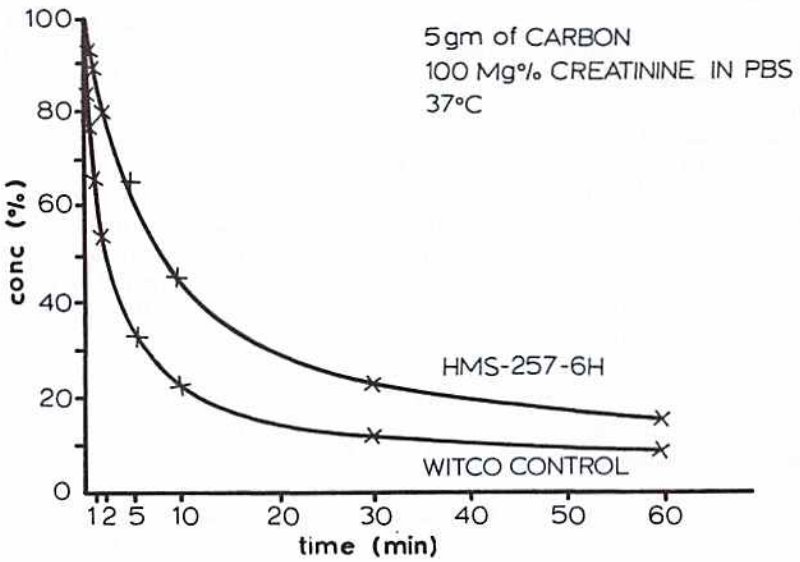

Figure 6. Stirred batch adsorption data for the adsorption of creatinine from 200 ml of a 100 mg% solution in PBS, pH 7.4, 37° C, onto 5 Gm of Witco 517 uncoated carbon (labeled as "control"), and onto 5 Gm of Witco 517 coated with Hydron Biomedical Polymer (labeled as HMS-257-6H).

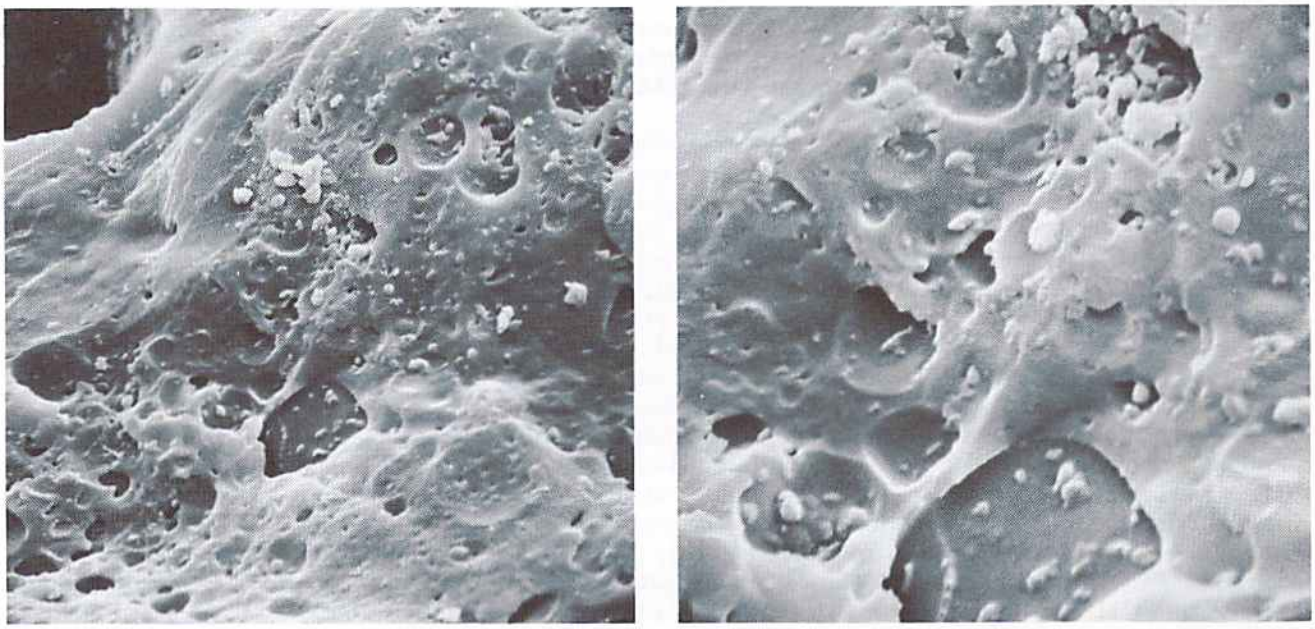

Figure 7. Representative Scanning Electron Micrographs of Witco 517 activated carbon with the HMS-257-6 coating. Compare with Figure 4 for uncoated carbon. Note that the coating covers some fines but that many others appear on the coating with little apparent adherence.

