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APPLYING "INTELLIGENT" MATERIALS FOR MATERIALS EDUCATION: THE LABLESS LAB®

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Introduction

A very large number of science and engineering courses taught in colleges and universities today do not involve laboratories. Although good instructors incorporate class demonstrations, homework, and various teaching aids, including computer simulations, the fact is that students in such courses often accept key concepts and experimental results without discovering them for themselves. The only partial solution to this problem has been increasing use of class demonstrations and computer simulations.

We feel strongly that many complex concepts can be observed and assimilated through experimentation with properly designed materials. We propose the development of materials and specimens designed specifically for education purposes.

"Intelligent" and "communicative" materials are ideal for this purpose. Specimens which respond in an observable fashion to new environments and situations provided by the students/experimenter provide a far more effective materials science and engineering experience than readouts and data generated by complex and expensive machines, particularly in introductory courses. Modern materials can be designed to literally communicate with the observer. Although some such materials can be obtained from commercial and research sources and are suitable for experiencing and learning certain materials phenomena and behavior, there has been no concerted effort to develop materials specifically for education application. We are embarked on a project to develop a series of Lableess Labs®, utilizing various degrees and levels of intelligence in materials. It is expected that such Lableess Labs® would be complementary to textbooks and computer simulations and be used to provide a reality for students in courses and other learning situations where access to a laboratory is non-existent or limited. Our initial project is the Lableess Lab® for Polymer Science.

Materials & Methods

Most students come into polymer courses with various concepts and preconceptions which lead them to conclude that the behavior and properties of polymers are counter intuitive. It is therefore important that they fully discover and observe the properties and behavior of polymeric materials for themselves. It is appropriate to begin our Lableess Lab® effort with polymeric materials because they are readily available for a wide variety of applications and because they exhibit a range of phenomena which are very easy to observe, experience, and discover.

There is considerable interest in effective polymer education [3]. The American Chemical Society Division of Chemical Education often includes polymer related articles in its Journal of Chemical Education [4, 5] and in its sessions at the American Chemical Society annual meetings. The ACS also has a Polymer Education Committee, as does the Society of Plastic Engineers (SPE). Polymer education is also of interest to the American Institute of Chemical Engineers (AICHE) and the Materials Research Society (MRS). There is a Polymer Education Center at the University of Wisconsin, Steven's Point and a Polymer Education Newsletter [3]. The Institute for Chemical Education at the Department of Chemistry at the University of Wisconsin, Madison, is also active in providing a variety of educational materials for discovery based chemistry and polymer education [6].

These activities are all helpful and indeed have greatly stimulated this project. However, the typical instructor, particularly in relatively large enrollment classes, often does not have the time or the inclination to assemble the materials, components, and equipment necessary to put together an effective discovery laboratory, particularly if the class is a lecture only course, which is typical for many introductory material science and polymer science courses.

Results

The following materials/topics/devices are being developed and will be discussed: Homopolymer Tg, Placticizer Gradient, Cross-link Gradient, Copolymer Gradients, Ionic/Temperature Responsive Polymers, Surface Property Gradients [7-9].

Conclusion/Summary

A Lableess Lab® for polymer materials is well on its way to becoming a real reality. Preliminary versions are being tested in a course at the University of Utah. A field test version keyed to several major textbooks in polymer science and engineering should be available by summer, 1995. Limited commercial distribution is expected in early 1996.

The goal is to develop a Lableess Lab® in polymer materials which could be made available in classroom quantities with prices comparable to those of existing textbooks, i.e., in the $40-$60 range per unit. It is anticipated that there will be two such products, an introductory polymer materials version, and a more advanced version.

We are interested in learning of additional materials and phenomena which could be incorporated into the Lableess Lab® in a very inexpensive manner. Lableess Labs® in other appropriate science and engineering courses are also under development. The Lableess Lab® is a trademark of Protein Solutions, Inc., Salt Lake City, Utah. *Acknowledgments:* This material is based upon work supported by the National Science Foundation under SBIR award number III-9361652. Our initial work in this area was funded by a small grant from the American Chemical Society's Division of Polymer Chemistry, Polymer Education Program. The application of the concept to a basic polymer materials science course at the University of Utah was funded by

the University of Utah Teaching Committee. Most of the existing work on the project is focused at Protein Solutions, Inc. in Salt Lake City, a science education products company, and funded by a small business innovation research (SBIR) grant from the National Science Foundation. J.D. Andrade acknowledges discussions on these topics with K. Caldwell, W. Callister, L. Feng, V. Hlady, and T. Matsuda.

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SMITH – request late

[Poster: **smith**.jessic]

Optimizing biosensor design with computer modeling: a case study involving creatine

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For many metabolites, there are several metabolic pathways that involve ATP and therefore, there are several pathways that can be potentially coupled to ATP-linked firefly luciferase to produce bioluminescence. The coupled reactions include two basic types, ATP production and ATP depletion. The change in ATP, which can be measured from the light produced via the firefly luciferase reaction, correlates with substrate concentration. A biosensor based on this principal, that could measure several metabolites at once, would be a great asset to both research and clinical and preventative medicine. However, for even the simplest metabolic pathways, biosensor design is a complex and costly endeavor. Computer modeling is a useful tool for evaluating the feasibility of using a bioluminescent-based biosensor to quantify specific metabolites. Computer models can be used to investigate several metabolic pathways to determine the optimal reaction scheme and kinetics for biosensor design. The purpose of this investigation was to develop a computer model for the ATP firefly luciferase reaction, present a case study of a homogeneous, ATP depletion assay involving creatine, and discuss the use of computer modeling for optimizing biosensor design.

[Talk: **squirrel**.davidj]

Rapid bacterial detection using adenylate kinase (AK) in a magnetic bead immunoassay

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Adenylate kinase (AK) is an essential enzyme catalysing the equilibrium reaction: Mg^{2+}.ADP+ADP <--> ATP+AMP. It can be used as a marker for the bioluminescent detection of bacteria with detection limits ~100 times better than can be achieved by measuring ATP alone. Fewer than 100 bacterial cells may be detected in a 5 min assay, with increased sensitivity being obtained from longer assay times. Immunoassays have been developed with target cells captured using antibody-coated magnetic beads. Detergents were used to release AK from captured cells so added ADP could be converted to ATP. The resulting ATP was measured using firefly bioluminescence. Salmonella at <1000 cells/ml and E.coli O157 at <100 cells/ml could be detected in ~10 mins. Optimisation experiments are described. Lysis using bacteriophages rather than detergents was investigated with the aim of imparting added specificity. Results from assays on foodstuffs are given.

[Poster: **sokolova**.irinav]

Formation of the myristic acid by bioluminescence of *Photobacterium leiognathi*

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Bioluminescence reactions of the luciferase with tetradecanal under different conditions were examined by monitoring quantum yields and formation of the myristic acid following the injection of chemically reduced $FMNH_2$. The fatty acid was measured quantitatively with a chromatho- masspectrometer. Bioluminescence activities were measured with a photometer, calibrated with the luminol reaction. One quantum of light is shown to be formed by 10 molecules of myristic acid. This correlation of quantum yield and fatty acid is not constant and can be varied by addition of organic solvents. So, added dimethilsulfoxid or aceton was shown to result in quantum yields decreased by 25-50%. In the formation of fatty acid the effect was opposite. The quantity of myristic acid increased and with addition of dimethilsulfoxid or aceton correlation became 30 and 50 molecules acid respectively for one quantum of light.

[Talk: **stewart**.cneal]

GFP in plant biotechnology and agriculture

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The ability to precisely manipulate plant genetics through transformation has revolutionized agriculture. However, several are concerned that ecological and health problems may accompany GM crops. One concern is that transgenes will be transferred from crops to weeds and create superweeds. The ability to track transgenes in space and time will be a helpful monitoring tool. We will present data showing a case study in which an insect resistance *Bacillus thuringiensis* transgene (Bt) is linked to a GFP gene, and the presence and expression of Bt is monitored by GFP. Also presented will be present and future applications of GFP in remote sensing, precision agriculture, and environmental biotechnology. For example, plant pathogen inducible promoters fused to GFP may be useful for precisely monitoring incidence and spread of plant diseases in real time. Finally, we believe that GFP will enter the human food supply because of its many useful qualities in agriculture. To that end we will present data that will address human allergenicity and toxicity issues. For example GFP appears to be digested in mammalian stomach and has no homology to known allergens.

AD-Smith-2

CELL ADHESION AS INFLUENCED BY SUBSTRATE SURFACE PROPERTIES

by

Lee Merrill Smith

A dissertation submitted to the faculty of The University of Utah in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Material Science and Engineering

The University of Utah

June 1979

ABSTRACT

A rotating shear disk is described for measuring short term (30 minute attachment) cell-substrate adhesion to selected substrates. Substrate surfaces are selected and prepared to obtain a wide variety of surface properties. Surface free energies are measured with captive air and octane angles on fully water extracted samples. Substrate surface chemistry is characterized via X-ray photoelectron spectroscopy for both bulk and spin cast thin (0.1 μm) films. Electrokinetic zeta potentials were measured via streaming potentials.

Human derived normal and SV_{40} transformed WI38 fibroblasts in serum containing media are used to elucidate substrate surface properties influencing short term cell adhesion. Contrasting adherence properties of SV_{40} transformed WI38 fibroblasts and SV_{40} transformed 3T3 mouse fibroblasts are detailed.

Short term adhesion of both normal and transformed WI38 fibroblasts appear to increase linearly with increasing substrate surface polarity (hydrophilicity). Substrate surface polarity as determined via air and octane contact angles more closely correlates with cell adhesion than X-ray photoelectron spectroscopy derived polarity determination. Air contact angles by themselves appear to be sufficient for predicting short term cell adhesion.

Substrate surfaces were modified by inhouse radio frequency glow discharging (RFGD) in Helium. For all surfaces, cell adhesion

increased on treated surfaces and was as good or better than adhesion to commercially prepared tissue culture substrates. X-ray photoelectron spectroscopy demonstrated commercially prepared surfaces to have more oxygen present on their surfaces when compared to inhouse RFGD treated substrates. Cell adhesion was influenced more by hydrophilicity than by total surface oxygen.

Normal and transformed WI38 fibroblasts demonstrated increased adhesion with increasing substrate negative zeta potential. However, good cell growth was observed with slightly positively charged substrates.

Short term adhesion correlated well with long term (24 hour) cell growth on most but not all surfaces.

Although substrate hydrophilicity, surface charge and chemical composition were characterized without serum proteins present, cell-substrate adhesion measurements made in the presence of serum demonstrated intriguing conclusions.

The rotating shear disk has been demonstrated to reproducibly measure cell adhesion to a variety of substrates. Short term cell adhesion on adherent surfaces is a more consistent parameter than short term plating efficiency. On fairly nonadherent substrates, a measure of plating efficiency is more useful.

Abstracts, World Biomaterials Ab-Smith-3

4.11.2 Congress, April 8-12, 1980

CELL ADHESION AS MODULATED BY SUBSTRATE SURFACE CHARACTERISTICS

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Most cellular activities and interactions occur with the cell in intimate contact with a substrate. Most normal mammalian cells will not grow or divide unless they are adherent to a substrate. An attempt is made to correlate important substrate surface parameters with the degree of short term (30 min.) cell adhesion for both normal contact inhibited cells as well as for virally transformed cell lines.

Substrate surface characteristics evaluated include surface charge evaluated via streaming potential, surface chemical composition detected with X-ray photoelectron spectroscopy and surface interfacial free energies measured using captive air and octane bubble contact angles (1). The extent of short term cell adhesion was determined by shearing cultured cells from test substrates on a rotating shear disc, and evaluating cell adhesion at an applied shear stress of 32 dynes/Cm^2 (2).