Comparative washing studies of 8 different activated carbons were carried out according to the procedure described. The Witco 517 carbon exhibited a clear wash solution after 4 washings. The other carbons were washed in exactly the same manner. A sample of the final wash solution was then taken and passed through a Millipore filter (Figure 4a). Clearly some carbons are much "dirtier" or "washable" than others. As a result of this and other studies, Witco 517 was chosen as the carbon for our future studies.

Fisher activated carbon has been used by Chang for a number of years[16-19]. We thus chose to more extensively compare the Fisher carbon with Witco 517. The Fisher material required 15 washings before the final wash solution looked clean. Fisher and Witco carbon, washed 15 times, were resuspended in fresh water, gently agitated, and samples taken 0, 10, and 20 min later. The carbon was then placed in a second fresh solution, and the solution sampled again. This was repeated a third time. The results are evident from Figure 4b. It is clear that the Witco carbon is the most easily washable of the carbons evaluated and quite superior to Fisher carbon in terms of washability and minimum fines after extensive washing - for the conditions described.

One might expect to reduce the fines content even more by more extensive washing, but a point of diminishing returns is rapidly reached. Beyond the optimum number of washings (4 to 5 for Witco, about 15 for Fisher), one apparently produces as many fines as he removes. This effect is largely dependent on the mechanical abrasion resistance of the carbon. The as-received Witco carbon looks terrible in the SEM photographs (Figure 3a), yet it is the easiest to clean. Other carbons which looked much better in the SEM study, were very difficult or impossible to clean.

In vitro studies. Figure 5 presents the in vitro adsorption results with creatinine. Three carbons, the Darco, Norit, and Fisher types, show substantially slower adsorption rates and a decreased capacity as compared with the other 4 grades. All the carbons look equally good for the adsorption of salicylate and nembutal[29]. It is quite fortunate that the carbon which, in our hands, can be cleaned the most effectively is also quite good for the adsorption of creatinine, salicylate, and barbiturate. This means that one carbon type could be used for both chronic uremia treatment and acute poisoning treatment.

One might expect that coated or encapsulated activated carbon would exhibit lower adsorption rates and perhaps even a lower ultimate capacity than uncoated carbon. This is partially true, in vitro. Just the opposite may occur in vivo. We have demonstrated that properly coated activated carbon retains its rapid adsorption rates and high capacity for creatinine and uric acid[20] and more recently for salicylate and barbiturate as well[29].

Figure 6 presents the results for the Hydro Med Sciences coated Witco carbon as contrasted with the uncoated control. There is a substantial decrease in rate of adsorption, but the decrease in ultimate capacity (say at t = 1 hr) is not very great. Figure 7a is a SEM of a relatively thick Hydron Biomedical Polymer coating, while Figure 7b is the coating we are presently using (also Figure 6). It is clear that a number of fine particles in the surface appear to be covered by the coating, but it is equally clear that a large number of fines appear completely uncoated. One must therefore conclude that the encapsulation of activated carbon does not necessarily solve the fine carbon emboli problem. Furthermore, it is unlikely that a heterogenous substrate such as activated carbons, with its sharp points, edges, etc., can be totally and completely coated with any reasonable assurance.

In vivo studies. A complete data presentation and analysis of the in vivo work is available[29]. Our earlier work[20] demonstrated that albumin and polyHEMA-encapsulated activated carbon were both quite blood tolerable as well as effective in removing creatinine.

The multi-compartment cartridge system was studied. It was clear that our original thesis, that maximum fluidization and agitation of the granules is necessary, was simply not correct. A series of experiments were performed employing 80 Gm of carbon per cartridge supported in one, 2, 3 and 6 separate compartments. There was no difference in clearance values for creatinine for the 4 configurations tested. These results were confirmed in 3 experiments. We, therefore, concluded that the highly agitated, multicompartment cartridge approach is not necessary and may even be disadvantageous as it necessitates a larger priming volume and exhibits a higher pressure drop.

A second series of experiments tested the effect of particle size in clearances and pressure drops. The size ranges evaluated were 12 x 16 mesh, 16 x 20, 20 x 24, and 12 x 24. There was no detectable difference, thus we selected the 12 x 24 size for all subsequent studies.