A linear, inverse correlation between short term cell adhesion and substra te surface air and octane contact angles was observed. A direct linear correlation was observed between increased cell adhesion and increasing substrate surface polarity. Normal, contact inhibited, WI38 cells and non-contact inhibited SV40 transformed WI38 cells displayed similar tendencies, the transformed cells were consistently less adherent.

Substrate zeta potentials were determined from flat plate streaming potentials (3). Cell adhesion was observed to increase on more highly negatively charged polymeric surfaces. Identical behaviour was observed for both normal and virally transformed cell lines. Normal, contact inhibited cells displayed a more dramatic increase in adhesion with increasing negative substrate zeta potential compared to transformed cells.

Long-term (24 hour) cell adhesion and growth agreed with short term results for all surfaces except PMMA. All surfaces displaying good short term cell adhesion were seen to have good long-term cell growth. Short term (30 min.) cell adhesion measurements appear to be helpful in determining successful long-term growth and adhesion. Short term adhesion is more readily influenced by surface wettability than by substrate surface oxygen content. Increased negative zeta potential has a direct influence on increasing short term adhesion, yet some investigators have reported good cell long-term growth and adhesion to positively charged substrates (4).

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QUANTITATIVE 2D GEL ELECTROPHORESIS OF BLOOD PROTEIN INTERACTION WITH MEDICAL DEVICES

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There may be over 600 proteins in plasma. They adsorb competitively to medical devices and artificial organs. Although there has been substantial progress in the qualitative understanding of protein adsorption, a quantitative, predicative understanding is still lacking. In some cases trace proteins, such as high molecular weight kininogen, dominate the adsorption process. We are evaluating 2D gel electrophoresis (isoelectric focusing and SDS-Polyacrylamide gel electrophoresis), using quantitative staining, densitometry, and computer-based pattern recognition and comparison techniques, to assess the competitive interaction of <u>all</u> plasma proteins with selected devices and surfaces. Initial results of plasmainteractions with high area devices will be discussed.

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Ab Lea-1

STM 91

2F/42

AFM IMAGING USING POLYETHYLENEOXIDE MODIFIED CANTILEVERS

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In aqueous systems, surfaces covered with polyethyleneoxide (PEO) repel each other when brought together due to the steric exclusion forces manifested by the extended polymer chains (1). AFM cantilever tips coated with PEO would enable imaging in aqueous environments to be accomplished using predominately steric exclusion forces. The potential for the use of these cantilevers to minimize the lateral translation of surface adsorbed entities (2) will be presented with emphasis on PEO surface coverage and molecular weight.

Silicon nitride cantilevers were modified by chemically coupling PEO of specified molecular weights to their surface. The coupling procedure was monitored by ESCA and electrophoretic mobility and PEO layer thicknesses were determined by ellipsometry. The force curves were similar to those obtained using the surface force apparatus (1): steric exclusion forces commencing at a separation distance of ~6 R_g (the solution mean radius of gyration of the random polymer coil) and dominating the attractive van der Waals forces at smaller separations. At large separations there is considerable loss of resolution due to the slow development of the exponential steric exclusion force (3). At smaller separations, the resolution is greater. We are currently investigating the potential of these tips to minimize perturbation of surface adsorbed gold particles and proteins.

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2F/43

A DETAILED ANALYSIS OF THE OPTICAL BEAM DEFLECTION TECHNIQUE.

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In Atomic Force Microscopy (AFM) minute displacements of a small cantilever are measured. The most widely used methods for the detection of the change in cantilever position are (fiber)interferometry and optical beam deflection. At first sight, most physicist would expect the interferometric based techniques to be much more sensitive than the beam deflection method. In practice, however, images with atomic resolution have been obtained while using the optical beam deflection method.

We will present a detailed theoretical analysis of the beam deflection method. Several optical configurations, e.g. laser beam focused at cantilever or at position sensitive detector, will be discussed within practical boundary conditions. The analysis explains that, while using a 1 mW laser diode, the signal-to-noise ratio in a 10 kHz bandwidth is sufficiently high to achieve atomic resolution. The theoretical predictions are compared with experimental results.

In all practical situations, the signal-to-noise ratio of the optical beam deflection method is comparable to that of the more complex interferometric techniques.

STM '91 Abstracts, International Conf. on STM, Interlaken, August 12-16, 1991 - under auspices of the European Physical Society

3F/29

AN AFM STUDY OF POLYSTYRENE LATEX PARTICLES AS A CALIBRATION STANDARD.

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Atomic Force Microscopy (AFM) has the ability to image conducting and, more importantly, non-conducting surfaces with atomic resolution. We have used AFM to investigate sub-monolayer to multiple layer coverage of polystyrene latex particles on various surfaces. Latex spheres with diameters ranging from 60 nm to 500 nm were studied. The distribution of the sphere sizes was consistent with independent measurements. The latex spheres were observed to pack into both ordered and disordered structures. The packing structure depends on such factors as particle concentration and size distribution. We have dispersed latex particles onto various substrates and have observed that "particle-substrate" interactions play an important role in film formation. The polystyrene latex spheres provide a simple way to calibrate AFM instruments in x, y and z directions simultaneously. AFM images of various latex spheres will be presented.

Supported by NSF (Dir 89-20053), ONR (N00014-90- J-1455), and the Vice President for Research, ASU.

Ab STroup-3

STM 91

3F/30

ELASTICITY MAPPING OF POLYMER SURFACES WITH AFM

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The elastic nature of samples being imaged with AFM is of great interest. If the sample is very elastic, it may deform under the force of the tip. This deformation may cause increased surface contact area between the tip and the sample thereby producing a convoluted topographical image. However, the elastic nature of the surface can be used as an advantage to map the "hardness" of the surface.

The AFM has been used to image polymer surfaces to produce a "hardness map" from differences in surface elasticities, in contrast to a topographical image of variances in height. To accomplish this imaging, the AFM is used in the force modulation mode. When activated, the piezoelectric crystal controlling the sample holder moves in the z direction and causes the cantilever to deflect from its original position, z_d. The deflection, Δz_d, is used in a ratio with the change in height of the piezo, Δz_m, to create an image. This mode allows the AFM to recognize soft sections in relation to hard sections, since the change in the deflection of the cantilever will be related to the hardness of the area under the tip (1). We have used this technique to image the surfaces of graphite fibers in a polycarbonate matrix, and copolymers of syrenebutadiene. The contrast in the elasticities of the components of the samples is revealed through the force modulation method.

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Abstracts

10 Years of STM

International Conference on
Scanning Tunneling Microscopy
Interlaken, August 12-16, 1991

Under the auspices of the European Physical Society

SURFACE DYNAMICS OF PLASMA TREATED POLYMERS

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The use of Radio-frequency glow discharge (RFGD) techniques has become widely accepted in modifying the surface chemistry of biomedical polymers to increase their biocompatability without affecting the bulk material. Reduction in bacterial colonization, plasma protein adhesion, or platelet adhesion can often be accomplished by reducing the hydrophobicity of the material surface. RFGD provides an effective technique to change the wettability of a polymer surface. It is well known that polymer surfaces treated by RFGD techniques are subject to aging phenomena in which the hydrophilicity produced by the RFGD treatment decreases with time. This aging is related to the time dependent surface dynamics of the material. In this work we investigate the changes in the surface dynamics of polycarbonate (PC) with RFGD treatments of Oxygen and Argon to determine the effects of surface crosslinking on the material's dynamic relaxation.

EXPERIMENTAL

Advancing and receding water contact angles were measured using the Wilhelmy Plate method. ESCA surface analysis was performed for the PC samples prior to RFGD treatment. The RFGD system consists of an RF power source inductively coupled to a flow-through Pyrex chamber. PC substrates were exposed to RFGD in an Oxygen environment. Other PC samples were treated with Argon RFGD followed by Oxygen RFGD and an "annealing" process in an Oxygen atmosphere. The advancing and receding contact angles were measured immediately following (within 15 minutes) the treatment. ESCA analysis was also performed following the treatment. The samples were then stored in air at room temperature. The analyses were then repeated after periods of time ranging from 1 hour to 7 days.

RESULTS AND DISCUSSION

The surface treatments of the PC produced a considerable decrease in the hydrophobicity as indicated by decreases in the measured contact angles. ESCA analysis indicated that carbonyl and carboxylate groups were formed as the carbonate and π-π^* features were reduced in intensity.[1] These results were more highly pronounced in the samples that were pretreated with Argon. This information suggests crosslinking of the PC surface. Figure 1 shows the Wilhelmy Plate advancing contact angle as it changes with time. The contact angle decreases upon RFGD treatment, but increases rapidly to a plateau level. Figure 2 shows the receding contact angle versus time. The differences in the behavior are related to the RFGD intensity of each treatment. Figure 3 shows the Wilhelmy Plate contact angle hysteresis for a sample prior to treatment (a), immediately following treatment (b), and after one day (c). The position of the slope for the advancing contact angle changes upon treatment reflecting the increased hydrophobicity. The slope position for the sample after one day of storage shows the relaxation of the surface to a more hydrophobic state. This relaxation in contact angle is probably due to the rotation of side groups into the bulk of the material in response to the surfaces micro-environment. These side group motions, which are activated near room temperature, occur to minimize the interfacial free energy. In air, nonpolar side groups will tend to orient themselves towards the interface while the polymer-water interface provides enough free energy to create a driving force for the restructuring of the polar side groups.[2]

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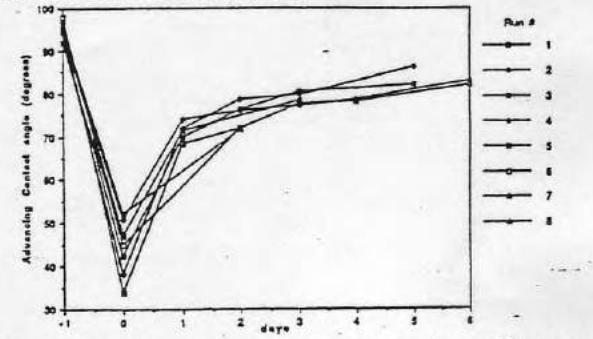

Figure1. Dynamic advancing water contact angle on PC as a function of time (-1 being before treatment) for 8 different RFGD treatments.

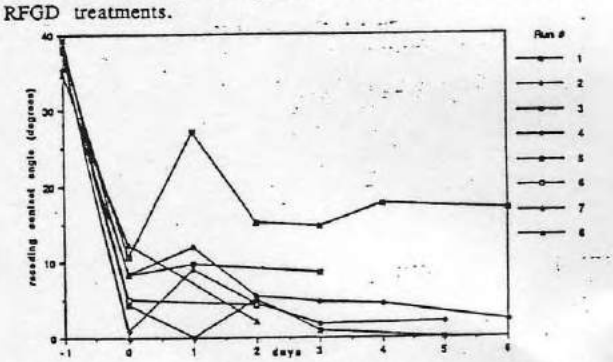

Figure2. Dynamic receding water contact angle on PC as a function of time (-1 being before treatment) for 8 different RFGD treatments.