Figure 8 presents the creatinine data for an acutely bilaterally nephrectomized sheep (about 70 Kg) given a loading dose of creatinine. Blood tissue equilibration appears to occur about 90 min after injection, at a high time. The first cartridge is connected. Flow rate is 100 ml/min. Clearance is 100 ml/min initially (the flow rate) and drops to about 80 ml/min after 90 min. These values are for 80 Gm of carbon. Though the high clearance can be maintained by the use of fresh cartridges (2, 3, 4 in Figure 8), the blood level does not drop much below 4-5 mg%. Other experiments exhibited initial creatinine clearances ranging from 100-150 ml/min initially, falling to 50-75 ml/min after 2 hr with a single 80 Gm cartridge. The initial clearance is roughly equal to the flow rate over the range of flows studied (80-150 ml/min).

Platelet studies indicated that an 80 Gm bed of uncoated carbon generally produces a 50-70% drop in platelets within one hour of perfusion. Our albumin coated carbons produced significantly less drop, but drops did reach 50% in some of the experiments. PolyHEMA-coated carbon (coated in our Laboratory by the technique

described earlier[20]) produced drops up to 30%. The Hydron Biomedical Polymer coated material produced platelet drops of 10-25% of initial values.

Uncoated carbon tends to pack, sludge, and channel, possibly due to pick-up of platelets and other cellular elements. Fibrin-like strands are also evident on uncoated blood-perfused carbon observed by SEM. Thus a perfusion with uncoated carbon requires substantially higher heparin levels than those used for coated carbon. One might expect that the clearances observed with coated carbon would be lower than with uncoated, due to the in vitro adsorption results (Figure 6 and Reference 20). However, because of the decreased cellular pick-up of the coated carbons, and the decreased packing, sludging, and channeling observed, the coated carbons are actually more effective in vivo than the uncoated. This is exactly opposite from the results quoted and assumed by some prominent editorial writers[23].

Studies with a 200 Gm capacity cartridge in constant creatinine infusion experiments indicate initial clearance values of 150 ml/min (the flow rate), dropping to 100 ml/min after one hour of perfusion and remaining at about 100 ml/min for up to several hours thereafter. The plateau clearance values are often substantially higher than 100 ml/min.

Our results showed no significant changes in phosphate, BUN, total protein, bilirubin, LDH, or SGOT during a 3 hr perfusion. Cholesterol does drop about 50 mg% initially to 35 mg%. High resolution plasma protein electrophoresis patterns showed no change during the course of our perfusions for both coated and uncoated carbons. Plasma amino acid chromatograms were obtained for 3 experiments - no differences or changes were observed.

A series of constant infusion experiments have been performed with salicylate. A 200 Gm bed of Witco 12 x 14 uncoated carbon exhibits an initial salicylate clearance of 130 ml/min dropping to 60 ml/min after 2 hr of perfusion (blood flow, 150 ml/min). Nearly 80% of the inflow salicylate can be removed.

A series of preliminary experiments using the single-needle access system[27] have been performed. Hemo-adsorption utilizing single needle access is as effective as conventional access methods. We are presently developing a charcoal hemo-adsorption system with single needle access suitable for small venous vessels and possibly for applications in acute pediatric poisonings[26].

A series of experiments on fine-particle emboli formation was also conducted. These results are very difficult to quantitate. It is clear, however, that perfusion of blood through a column of unwashed or poorly washed uncoated carbon results in readily detectable carbon particles in outflow blood smears and in the organs.

Uncoated Witco carbon washed according to the procedure described (see METHODS) does not generally produce readily detectable particles in the organs, though we have seen some. Poorly washed but coated carbon does produce detectable particles, though much less than for the uncoated material. Thoroughly and carefully washed carbon which has been coated by our procedures has not produced detectable particles in the organs. These are still tentative results; they are based on a fairly comprehensive multiple section analysis of the organs. This work is continuing.

It must be emphasized that all the in vivo data reported was obtained in acute experiments utilizing a circuit constructed of conventional components (silastic tubing, tygon tubing, polypropylene, polycarbonate, etc.), with no surface treatments. Only the carbon is coated.

Other. J. P. Merrill recently published an editorial[23] which merits some discussion. He implied that activated carbon-based hemo-adsorption systems do not deserve continuing clinical trial. The reasons given were platelet and WBC damage, and charcoal emboli. He further stated that "... the encapsulating agent also decreases exchange and absorption and, therefore, the efficacy of the system." That statement is simply not always true.

Our work has clearly shown that properly coated activated carbon can greatly reduce the platelet and WBC problems exhibited by uncoated carbon. The coating may decrease the kinetics and even ultimate capacity of adsorption in vitro. However, our coatings often lead to even more effectiveness in vivo than uncoated carbon. The reason is that uncoated carbon has a tendency to pack and sludge, thereby inducing channeling and decreasing the effectiveness of the system. Properly coated carbon does not pick up large quantities of platelets, it does not tend to pack or sludge, thus the effectiveness of the circuit is often higher than with uncoated carbon - this result is in direct opposition to Merrill's statement[23].