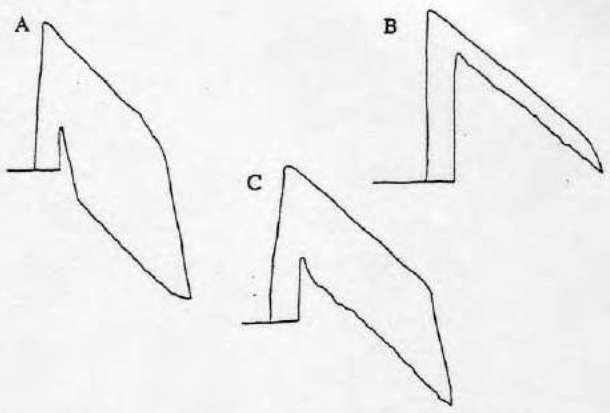

Figure 3. Hysteresis plots of dynamic water contact angles on PC as measured by Wilhelmy plate method. (a)PC sample before treatment. (b)Sample immediately following RFGD treatment. (C)One day after treatment.

Ab Suci 1
Fluor. 188

Variable Angle Total Internal Reflection Fluorescence.

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Variable angle TIR should allow one to measure the concentration and thickness of the fluorescently labeled protein as it adsorbs from solution to a solid surface without having to make assumptions about the physical nature of the adsorbed layer. In this experimental design the solid transparent medium is a hemi-cylindrical prism and the liquid medium is a protein solution delivered to the interfacial region via a flow chamber . For modeling purposes a protein layer can be thought of as parallel planes of fluors, each with an average fluor density . These planes (which make up the concentration-distance profile of the adsorbed layer) fluoresce in proportion to the product of the evanescent field intensity and the fluor density in that plane. Because the rate of exponential decay of the evanescent field increases with increasing angles of total reflection, one can change the field experienced by each fluor plane by varying the angle of incidence of the totally reflecting beam . For a range of angles of incidence and a fixed angle of observation one obtains a fluorescence data curve representative of the shape of the fluor concentration-distance profile (1). Expressed mathematically, protein fluorescence excited sequentially by a series of evanescent fields with different exponential decay rates (variable incident angle) and collected at a fixed observation angle is the Laplace transform of the fluor concentration-distance profile. In rigorous terms, this functionality means that: 1) each fluor data curve is a unique representation of a particular concentration-distance profile and 2) numerical inverse Laplace transform methods exist to directly calculate the profile contained within the fluorescence curve. These two points form the basis of the variable angle TIRF (VA-TIRF) technique for calculating protein film thickness and concentration directly from the fluorescence data.

One can also obtain a fluorescence curve equivalent to the variable incidence angle data by holding the angle of incidence constant (greater than the critical angle necessary for TIR) and collecting the angular distribution of the emitted fluorescence by varying the angle of observation (2,3). The equivalent variable observation approach is more complicated theoretically but much simpler experimentally and is thus the technique of choice. Experimental and theoretical feasibility of the VA-TIRF technique in the variable observation angle mode was demonstrated by the collection and numerical analysis of data from an immunoglobulin (IgG) protein film adsorbed to a quartz hemi-cylinder (4). A step function representation for the concentration-distance profile of the adsorbed dye labeled protein film was assumed. This "simplest case" assumption produced an analytical expression for the inverse Laplace transform. A surface concentration of 25 mg/ml and a thickness of 150 Å was obtained which are in agreement with the literature values (5,6) and the predicted model of adsorbed IgG.

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research. It was found that if the electron discharge machining is terminated by a large final discharge, microcracks form at the root of the EDM notch.

The experimental method has been justified both theoretically and experimentally. A correction factor has been derived relating the overstated K_Q of the notch-microcrack system to K_{IC}. The notch-microcrack system used satisfies the Novak and Barsom condition. K_{IC} values, measured by this method on specimens cut from stock tested by the wedge loaded double cantilever beam method, differ by less than 7% from the corresponding DCB value.

Fracture toughness has been measured on twenty-eight alloys of varying composition. Transmission electron microscopy (TEM) has been used to measure mean linear intercept grain size and contiguity, and both nominal and true mean free paths were computed. The fracture surfaces of four alloys were investigated by SEM and TEM to gain insight into the fracture mode so the fracture toughness could be related to the aforementioned microstructural parameters.

A theoretical model is proposed in which the large stresses ahead of the crack tip are envisioned to cause WC–WC and/or WC–Co interfacial decohesion, leaving binder ligaments whose size is related to the true mean free path. When the crack opening displacement equals the size of the ligaments, fracture occurs.

TRANSPORT PROPERTIES OF CALCIA AND YTTRIA DOPED CERIUM OXIDE Order No. 7801931

REDDY, S.N. Srinivasa, Ph.D. Marquette University, 1977. 149pp.

The transport properties of doped cerium oxide are studied by measuring the electrical conductivity and ionic transference numbers. The main purpose of the study is to determine the effect of dopant concentration and non-stoichiometry on the electrolytic properties of cerium oxide. The electrical conductivity is measured as a function of temperature and oxygen pressure for CeO_2 samples containing 1, 7 and 10 mole percent CaO and 5 mole percent Y_2O_3. The ionic transference numbers are measured as a function of temperature and oxygen pressure for CeO_2 samples containing 1 and 7 mole percent CaO. The results are combined with the non-stoichiometric data available to obtain partial ionic and electronic conductivities as a function of temperature and non-stoichiometry.

In the temperature range 700–1000°C, cerium oxide doped with 7 mole percent CaO is essentially an ionic conductor at high oxygen pressures and becomes increasingly electronic as the oxygen pressure is decreased. The isothermal ionic conductivity increases linearly with the increasing oxygen vacancy concentration indicating constant ionic mobility. The isothermal electronic conductivity increases linearly with increasing non-stoichiometry when the non-stoichiometry is small. When the deviation from stoichiometry is large, the electronic mobility becomes a function of non-stoichiometric composition.

INTERACTION OF WATER WITH HYDROPHILIC METHACRYLATE POLYMERS Order No. 7808319

~~SUNG,~~ Yong Kiel, Ph.D. University of Utah, 1978. 177pp. Chairman: Joseph D. Andrade

Hydrophilic three-dimensional methacrylate polymer networks (hydrogels) were prepared from 2-hydroxyethyl methacrylate (HEMA) and 2,3-dihydroxypropyl methacrylate (DHPMA). Highly isotactic poly(2-hydroxyethyl methacrylate) [p(HEMA)] was prepared by anionic polymerization of HEMA benzoate ester at -10° C and by hydrolysis of the resulting poly(HEMA benzoate). Highly syndiotactic p(HEMA) was prepared by free radical polymerization at -40° C using ultraviolet photolysis of HEMA monomer in methanol. Ethylene glycol dimethacrylate (EGDMA), tetraethylene glycol dimethacrylate (TEGDMA), and hexamethylene diisocyanate (HMDIC) were used as crosslinkers.

The interactions of water with the hydrophilic methacrylate polymers have been studied. The techniques employed in this study are differential scanning calorimetry (DSC), water vapor sorption measurements, and pulse NMR relaxation spectroscopy.

The DSC studies showed no endotherm peak for ice melting in the low water content (bound water region); there were two endotherm peaks for high water content hydrogels near 0° C. The amounts of bound water, intermediate water, and bulk-like or free water in hydrogels [p(HEMA)-H_2O, p(HEMA)-EGDMA-H_2O, p(HEMA)-TEGDMA-H_2O] were determined from a quantitative analysis of the endotherms of the water melting transitions. The water structure ordering in the hydrogels has also been discussed in terms of the heat of fusion, volume contraction, and the entropy of fusion obtained from the endotherms.

The effect of water on the glass transition temperature of different tactic methacrylate polymers has been studied. The results show that the Tg of p(HEMA) is markedly affected by the water content and tacticity. The measured Tg value is 120 + 2° C for the 60% syndiotactic p(HEMA) – 1 mole % EGDMA sample and 38 ± 2° C for 80% isotactic p(HEMA) sample.

The sorption of water vapor in the different gels has been measured as a function of water activity. The results have been interpreted in terms of Anderson's modified B.E.T. theory and the Hailwood-Horrobin theory of sorption. Anderson's modified B.E.T. theory predicts a fit to most of the experimental isotherms up to 0.7 equilibrium relative humidity, while the solution theory of Hailwood-Horrobin gives a somewhat better fit to most of the experimental isotherm over the entire range of equilibrium relative humidity. Water vapor sorption by the methacrylate polymers is greatly affected by tacticity and the number of hydrophilic sites in the molecules. The amount of water vapor sorbed by isotactic p(HEMA) and p(DHPMA) was found to be greater than that by syndiotactic p(HEMA).

The pulse NMR relaxation studies showed that the spin-lattice relaxation times of low water content (bound water region) gels show a double environments resulting in two spin-lattice relaxation times (T_{1a} and T_{1b}). The data indicate that there are two different kinds of bound water, i.e., strongly bound water and weakly bound water. The spin-lattice relaxation time of bound water in p(HEMA) hydrogels is much less than that of water portions in ice. As the water content in the gels is increased, the pulse amplitude intensities show a single exponential behavior. The measured T_1 values for the water protons in the hydrogels are greatly reduced compared to that of liquid water (T_1 = 4.50 sec at 34° C).

The measured values of spin-spin relaxation times (T_2) of the water protons are approximately ten times less than that of T_1 and are almost constant in the region of bound water content. Beyond the bound water content region, the T_2 values rapidly increase as the water content increases.

It is proposed that the interactions of water with hydrophilic polymers occur in four ways: (1) Water molecules are strongly bound to specific sites such as hydroxyl or carboxyl groups within the polymer network; dynamically and thermodynamically they behave as part of the chains. (2) Water molecules are weakly bound to the hydrophilic sites and/or preferentially structured around the polymer network. (3) Water molecules behave as though they are in the presence of a high concentration of solute, i.e., freezing point depression. (4) Water molecules behave dynamically and thermodynamically as bulklike or "free" water.

Dissertation Abst. 38 (12) pt. 1B. (1978)

Abs-Tingey-3

MODEL POLYURETHANE DOMAIN EFFECTS ON PROTEIN ADSORPTION

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Salt Lake City, Utah 84112

INTRODUCTION

The morphology of multiphase block copolymers has long been correlated to platelet adhesion(1). The importance of the adsorbed protein layer in conditioning artificial surfaces in the body has been established. Structure, as well as composition, are important material surface parameters which determine the adsorption of proteins to multiphase polyurethanes. The domains of segmented polyurethane block copolymers are approximately the size of the domains of plasma proteins. This size similarity will affect the interaction of proteins with the surface on a sub-molecular scale and may tend to stabilize the native conformation of the adsorbed protein. We are currently investigating the effect of polyurethane surface morphology and composition on the adsorption of multi-domain proteins.

EXPERIMENTAL

Polyurethane particles formed from methyl-diphenyl-diisocyanate and butanediol hard segments, and poly(tetra-methylene oxide) soft segments which vary in molecular weight from 650 to 2900 g/mole were supplied by Becton Dickinson Polymer Research. Their bulk properties have been characterized using infrared spectroscopy, differential scanning calorimetry, and elemental analysis. The surface of these polyurethanes have been studied with electron microscopy, X-ray photoelectron spectroscopy, contact angle, and inverse chromatography methods (2,3).

In vitro protein adsorption on these particles has been studied by exposing pooled human plasma to the particles and measuring the specific depletion of specific proteins from the solution as a function of time, polymer composition and temperature. Two dimensional gel electrophoresis has been used to semi-quantitatively determine the depletion of several proteins from plasma as previously described(4).

Factor analysis has been applied to the material characterization and protein data to establish correlations between the specific polyurethane hard segment domain properties and protein adsorption.

RESULTS AND DISCUSSION

The particle surface composition and energetics were observed to vary as a function of bulk composition using ESCA, and contact angle methods respectively. Increasing soft segment molecular weight was also observed, using differential scanning calorimetry, to change the purity of the hard segment domain. The hard segment long range order temperature, similar to the melt temperature in a thermoplastic polymer, is indicative of polyurethane hard segment structure. An increase in the temperature associated with the loss of order (T_{ord}) indicates an increase in hard segment phase purity. Total adsorbed protein was observed to decrease with increasing T_{ord} as seen in Figure 1. This may indicate that protein adsorption is related to the morphology of the surface layer. These results further suggest that surfaces having hard segment domains of higher phase purity are less protein attractive.

Hypotheses relating material morphology or reorientation properties to protein adsorption are being developed. The decreased adsorption of plasma proteins on well-phase-separated polyurethane copolymers may be related to the disorder or non-cooperativity of neighboring proteins dictated by the heterophase nature of the substrate surface. Alternative mechanisms associated with the dynamic response of the substrate material may also play a significant role in modulating protein adsorption and will be discussed.

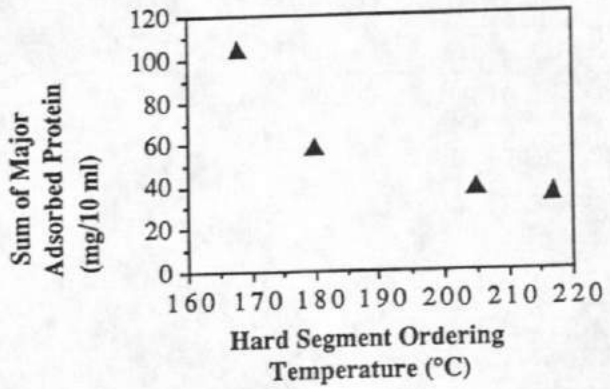

Figure 1. Relationship between hard segment T_{ord} and the sum of the amount of the major proteins adsorbed.

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ACKNOWLEDGEMENTS

The authors wish to acknowledge the assistance of Mr. C-H. Ho in the protein studies, and Becton Dickinson Polymer Research for their support and for the preparation of the polyurethanes.

† Becton Dickinson Polymer Research, Dayton, Ohio 45401

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THE EFFECT OF HYDRATION ON BIOMEDICAL POLYURETHANE (PU) SURFACES

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INTRODUCTION

The mechanisms for biocompatibility may be related to surface composition and morphology. The surfaces of PUs are dynamic and change to minimize the interfacial free energy initiated by interaction of the surface with aqueous solutions[1] or perhaps with adsorbed protein layers. This effect may be important in determining protein sorption kinetics and the thrombotic potential of the material.

EXPERIMENTAL METHODS

Commercial block copolyurethanes including Biomer®, Vialon® 510X-65, and Cardiothane®; and Becton Dickinson experimental PUs with PEO/PTMO (BDH-1) and fluorinated (BDF-1) soft segments were analyzed in the dessicated and water hydrated states. The PUs studied vary primarily in soft segment chemistry.

FTIR spectra of thin spin cast PU films from up to 2000 coadded scans of 2 wavenumber resolution were obtained using the Digilab FTS 15 and 3200/60 spectrometers. Spectra were collected under vacuum, ambient room conditions, and under 37°C deionized water at flow rates of 30 ml/minute.

Wilhelmy Plate contact angle analysis was performed as a function of hydration or vacuum time on PU samples cast against clean glass. Hydration degree was determined gravimetrically.

X-Ray photoelectron spectroscopy (XPS) was performed on wetted and dried samples using the Hewlett Packard 5950B photoelectron spectrometer with variable temperature or variable angle probes. The effect of hydration was studied by sublimation of the frozen water on hydrated films.

RESULTS & DISCUSSION

The frequency shift of these PUs on hydration, redrying, rehydration, and redrying for the hard segment 1530 band and the soft segment 1110 band suggest interphase mixing. Intensity changes suggest phase translations at the surface as hard segment bands increase on drying and decrease on wetting.

Their dynamic wetting character is presented as a function of decrease in advancing contact angle upon hydration in Table 1. Table 2 indicates the contact angle change upon wetting, drying, rewetting, and redrying the fluorinated PU. The consistent hydration cycling indicates the surface's dynamic environmental response. This is further demonstrated with variable angle XPS data which shows the compositional difference between the PU surface relative to the bulk, this primarily due to the interfacial environment. Variable temperature XPS allows us to observe semiquantitatively the surface reorientation as a function of the interfacing medium.

Table 1
Decrease in Equilibrium Advancing Contact Angle

Polymer	Ødry	Øwet	ΔØ(±2°)
Biomer®	102	100	2
Vialon® 510X-65	82	75	7
Cardiothane®	115	112	3
BDH-1	55	40	15
BDF-1	129	119	10

Table 2
Hydrated BDF-1 Contact Angle

Hydration State	Ø adv (±1°)	Ø rec (±1°)
Cast, cured dry	129	38
Equilibrium wetted	119	37
Vacuum dried	124	39
Equilib. rewetted	119	39
Vacuum redried	123	39

CONCLUSIONS

Small deviations in the IR and photoelectron spectra coupled with changes in surface energetics suggest that the surface composition and morphology of these PUs are dynamic and indicate that characterization in a hydrophobic state may not accurately describe the surface in an aqueous blood-like environment.

This research was funded by grants from Becton Dickinson Polymer Research and Battelle's National Center for Biomedical FTIR NIH-RR011367.

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†National Center for Biomedical Infrared Spectroscopy, Battelle, 505 King Ave., Columbus, Ohio 43201

Keyword (Primary Category)
Polyurethane
Keyword
Protein Adsorption
Keyword
Platelet
Keyword
Surface Analysis

Ab-Ting-2

Platelet and Specific Plasma Protein Adsorption on Model Polyetherurethanes

K. G. Tingey, K. Sibrell, J. M. Lambert†, D. D. Solomon†, K. D. Caldwell, J. D. Andrade

Dept. of Bioengineering, University of Utah, Salt Lake City, Utah 84112

INTRODUCTION

The complex microphase separated structure and environmentally induced surface compositional reorientation of phase separated polyetherurethanes (PEUs) causes difficulties in establishing polymer surface character. Model PEU surfaces have been developed in a high surface area form by stepwise emulsion polymerization techniques to study their dynamic surface character and adsorption properties in contact with blood. Through utilization of two-dimensional gel electrophoresis the individual protein depletion from diluted plasma can be evaluated to enhance our understanding of the adsorption characteristics of appropriate plasma proteins on these polyurethanes. This method allows for the consideration of the important protein-protein interaction as well as the protein-material interaction. The interaction of platelets from whole heparinized blood is also being tested.

EXPERIMENTAL

Experimental block copolyurethanes based on MDI and BDO hard segment and PTMG soft segment of varying molecular weight (650-2900) have been synthesized through emulsion polymerization techniques in an aqueous environment as previously described[1]. The emulsion technique results in PEU beads ranging from 10-25 microns in diameter (surface area of 2600 cm^2/gm).

Bulk and surface analytical techniques including thermal analysis, infrared spectroscopy, inverse chromatography, X-ray photoelectron and fluorescence spectroscopy have been used to characterize the material properties of the surface relative to the bulk.

Specific protein depletion was evaluated by exposing plasma in a 30:1 Tris buffer dilution to 25 wt% beads, at room temperature for 4 hours, relative to a control dilution unexposed to beads. An aliquot of the solution was withdrawn from the bead suspension and the control and separated by 2-dimensional electrophoresis as previously described[2]. The depleted protein concentration was semi-quantitatively evaluated directly from the gel using computer graphics techniques.

Platelet adhesion on these PEUs was evaluated in static exposure tests with platelet rich blood spiked with ^{111}In labeled platelets. The beads and thin PEU films were exposed to the spiked blood for 1 hour, rinsed, and followed by detection of the In isotope.

RESULTS & DISCUSSION

Surface characterization of the PEU beads reveals variable surface composition and dynamic properties correlating with the soft segment molecular weight. The results suggest surface soft segment enrichment is at a maximum at intermediate soft segment molecular weight. The surface concentration of hard segment is also shown to decrease with increasing soft segment molecular weight.

Specific protein depletion due to adsorption on the suspended beads indicates strong adsorption of specific proteins. Table 1 presents relative depletion of selected proteins exposed to PEU beads composed of 1000 Mw soft segment blocks containing 5% polymeric silicone. Hemopexin and fibrinogen were strongly depleted in both denatured and native protein solutions while albumin , haptoglobins and others were only weakly depleted.

Platelet adhesion appears to plateau at low and intermediate soft segment molecular weights and dramatically decrease with high soft segment molecular weight. Due to surface induced phase orientation the data suggests that even low concentrations of hard segment at the surface induce platelet adhesion.

Table 1
Protein Depletion

PROTEIN	%DEPLETION
Haptoglobin	6
Glycoprotein	6
Antitrypsin	22
Apo A1 Lipoprotein	47
Albumin	7
Hemopexin	83
Fibrinogen	90
Macroglobulin	63
Antithrombin III	25

CONCLUSIONS

Protein adsorption from diluted plasma on polyurethane particles exhibits some specificity and adsorbs in approximate monolayers in long term exposure. Platelet adhesion on PEUs correlates with apparent surface hard segment concentration suggesting some specificity for the more polar hard segment domains.

ACKNOWLEDGEMENTS

The authors wish to acknowledge, from the University of Utah, the expertise and assistance of C. H. Ho and G. Nyquist, in the two dimensional electrophoriesis work and B. Nelson and Dr. F. Mohammed in the platelet studies. Becton Dickinson Polymer Research is gratefully acknowledged for their funding support and synthesized polymers.

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Ab-Tingey-2

Platelet and Specific Plasma Protein Adsorption on Model Polyetherurethanes

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†. Becton Dickinson Polymer Research, 11125 Yankee St., Dayton, Ohio 45401

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Abstract
for submission to
Utah Academy of Arts and Sciences
Annual Meeting
May 1992

Culture of Pyrocystis Lunula in Sealed Polyethylene Bags

(18)

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Pyrocystis lunula is a bioluminescent, non toxic dinoflagellate which is beginning to be utilized for science education purposes. NIGHT-LIFE™, a commercial science education kit, utilizes pyrocystis lunula and conventional tissue culture flasks using a modified Guillard F/2 culture medium. Although this approach works very well, it is also desirable to maintain the cultures under totally sealed conditions. A totally sealed culture permits students to observe the development of the culture without having to take samples, pour solutions, or otherwise disturb the colony. As these organisms are photosynthetic, and require CO2 and O2 exchange to live, it has always been doubtful whether they could exist in a sealed environment.

Experience with the NIGHT-LIFE™ product demonstrated a 3 month shelf life with pyrocystis lunula flasks which were completely sealed with no gas exchange with the surrounding environment. Experience with the gas transfer characteristics of membranes led to a study where the colony was completely sealed in thin polyethylene bags. The colonies have survived in this sealed culture environment for several months with little problem.

There is an increase in salinity due to progressive water loss by water vapor permeation through the bags. The bags always feel cool, presumably due to evaporative cooling. Though there are other polymers, such as polydimethyl siloxane, with better gas transfer characteristics, they also have greater water loss.

The bags have the advantage that the organisms in the colony can be observed readily. They can be placed directly in an optical microscope. A variety of temperature, light intensity, light period, and related experiments can readily be performed without any direct contact with the culture or the organisms. Completely sealed pyrocystis lunula colonies in polyethylene bags are now the basis of a new product development, LIGHT-BAG™.

This work was partially supported by Protein Solutions, Inc., Salt Lake City, Utah. NIGHT-LIFE™, LIGHT-BAG™, LIGHT-POUCH™, FLIGHT BAG™, are trademarks of Protein Solutions, Inc.