Our studies on the washing of carbon clearly show that proper washing greatly reduces the incidence of charcoal emboli - though they may still be a problem. The results reported in this paper on the washability of various carbons clearly indicate that some carbons are cleaner than others, and, more importantly, that some carbons are more washable than others.

Properly washed, properly coated, activated carbon may have an important place in clinical treatment of chronic uremia and certain acute poisoning episodes.

SUMMARY AND CONCLUSIONS

1. Perfusion of blood over about 200 Gm of coated activated carbon can reduce blood creatinine values in 70-80 Kg sheep from 15 to 4 mg% in less than 3 hr of perfusion. Clearances for creatine can be maintained at over 100 ml/min for up to 3 hr. Salicylate clearances can be maintained at over 60 ml/min for up to 2 hr.
2. Uncoated carbon tends to pack and sludge, decreasing column effectiveness.
3. Albumin and PolyHEMA coated carbon (as prepared in our labs) is relatively resistant to packing and sludging, albumin being better than the PolyHEMA. Hydron Biomedical Polymer coated activated carbon is also resistant to packing and sludging.
4. Uncoated carbon produces a platelet drop of 50-70% in the first hour of perfusion.
5. Albumin coated carbon produces a platelet drop of 20-50% in the first hour of perfusion.
6. PolyHEMA and Hydron Biomedical Polymer-coated carbon produces a platelet drop of the order of 20% or less in one hour of perfusion.
7. There is no optimum particle size in the 12-24 mesh range.
8. A single compartment cartridge is just as effective as a multi-compartment-fluidized one.
9. Charcoal which is properly and thoroughly washed does not produce readily detectable charcoal emboli - particularly when such charcoal is encapsulated. This is a tentative conclusion.
10. High resolution electrophoresis patterns of perfused blood reveal no protein changes. Inflow and outflow samples show no differences. Serum amino acid chromatograms also show no differences.
11. Activated carbon which is encapsulated can be even more effective than uncoated carbon in removing material from blood due to the lack of sludging and packing.
12. Witco 517 activated carbon appears to be superior to all the others tested with respect to cleanliness and washability. It is comparable to many others for the in vitro adsorption of creatinine, salicylic acid, and pentobarbital.
13. Hydron Biomedical Polymer-coated activated carbon (Witco) may be suitable for use in clinical adsorbent hemoperfusion applications.

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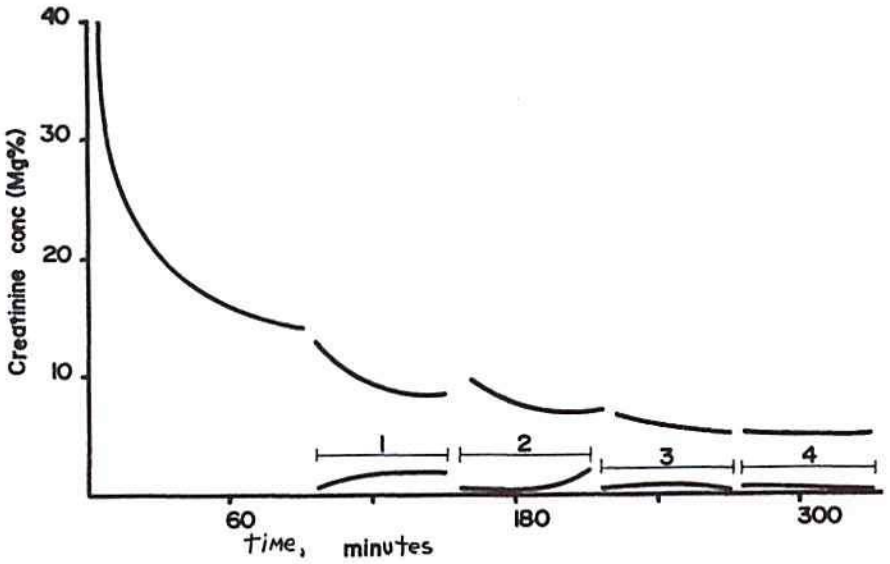

Figure 8. Creatinine data for a bilaterally nephrectomized animal given a loading dose of creatinine. 90 min was allowed blood-tissue equilibration. Hemoadsorption through cartridge 1 was then begun and continued for 60 min, at which time flow was switched to cartridge 2, etc. Eighty Gm of albumin-coated carbon per cartridge. Flow rate is 100 ml/min. Note the very good clearances obtained during the perfusion.