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Abstract

Utah Academy of Sciences, Arts, and Letters
Spring Meeting, May 10, 1991
Westminster College, Salt Lake City, Utah

Culture of Bioluminescent Dinoflagellates in Non-Traditional Media

by J. Tobler and J. Andrade, Department of Bioengineering
University of Utah, Salt Lake City, Utah 84112

Certain marine microalgae, dinoflagellates, give off light when mechanically stimulated. This natural bioluminescence is responsible for the light seen on the surfaces of oceans, bays, and estuaries throughout the tropical and sub-tropical regions of the world, and often in temperate climates as well. Pyrocystis lunula and Pyrocystis noctiluca are two such dinoflagellates which have high bioluminescence intensities and are relatively easy to grow and maintain in laboratory environments. When mechanically stimulated, they produce a bright bioluminescent flash. We have been evaluating these organisms as possible science education aids for discovery oriented science curricula.

Historically the organisms have been grown in natural seawater, supplemented with the Guillard F/2 medium, a medium which was designed for the culture of marine phytoplankton. Because Utah is far from natural ocean waters, and because natural sea waters vary dramatically at various times of the year in various locations, we are evaluating various synthetic, artificial sea waters, and Great Salt Lake waters for dinoflagellate culture applications.

Progress on the work to date will be reviewed, including a listing of the artificial sea salts employed, dinoflagellate growth rates, bioluminescence intensities, and culture stability and longevity.

Acknowledgements:

This work has been supported by Protein Solutions, Inc., Salt Lake City.

STREAMING POTENTIAL STUDIES OF GLASS AND CELL SURFACES

by

Richard Allen Van Wagenen

A dissertation submitted to the faculty of the University of Utah in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Materials Science and Engineering

University of Utah

August 1976

ABSTRACT

Two problems exist regarding the electrokinetic surface characterization of biomedical materials and cells proliferating *in vitro* on biomedical surfaces, e.g., tissue culture substrates and prosthetic materials. First, conventional microelectrophoresis requires a monodisperse suspension of cells. Consequently, cells adherent to a surface must be removed prior to analysis using enzymatic, chemical or physical techniques. Evidence indicates that such treatments alter the properties of the cell surface one is attempting to study. The solution to this problem was to evaluate cell monolayers cultured in capillary lumens using the streaming potential technique.

A review of the streaming potential literature indicated a considerable amount of discrepancy in regard to accuracy and reproducibility of the streaming potential. This was particularly evident in several attempts to correlate biocompatibility with electrokinetic data. Discrepancies were usually attributed to sample heterogeneity, contamination, surface conductance, etc. The solution to this problem was to investigate various factors which could conceivably cause errors in the measurement of the streaming potential, e.g., sample heterogeneity and electrokinetic stability, electrode polarization potentials, temperature, hydrodynamic conditions required by theory, and surface conductance.

It was found that sample heterogeneity was not normally a problem as long as the glass substrates were obtained from a single source.

Also, electrokinetic potentials were stable and reproducible over a period of several days if care was exercised. Electrode asymmetry and polarization flow potentials could be easily detected and eliminated by a cathodic 'cleaning' and rechloridizing. There was no significant effect of temperature on the ζ-potential over the range 20.5°C-36.5°C as long as the temperature variations in bulk viscosity, dielectric constant and specific conductance were accounted for. It was also found that the effect of surface conductance was minute at electrolyte concentrations greater than 1×10^{-3} molar univalent electrolyte.

Accurate streaming potential measurements require fluid flow to be of the Poiseuille type, i.e., steady, incompressible, laminar and established. This research indicates that if the length required to develop an equilibrium velocity profile, L_e, comprises more than 10 percent of the total streaming conduit flow length, the measured potential, E_{str}, and subsequently calculated ζ-potential will be anomalously low. An analysis of the hydrodynamic conditions utilized by several investigators attempting to study the relationship between thrombogenicity and surface potential indicated a lack of awareness concerning the hydrodynamic stipulation of established flow. Consequently, the results of such investigations are most likely a reflection of nonequilibrium flow properties, particularly a lack of flow development. It is concluded that previous attempts to correlate electrokinetic potentials with biocompatibility must be held circumspect unless the flow conditions utilized in such research can be shown to be valid.

The electrokinetic properties of murine fibroblasts cultured to monolayer confluency *in vitro* were evaluated as a function of pH and

ionic strength. The ζ-potential of tumorigenic, noncontact inhibited BALB/3T12 cells was significantly higher than that of normal, contact inhibited SWISS/3T3 fibroblasts. Both cell types exhibited ζ-potential-pH curves qualitatively similar to those of blood cells obtained on the basis of microelectrophoresis. SWISS/3T3 fibroblasts apparently have a considerable number of positive ionogenic groups at the electrokinetic cell surface as indicated by a positive branch to the ζ-potential-pH data. BALB/3T12 fibroblasts lack a significant number of positive species. Electrokinetic studies at low ionic strength suggest that both cell types may possess positive ionogenic species deeper within the cell membrane periphery which are not normally detectable at physiological ionic strength.

The streaming potential technique was also utilized to study the electrokinetic changes that occurred when normal or stimulated macrophages were modified to become activated macrophages capable of nonimmunologically recognizing and destroying tumor cells. It was found that cytotoxic macrophages had a substantially lower ζ-potential than macrophages incapable of tumor cell destruction. The decrease in macrophage ζ-potential seems to be the result of exposure to protease inhibitors, i.e., fetuin and soybean trypsin inhibitor. It is conceivable that this reduction in cell surface potential and thus charge is an attempt by the macrophage to lower the electrostatic barrier and make intimate contact with the tumor target cell. Following contact the target cells are destroyed.

7090

NITRENDIPINE MAY STIMULATE A CALCIUM PUMP IN VASCULAR MUSCLE. Kent Hermsmeyer and Ronald Mason*. The Cardiovascular Center, University of Iowa, Iowa City, IA 52242

Nitrendipine is a new dihydropyridine calcium (Ca^{++}) antagonist proposed to block contraction by blocking entry of Ca^{++} into vascular muscle cells. Recording of membrane potential (E_m) and contraction stimulated by norepinephrine (NE) in the rat caudal artery showed that nitrendipine (10 nM to 1 μM) rapidly induced hyperpolarization and relaxation. In NE concentrations up to 1 μM, 10 nM nitrendipine hyperpolarized by 2-3 mV accompanied by larger relaxation than the voltage-tension relationship would predict. In other experiments, NE was added to caudal arteries in either 0 K^+ solution or 0 Ca^{++} solution. 0 K^+ solution completely abolished the action of nitrendipine on E_m and relaxation. Contractions to NE were larger in 0 K^+ solution than in control solution, and were not blocked by up to 1 μM nitrendipine. In solutions with no added Ca^{++} (22 μM Ca^{++} by Ca^{++} ion electrode), nitrendipine more strongly blocked NE contractions (from 30% in control to 60% in low Ca^{++}). Enhanced action of nitrendipine in low Ca^{++} and its blockade in 0 K^+ suggest that nitrendipine acts on K^+-sensitive Ca^{++} transport important for relaxation of vascular muscle. This hypothesized stimulation of a Ca^{++} pump by Ca^{++} antagonists might be difficult to differentiate from block of Ca^{++} entry in ion flux experiments (same net result). Further E_m experiments will be needed to test the Ca^{++} pump hypothesis.

7091

NIMODIPINE, A NEW C[illegible] SEPARATE VASCULAR BEDS. ACTIVATED ATPase OF [illegible], Univ. of
Ronald A. Janis. M[illegible]
1956, New Haven, CT [illegible]

The effects of nim[illegible] 1483
nitrophenyl)-3,5-pyridi[illegible]
methylethyl ester), a[illegible]
triphosphatase (ATPase) ac[illegible]
deoxycholate-treated rat a[illegible]
ed ATPase was measured as tha[illegible] and
activity, measured in the presence Box
and Mg^{++}-ATPase, measured in th[illegible]
cations. All assays were carrie[illegible]
MgATP, 0.2 mM EDTA, 32 mM Tris (p[illegible]
The ATPase activities (in μmol Pi/hr

	Total-ATPase	Mg-[illegible]
Control	189 ± 10	169 [illegible]
Nimodipine (1.5μM)	207 ± 13	162 ±

(Mean ± S.E. for duplicate measurement[illegible]
each of which contained aortic membran[illegible]
Nimodipine was found to stimulate this enzym[illegible]
microsomes from rat vas deferens. For both [illegible]
smooth muscle the concentration of nimodipi[illegible]
half-maximal stimulation of Na^+,K^+-activated A[illegible]
than 15 nM. Verapamil at high concentrations [illegible]
stimulated Na^+,K^+-activated ATPase. The relations[illegible]
the effects of these calcium entry blockers on smo[illegible]
tone and on ATPase activities has yet to be defined.

7092

AGONIST-RELEASABLE INTRACELLULAR CA^{2+} POOL MAY MODULATE THE CONTRACTILE RESPONSE TO CA^{2+} INFLUX. Rodger D. Loutzenhiser* and C. van Breemen. Department of Pharmacology, University of Miami, Miami, FL 33101

Several agonists induce the release of an intracellularly bound Ca pool in arterial smooth muscle. This releasable Ca pool refills only in the presence of extracellular Ca. Refilling of the release pool of isolated rabbit aorta was determined by first depleting the pool and then measuring the contractile response to agonists under Ca-free conditions, after reloading for various times in Ca containing buffer. Lanthanum which blocks both stimulated Ca influx and the nonspecific Ca leak, prevented refilling, while D-600, which blocks only stimulated Ca influx did not, suggesting that the pool refills via the Ca leak pathway. However, the rate of refilling and size of the pool were increased by KCl depolarization, indicating that the Ca in this pool may also enter via the stimulated Ca influx (ie. D-600 sensitive) pathway. ^{45}Ca uptake measurements demonstrated that the extent of refilling was only dependent upon the amount of Ca which entered the cell and not the entry pathway. The rate of tension development in response to KCl was slowed when the release pool had been depleted and accelerated in the presence of an agonist capable of releasing this pool. These data indicate that the agonist-sensitive pool refills using Ca which has just entered the cell. Furthermore, an agonist-induced reduction in the sequestration capacity of this pool may facilitate the entry of Ca into the myoplasm during agonist-stimulation of Ca influx.

7093

THE LOSS OF NE STIMULATED Ca UPTAKE DURING WASHING OF RABBIT AORTA IN ICE-COLD La-CONTAINING AND ZERO Ca MEDIA. Philip Aaronson*, Cornelis van Breemen, and Pauline Zera*. University of Miami, Department of Pharmacology, Univ. of Miami, Miami, FL 33101

The quantitation of cellular Ca in smooth muscle tissues generally involves washing tissues labeled with ^{45}Ca in warm or cold media containing La or EGTA (ethyleneglycol-bis-(β-aminoethyl ether)N,N' tetraacetic acid) in order to remove extracellular bound label. Cellular Ca is then estimated by extrapolating the monoexponential slow efflux component. In order to investigate whether stimulated Ca influx can be accurately measured using this technique, rabbit aorta rings were labeled for 15 min in the presence or absence of 10^{-5} norepinephrine (NE) and then allowed to wash out into ice cold Ca free media containing 2 mM EGTA or 10 mM La. Comparison of washout curves from control and stimulated tissues indicated that NE induced a loss of Ca from a rapidly exchanging tissue pool, as well as an uptake of Ca into a more slowly exchanging pool. During washing, the stimulated Ca uptake was progressively lost. The half time for this loss was approximately 12 min in the La containing media and 24 min in the EGTA containing medium. These results suggest that NE induces a release of extracellular Ca, and further indicate that extended washing of tissues in cold media may lead to an underestimate of NE-stimulated Ca uptake, especially in La-containing media. (Supported by NIH grants HL 07188 and HL 27559)

Ab-Van W-4

BIOCHEMICAL TRANSDUCERS (7094–7095)

7094

POTENTIAL SENSOR APPLICATIONS OF TOTAL INTERNAL REFLECTION FLUORESCENCE (TIRF) SPECTROSCOPY. Richard A. Van Wagenen* and Joseph D. Andrade, Department of Bioengineering, Univ. of Utah, Salt Lake City, Utah 84112

Conventional immunoassay technology requires the use of a labeled antigen (Ag) or antibody (Ab) and a process for separating the complex (bound fraction) from the unbound label (free fraction). Total internal reflection (TIR) [1] at an interface containing immobilized Ab or Ag permits direct monitoring of fluorescently-labeled (extrinsic fluorescence) Ag* or Ab* (the * denotes the molecule is fluorescently labeled) due to the formation of the surface-bound Ab-Ag* or Ag-Ab* complex [2-3]. Many of the Ag and all of the Ab of interest are intrinsically fluorescent in the ultraviolet due to tyrosine and/or tryptophan amino acid residues. We have employed this intrinsic fluorescence property in the total internal reflection mode to monitor the adsorption of proteins at solid-liquid interfaces [4]. Preliminary studies suggest that total internal reflection intrinsic fluorescence (TIRIF) can be used as a direct assay of Ag-Ab interactions and thus as a reagent-less, label-less immunoassay probe.

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7095

DESIGN FACTORS IN ENZYME ELECTRODES FOR GLUCOSE. David A. Gough* and John K. Leypoldt* (SPON: B.W. Zweifach). Univ. of California, San Diego, La Jolla, CA. 92093

We are developing a potentially implantable glucose sensor for possible use in diabetes. The sensor is based on the "enzyme electrode" principle in which a membrane containing immobilized glucose oxidase is mounted over an oxygen sensing electrode. Glucose and ambient oxygen diffuse into the membrane and react enzymatically, with the unconsumed oxygen being detected at the electrode and indicating the glucose concentration. Progress in the development of this sensor has previously been hindered by the lack of a detailed understanding of the processes in the membrane. We have developed a model of mass transport and reaction within the membrane, taking into account such features as the concentration boundary layer in the solution adjacent to the membrane, substrate partitioning, nonlinear two-substrate reaction kinetics, and enzyme inactivation. The model predicts the sensor response to glucose concentration under various conditions of oxygen concentration, enzyme loading, internal and external mass transfer resistances, and is therefore useful in sensor design. We have tested this model with a novel, membrane-covered rotated disc electrode that we have developed which allows control of the external mass transfer resistance and independent evaluation of the internal mass transfer and the reaction kinetics. (Supported by the Juvenile Diabetes Fdn. and NIH Grant AM 27541).

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Ab-VanW-2

Abst 4th Int Conf S&CS 7/81

PROBING INTERFACIAL PROTEIN CONFORMATION VIA INTRINSIC FLUORESCENCE SPECTROSCOPY

R.A. VAN WAGENEN and J.D. ANDRADE
Department of Bioengineering, College of Engineering, University of Utah, Salt Lake City, Utah 84112 U.S.A.

Evanescent wave induced, interfacial protein fluorescence is of great utility in studying protein adsorption on flat surfaces. The methodology employed consists of a single pass quartz dovetail prism adjacent to a flow cell. Radiation produced by a HgXe lamp is isolated via an excitation monochromator (285nm) and directed into the prism where it undergoes internal reflection at the prism-electrolyte buffer interface. Alternatively, protein adsorption on polymer thin films deposited on the prism surface may be studied if polymer fluorescence is low and internal reflection still occurs. A portion of the emitted fluorescence (300-400 nm) normal to the interface is collected via an emission monochromator, amplified in a photomultiplier and quantitated in a photon counting system. Data is recorded as fluorescence signal intensity as a function of adsorption time and bulk protein concentration, C_B. This approach has several advantages. It is an in situ sensing method for directly following protein adsorption in real time with 0.1 second resolution. Thus, adsorption and desorption kinetic studies are greatly facilitated. The flow cell configuration provides well quantitated temperature and hydrodynamic flow conditions. Fluorescence is intrinsic, i.e., it arises from tryptophan or tyrosine moieties integral to the protein. This is in contrast to previous surface fluorescence research which relied upon an extrinsic fluor covalently linked to the molecule. Such binding may alter the physical properties of the protein under study. Also, intrinsic tryptophan fluorescence is much more sensitive to local microenvironmental changes which may result as protein molecules undergo conformational changes during adsorption. Such information can be derived from an analysis of the fluorescence emission spectra. The quantitation of the amount adsorbed, Γ, can be calculated on the basis of an internal standard arising from evanescent wave excited bulk protein fluorescence adjacent to the adsorbed film. Competitive adsorption may be studied by extrinsically labelling one of the competing macromolecule types. The adsorption of bovine serum albumin (BSA) and gamma globulins (γG's) on quartz has been studied. The γG's adsorbed to an equilibrium value of Γ=3.6μg cm^{-2} on hydrophilic quartz at a C_B exceeding 1 mg ml^{-1}. The adsorption isotherm for BSA on quartz was linear and Γ was directly proportional to C_B between 0 and 5 mg ml^{-1}. At a C_B of 1.0 mg ml^{-1}, Γ was 0.08 μg cm^{-2} for BSA versus 3.6 μg cm^{-2} for γG's. The initially rapid desorption kinetics followed by a second distinct phase in desorption rate argue strongly in favor of multiple layers and/or multiple binding sites for both BSA and γG's. In light of the large amount adsorbed in both cases, multilayer adsorption seems to be highly likely. Fluorescence emission spectra for adsorbed γG's and BSA are substantially different than spectra for bulk protein suggesting significant changes in local microenvironment upon adsorption. Two major problems complicating the interpretation of such research are interfacial photochemistry due to high radiation intensity at the interface and fluorescence quenching in the protein film where energy transfer is facilitated by close molecular association.

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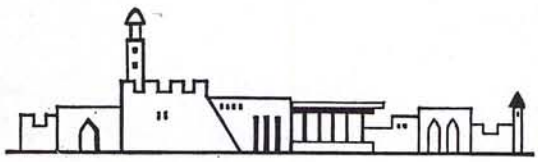
ABSTRACTS



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PROBING INTERFACIAL PROTEIN CONFORMATION VIA INTRINSIC FLUORESCENCE SPECTROSCOPY

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Evanescent wave induced, interfacial protein fluorescence is of great utility in studying protein adsorption on flat surfaces. The methodology employed consists of a single pass quartz dovetail prism adjacent to a flow cell. Radiation produced by a HgXe lamp is isolated via an excitation monochromator (285nm) and directed into the prism where it undergoes internal reflection at the prism-electrolyte buffer interface. Alternatively, protein adsorption on polymer thin films deposited on the prism surface may be studied if polymer fluorescence is low and internal reflection still occurs. A portion of the emitted fluorescence (300-400 nm) normal to the interface is collected via an emission monochromator, amplified in a photomultiplier and quantitated in a photon counting system. Data is recorded as fluorescence signal intensity as a function of adsorption time and bulk protein concentration, C_B. This approach has several advantages. It is an in situ sensing method for directly following protein adsorption in real time with 0.1 second resolution. Thus, adsorption and desorption kinetic studies are greatly facilitated. The flow cell configuration provides well quantitated temperature and hydrodynamic flow conditions. Fluorescence is intrinsic, i.e., it arises from tryptophan or tyrosine moieties integral to the protein. This is in contrast to previous surface fluorescence research which relied upon an extrinsic fluor covalently linked to the molecule. Such binding may alter the physical properties of the protein under study. Also, intrinsic tryptophan fluorescence is much more sensitive to local microenvironmental changes which may result as protein molecules undergo conformational changes during adsorption. Such information can be derived from an analysis of the fluorescence emission spectra. The quantitation of the amount adsorbed, Γ, can be calculated on the basis of an internal standard arising from evanescent wave excited bulk protein fluorescence adjacent to the adsorbed film. Competitive adsorption may be studied by extrinsically labelling one of the competing macromolecule types. The adsorption of bovine serum albumin (BSA) and gamma globulins (γG's) on quartz has been studied. The γG's adsorbed to an equilibrium value of Γ=3.6μg cm^{-2} on hydrophilic quartz at a C_B exceeding 1 mg ml^{-1}. The adsorption isotherm for BSA on quartz was linear and Γ was directly proportional to C_B between 0 and 5 mg ml^{-1}. At a C_B of 1.0 mg ml^{-1}, Γ was 0.08μg cm^{-2} for BSA versus 3.6μg cm^{-2} for γG's. The initially rapid desorption kinetics followed by a second distinct phase in desorption rate argue strongly in favor of multiple layers and/or multiple binding sites for both BSA and γG's. In light of the large amount adsorbed in both cases, multilayer adsorption seems to be highly likely. Fluorescence emission spectra for adsorbed γG's and BSA are substantially different than spectra for bulk protein suggesting significant changes in local microenvironment upon adsorption. Two major problems complicating the interpretation of such research are interfacial photochemistry due to high radiation intensity at the interface and fluorescence quenching in the protein film where energy transfer is facilitated by close molecular association.

Joe

Ab- Wang - # I

STUDY OF PEO ON LTI CARBON SURFACES BY ELLIPSOMETRY AND TRIBOMETRY

Jinyu Wang, Eric Stroup, Xingfa Wang and J. D. Andrade
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Key words: Ellipsometry, Tribometry, Polyethylene Oxide, Low Temperature Isotopic Carbon.

Abstract

Proteins adsorb to almost all surfaces during the first few minutes of exposure [1]. Surfaces which show minimal protein adsorption are important in many biomedical applications. Moreover, patient discomfort due to poor lubricating action between tissue and various medical devices, especially contact lenses, is a serious medical problem. An effective polymer for protein-resistant surfaces and super lubrication properties appears to be polyethylene oxide (PEO) [2] [3]. Here we report a study of PEO films on low temperature isotropic (LTI) carbon surfaces, including preparation using a photochemical reaction, characterization of the thickness of the PEO layer by ellipsometry and measurement of coefficient of friction with a custom built tribometer.

The UV induced-reaction of PEO onto carbon surfaces has been studied. The LTI carbon surfaces examined under optical microscopy are smooth. The cleaned sample is submerged in methanol before treatment in a PEO/H_2O solution [BBE-PEG(3350)-OH in H_2O, 5 mg/ml] for 10 minutes [4]. The sample is rinsed using deionized (DI) water and is dried in air. The sample surface is exposed to UV light of 4.7 mw/cm^2 for 5 minutes and then stored in DI water.

The samples are air dried, and then ellipsometry measurements (RUDOLPH RESEARCH RR2000 Automatic Ellipsometer) made to determine the optical parameters of PEO layer. A series experiments have been done to analyze the film thickness. The results of ellipsometric measurements suggest that the UV induced-reaction PEO on the LTI carbon surface is of about 25Å thick, and is very difficult to remove. These suggest that a

photochemical reaction resulted in the covalent binding of the PEO molecules to the carbon surface.

Friction and lubrication phenomena on the PEO film have also been investigated. Friction forces were measured in air for both the LTI carbon surface and the LTI carbon surface coated with a PEO layer using the same process. The coefficient of friction of the LTI surface with a PEO layer, about 0.17, is less than that of the LTI surface alone, about 0.35. This suggests that an added PEO layer is helpful in improving lubrication.

Photoactive PEO has been used for glass eyes [5]. Patients feel more comfortable wearing artificial eyes coated a layer of PEO due to better lubrication of the eyes. PEO will be a very useful coating for contact lens and other various medical devices in the future because of better lubrication properties and biocompatibility.

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ADSORPTION OF MODEL PROTEINS AT THE AIR-WATER INTERFACE:ROLE OF STABILITY AND EFFECTIVE HYDROPHOBICITY. Ai-Ping Wei, James N. Herron, Joseph D. Andrade. Department of Bioengineering, The University of Utah, Salt Lake City, Utah 84112

A set of small,single domain proteins, including cytochrome-c, myoglobin, lysozyme, ribonuclease-A and superoxide dismutase, were chosen as model proteins to study the relationship between their structures and adsorption behavior. The surface tensions of solutions (0.1 mg/ml in PBS buffer) of these proteins were measured as a function of time by the Wilhelmy Plate technique. The intrinsic stabilities were fluorimetrically determined by guanidinium hydrochloride(GdnHCl) denaturation in solution. The effective surface hydrophobicities were probed by a fluorescent hydrophobic probe, cis-parinaric acid. The measured surface tension values at the adsorption time of less than one minute correlate very well with the probed effective surface hydrophocities. The surface tensions under denaturing conditions(8M GdnHCl) correlate well with the apolar amino acid residues content, while the equilibrium surface tensions did not show any significant correlation with either of these two parameters. It is proposed that both the intrinsic stability and effective surface hydrophobicity of a protein are significant in determining the extent to which it adapts its conformation to a surface. Experimental results are examined by computer molecular graphics and calculations of accessibility based on the x-ray coordinates.

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Ab-WINT-2

ADSORPTION OF ANTITHROMBIN III

S. Winters, D. E. Gregonis, D. Buerger, and J. D. Andrade

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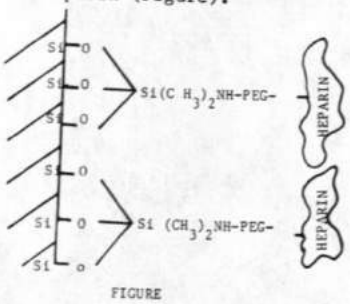
Protein adsorption is important in the interaction of materials with biological systems. Hydrophilic surfaces induce less total protein adsorption which has been proposed to be a critical factor in eventual thrombus formation. A large number of modifications to the surfaces have been attempted to prevent thrombosis. One method has been to heparinize the polymeric materials to provide antithrombotic activity at the implant-blood interface (1). Heparin is by far the most commonly used anticoagulant. However, due to the complex nature of this mucopolysaccharide and its many interactions (i.e. platelet factors, antithrombins, lipoproteins, etc.) its mechanism of action is not well understood. Here, we combine the advantages of a hydrophilic surface with those of heparin and study the specific interaction of immobilized heparin with antithrombin III (ATIII).

Total internal reflection intrinsic fluorescence spectroscopy (TIRF) provides a sensitive technique for studying interfacial phenomena between the immobilized heparin and blood protein (2). The intrinsic fluorescence of tryptophan residues in ATIII provides information on the molecular level events occuring when this blood clotting protein interacts with heparin. It has been suggested that heparin binds to lysine residues on the ATIII molecule, thereby inducing a conformational change and making its active site more accessible to thrombin (3).

Intrinsic TIRF has a number of advantages for this type of application, including quantitation of adsorbed protein, use of low surface area substrates, real time sensing, and it requires no extrinsic labels which could alter the activities of materials used (2).

Hydrophilic surfaces used for the covalent attachment of heparin have been developed using four different derivatives of polyethylene glycol (PEG-MW3400) (4). These include PEG-bisthiochloroformate, PEG-diisocyanate, and PEG-diisothiocyanate by reaction of the PEG with phosgene and thiophosgene. The derivatives are coupled to quartz slides via a urethane linkage using the amino group of aminopropyltriethoxysilane (APS) coupling agent. Heparin is then covalently bound to the PEG derivatives to form a hydrophilic substrate with active heparin (Figure).



FIGURE

The Heparin is probably several angstroms from the surface and therefore relatively free to interact with proteins flowing past the surface. The tritiated or C^{14} heparin is quantitated using a surface planchet counter. A flow cell used for the TIRF studies was developed to provide constant laminar flow with a well characterized flow profile. The volume of the cell is 100 ul. The hydrophilic surfaces are stable under aqueous conditions as determined by X-ray photoelectron spectroscopy, Wilhelmy plate contact angle measurements, and TIRF using a fluoresceinamine label.

The adsorption of ATIII is monitored in real time on the TIRF system. Excitation energy at 280 nm is used to follow the fluorescence emission at 334 nm by optically coupling the quartz slide to a quartz prism. The kinetics of ATIII adsorption onto clean hydrophilic quartz is relatively slow, taking approximately 20 minutes before a plateau is reached. This surface retains greater than 90% of the adsorbed protein after a buffer flush. Adsorption onto clean quartz, APS treated quartz and PEG/quartz without heparin are used as controls. The interaction of ATIII and PEG surfaces with the immobilized heparin can be seen in the emission spectra and can be correlated with reported fluorescence emission of buried tryptophan residues resulting from a conformational change in the ATIII molecule upon binding (5).

Evidence from TIRF data suggests that immobilizing heparin onto hydrophilic PEG surfaces provides an effective method for improving the blood compatibility of implantable devices.

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Title:

Pursuing Point-of-Care Testing:
An ImmunoChip Prototype for Antiepileptic Drugs

Authors & affiliations:

Xiaoyun Yang, Jarmila Janatova, Joseph D. Andrade
Department of Bioengineering, University of Utah,
Salt Lake City, UT 84112, USA

Abstract: (Your abstract must use **Normal style** and must fit in this box. Your abstract should be no longer than 300 words. The box will 'expand' over 2 pages as you add text/diagrams into it.)

Introduction: Therapeutic drug monitoring through a microarray platform offers considerable advantages over conventional analytical methods. Using a one-step homogeneous immunoassay as the platform [1], the multi-analyte ImmunoChip developed herein provides a simple and rapid technique for parallel quantitative detection of multiple antiepileptic drugs (AEDs).

Methods: A one-step bioluminescence immunoassay for quantitation of AEDs was developed and optimized in our laboratory [1]. The analytical chemistry is based on CEDIA [2] kits designed by Microgenics Corp. To produce the 3 × 3 multi-well ImmunoChip, a novel microfabrication method [3] was employed. Each ImmunoChip, after individual wells were filled via a solenoid dispensing system with assay constituents, containing respective capture antibodies, was lyophilized. Serum samples containing three AEDs (CBZ, PHT and VPA) were dispensed onto the ImmnoChip. Luminescent signal was detected and quantified across the area of each well by a CCD camera. The CCD counts, integrated for a 5-minute exposure in each well, were plotted as a function of AEDs concentrations.

Results: The luminescence patterns demonstrated in Figure A were obtained after serum samples containing a mixture of all the three AEDs were dispensed over the arrayed assay reagents. A comparison of 6 chips (from left to right in Figure A) reveals that the light intensity in each well increases with higher concentrations of the respective drugs dispensed (from top to bottom row: VPA; PHT; and CBZ). Calibration curves in Figure B were plotted by taking average integration of CCD counts across multiple chips tested at different sample concentrations of the three AEDs.

Discussion: This is an innovative study to demonstrate the feasibility of a microarray platform to achieve simultaneous detection and quantitation of multiple AEDs [4]. Further optimization is required to achieve better reproducibility between different batches of Immunochips and to increase homogeneity of signal detected from each well. This multianalyte microarray, when fully developed, will be suitable for application in on-site testing [5].

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Figure A. CCD images for VPA, PHT and CBZ assays performed on ImmunoChips. Serum containing different concentration of the three AEDs (listed below each chip) reconstituted the lyophilized reagents in each well to yield luminescent signal.

Figure B. Calibration curves of integrated CCD counts as a function of AED concentrations. CBZ (open diamond) and PHT (open square) employ lower X-axis; VPA (open circle) uses upper X-axis. Each rectangular bar designates therapeutic range of the respective AED.

It is well known that two component urethanes represent an excellent approach to provide durable coatings with good physical and chemical resistance combined with low temperature cure. To replace this technology with coatings based on novel curing mechanisms would indeed represent a leapfrog achievement. One approach to this is through other addition reaction mechanisms which provide ambient cure coatings with good properties.

Other leapfrog objectives for the future include development of 100% solids liquid coatings that cure at room temperature without releasing volatiles and the development of coatings with a greater tolerance to variations in substrates. Also, durable high solids automotive topcoats that cure at 180°F. is an important objective in view of the inroads being made by plastic exterior parts into the automotive industry.

To achieve leapfrog successes, a new philosophy in coatings research is required. We need to invest 20% of our resources in leapfrog research. We must encourage risk taking in an atmosphere of enterprise rather than of punishment for failure. This healthy balance of leapfrog research and incremental research commitment will transform the coatings industry into an exciting, high tech, growth industry.

HB-Yen-2

SYNTHETIC WATER SOLUBLE COPOLYMERS FOR OPTICALLY CONTROLLED LIGAND DELIVERY

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Abstract : To verify the possibility of developing a ligand delivery system which is controlled by light pulses, we have synthesized N-(2-hydroxypropyl)methacrylamide copolymers containing side-chains terminated in ligands (BOC-Gly, fluorescein, tetramethylrhodamine) bound via photocleavable 2-nitrobenzyl groups. Copolymers in solution were exposed to light of wavelength ~ 360 nm which resulted in release of the bound ligand. Depending on the experimental conditions (type of solvent, presence of oxygen) changes in the structure of released fluorochromes were observed (photofading effect). These effects were quantified by determining the binding constants of released modified fluorochrome with monoclonal antifluorescyl antibodies.

INTRODUCTION

The specificity of antibody (Ab) - antigen (Ag) interactions and competitive binding reactions using labeled Ag or labeled Ab have permitted the development of immunoassays with very high sensitivity and specificity. Such techniques can detect Ag and haptens at 10^{-11} M concentrations in such complex solutions as blood and serum (Ref.1). However, modern competitive binding immunoassay kits are totally unsuitable for continuous and/or remote measurements. There is a possibility to use the competitive binding immunoassay principle in fiber optic and thin film wave guide sensors. In our laboratory an effort is made to use interfacial excitation and photochemically controlled delivery to develop truly remote, nearly continuous sensors. The development of such sensors requires a number of basic and applied science studies (Ref.2) including polymer synthesis and ligand delivery, modulation of Ag-Ab binding constants (Ref.3), antibody orientation and immobilization (Ref.4), non-specific binding and bio- or blood compatibility.

This communication is focused on one key part of the overall sensor problem : optically controlled release of ligands bound to side-chains of N-(2-hydroxypropyl)-methacrylamide (HPMA) copolymers via photocleavable 2-nitrobenzyl bonds. For this first study we have chosen water soluble HPMA copolymers. In solution the structure of the copolymers synthesized can be better evaluated and the factors influencing the photocleavage of bound ligands can be determined.

RESULTS AND DISCUSSION

Synthesis of copolymers (Tab. 1) : By copolymerization of HPMA with N-methacryloylglycylglycine p-nitrophenyl ester we have synthesized a copolymer (1)

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containing reactive p-nitrophenyl ester (ONp) groups (M_w=27 000; M_w/M_n=1.3). By reaction of (1) with an excess of ethylenediamine ONp groups were converted into terminal NH_2 groups (2). Polymer (2) was reacted with 3-nitro-4-bromomethylbenzoic acid to produce copolymer (3) which side-chains terminated in a benzene ring substituted with bromomethyl groups in ortho-position to NO_2 groups. To polymer (3) BOC-glycine (model ligand) was bound by a modified method of Rich and Gurwara (Ref.5), forming polymer (4). Polymer (4) was deprotected by HCl/CH_3OH forming polymer (5). Fluoresceinisothiocyanate (FITC) and tetramethylrhodaminelsothiocyanate (MRITC) were bound to the terminal NH_2 groups of polymer (5), yielding copolymers (6) and (7), respectively.

Photochemical release of BOC-Gly : Photochemical release of BOC-Gly from copolymer (4) was carried out in methanolic solution in a quartz cuvette in nitrogen atmosphere. After 96 hours 80 % of BOC-glycine bound via 2-nitrobenzyl groups was released from the polymeric support (Fig. 1) as determined by the ninhydrin method. The cleavage was approx. linear to 50 % of ligand released. A similar interval of linearity was observed with poly(ethylene glycol) support (Ref.6). In this interval of cleavage the polymer synthesized should be suitable for continuous release of the bound ligand.

Photochemical cleavage of copolymers (6) and (7) : In these copolymers MRITC and FITC are separated from the photocleavable bond by a glycine unit. Thus after photocleavage a glycine modified fluorochrome should be released (N-carboxymethyl-N'-tetramethylrhodamyl thiourea [MR-Gly] and N-carboxymethyl-N'-fluorescyl thiourea [FL-Gly], respectively). This should have a minimum effect on their binding constants with antibodies, since it was shown (Ref.7) that the amino acid residues attached to the fluorescein molecule in the same location had minimal effect on ligand binding.

Three different reaction condition were used in the study of the photocleavage of copolymers (6) and (7) : a) methanol as a solvent and removal of oxygen by bubbling with nitrogen; b) water as a solvent and removal of oxygen by bubbling with nitrogen; and c) water as a solvent and the freeze thaw technique to remove oxygen. In all three cases photocleavage took place and glycine modified fluorochromes were released. However, in cases a) and b) photofading was observed during the process of irradiation and photocleavage. The absorption spectra of MRITC and low molecular weight products separated by column chromatography after photocleavage of copolymer (6) are compared in Fig.2. Only in case c) practically no changes in the spectra were observed. FITC appers to be a more stable molecule. For case a) major changes in the absorption spectra of the released ligand compared to the free one (Fig.3) were observed, whereas in cases b) and c) the spectra of released and free ligand are similar (Fig.4).

To characterize changes in the structure of released FL-Gly the association constants (K_a) of released fluorochromes with monoclonal antifluorescyl antibodies (clone

4-4-20) were compared with the K_a of fluorescein (Table 2). The association constants of the released ligands and of fluorescein are within one order of magnitude if the photocleavage was performed in aqueous solution.

Fluorescein, and phtaleins generally, owe their photochemical reactivity to the relatively long life time of the dye triplet (Ref.8). Reactions available to the dye (D) triplet were characterized (Ref.9) as D-O; D-R and D-D types, according to whether the primary step involves interactions of the dye triplet with an oxidizing agent (oxygen), reducing agent (methanol) or another molecule of dye. During irradiation of copolymer (7) in methanolic solution we have observed diminishing of the color of the solution. The absorption spectrum (Fig.3) is accordingly changed. This is in accordance with the observation that photoreduction to the colorless leuco-dye is one of the principal modes of photodecomposition of phtalein dyes in oxygen-free alcohol solutions (Ref.9). Experiments performed in aqueous solution gave much better results (Fig.4; Tab.2) in accordance with the observed stability of phtaleins in oxygen-free aqueous solutions. In this connection it is important to note that the rates of photofading should be different for free and bound dye. It was shown (Ref.10) that rose bengal is irreversibly photooxidized in aqueous solutions, whereas when bound to poly(vinyl pyrrolidone) the dye resists fading, even when exposed to sun light for a month.

In conclusion it may be stated that we have demonstrated the possibility of optically controlled release of ligands from water soluble HPMA copolymers. Since the structure of HPMA copolymers can be varied over a wide range and permits the introduction of different reactive units into one polymeric chain (Ref.11), it is our intention to attach these copolymers to functionalized surfaces in the next step of our studies.

ACKNOWLEDGEMENT

The work was partially supported by NSF Grant ECS-85-02107. We thank Dr. J. Herron and J.-N. Lin for advice and assistance.

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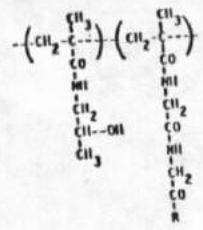
Table 1. Structure and characterization of copolymers



Copolymer No.	R	Content of side chains (mol%)
1	O-(C6H4)-NO2	5.2
2	NH-CH2-CH2-NH2	3.9
3	NH-CH2-CH2-NH-CO-(C6H3(NO2)-CH2Br)	3.7
4	NH-CH2-CH2-NH-CO-(C6H3(NO2)-CH2-O-CO-CH2-NH-CO-O-C(CH3)3)	2.8
5	NH-CH2-CH2-NH-CO-(C6H3(NO2)-CH2-O-CO-CH2-NH2)	2.8
6	NH-CH2-CH2-NH-CO-(C6H3(NO2)-CH2-O-CO-CH2-NH-CS-NH-C13H8O3-N(CH3)2)	0.6
7	NH-CH2-CH2-NH-CO-(C6H3(NO2)-CH2-O-CO-CH2-NH-CS-NH-C13H8O3-OH)	2.2

Table 2. Characterization of the fluorochromes by their association constant with monoclonal antifluorescyl antibody (clone 4-4-20).

Compound	Condition of photocleavage	Ka (M^{-1})
Fluorescein	——	5×10^9
FL - Gly[a] released	H_2O, bubbled with N_2	5×10^8
FL - Gly released	H_2O, freeze and thaw technique	2×10^8

[a] N - carboxymethyl - N' - fluorescyl thiourea

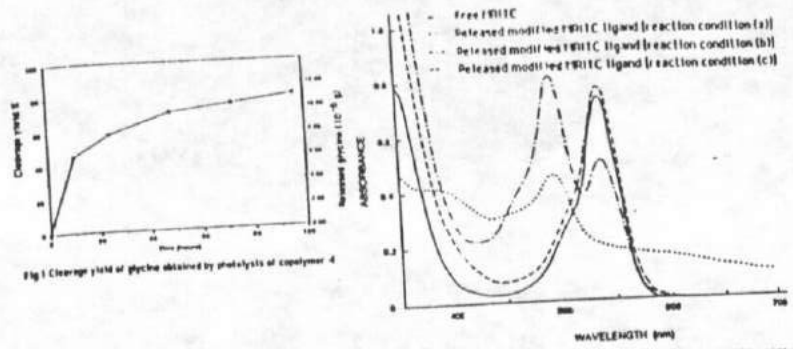


Fig 1 Cleavage yield of glycine obtained by photolysis of copolymer 4

Fig 2 Comparison of absorption spectra in MeOH of the free FITC and the released modified FITC ligands obtained under different conditions

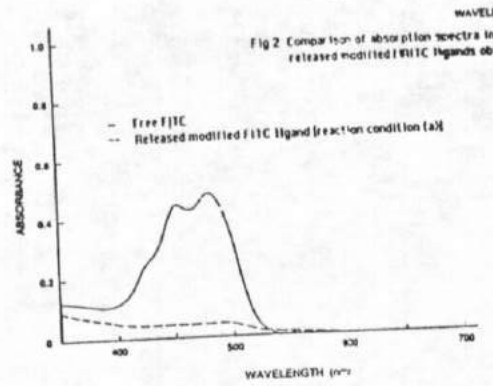


Fig 3 Comparison of absorption spectra in MeOH of the free FITC and the released modified FITC ligand

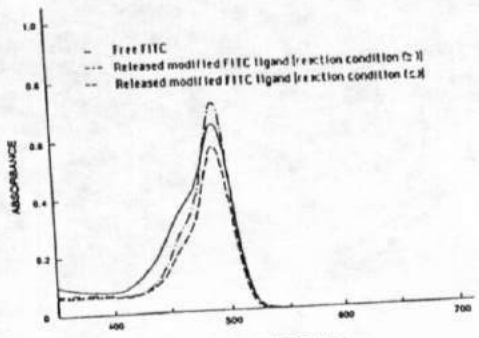


Fig 4 Comparison of absorption spectra in H_2O of the free FITC and the released modified FITC ligands obtained under different conditions

TOTAL INTERNAL REFLECTION FLUORESCENCE SPECTROSCOPY STUDIES OF PROTEIN ADSORPTION

by

Barbara Jean Zdasiuk

A thesis submitted to the faculty of The
University of Utah in partial fulfillment of the requirements
for the degree of

Master of Science

Department of Bioengineering

The University of Utah

August 1980

ABSTRACT

Protein adsorption is considered to be an important process in thrombus formation. Consequently, to clearly evaluate the potential of a biomaterial, a detailed knowledge of protein adsorption is essential. Many present techniques suffer the inability to obtain in situ, real time measures of protein adsorption. Total internal reflection fluorescence (TIRF) is a technique which is capable of yielding greater understanding of protein-substrate interactions. It is a technique in which in situ, real time adsorption can be monitored yielding kinetic and equilibrium adsorption and desorption information. The adsorption of fluorescently labeled bovine serum albumin, γ-globulins and fibrinogen onto hydrophilic and hydrophobic substrates was evaluated in order to assess the feasibility of total internal reflection fluorescence as a technique for the study of protein adsorption.

Zhang-2

ThP41 CROSS-LINKABLE PEO-CONTAINING POLYMERIC SURFACTANTS

J. Zhang, Z. Yang, J. Kopecek, and J. Andrade

Dept. of Bioengineering, Univ. of Utah, Salt Lake City, Utah 84112, USA

A series of PEO-containing polymeric surfactants has been synthesized. Surface treatment via adsorption and cross-linking of surfactants on polyethylene has been studied for the purpose of obtaining a stable protein-resistant coating. The surfactants are methacrylate copolymers with three different side-chains: 1) alkyl chains to enhance hydrophobic interaction between the surfactants and the hydrophobic polymer surface; 2) long PEO chains to form a liquid-like layer in aqueous solution, which is expected to prevent protein from adsorbing on the surface; 3) side-chains terminated with NH2 functional groups to react with cross-linking agents and to form a surfactant network. The molecular weight and the composition of the surfactants were estimated by GPC ~~and FTIR.~~ Spectrometer and NMR. Adsorption and desorption of the surfactants on polyethylene surfaces studied by XPS showed an increased stability of the adsorbed and cross-linked surfactants on the surface compared with the uncross-linked ones. The amount of lysozyme adsorbed on the surfactant-treated surface was significantly lower than that on the bare polyethylene surface.

The authors gratefully acknowledge the support of Polymer Technologies, Corp. and the Center for Biopolymers at Interfaces, University of Utah.

Abstracts — 8th Intern. Symp. on Surfactants in Solution, June 10-15, 1990 Gainesville, FL.

ThP42 DIRECT AND INVERSE MICROEMULSION COPOLYMERIZATION OF STYRENE AND ACRYLIC ACID

J. E. Puig, S. Corona-Galvan, P. C. Schulz, B. E. Rodriguez, A. K. Murthy and E. W. Kaler

Facultad de Ciencias Quimicas, Universidad de Guadalajara, Guadalajara, Jal. 44430, Mexico, and Department of Chemical Engineering, University of Delaware, Newark, DE 19716 USA

We report the copolymerization of acrylic acid and styrene in water-in-oil (w/o) and oil-in-water (w/o) microemulsions. In the o/w microemulsion, acrylic acid is mostly in the continuous phase whereas styrene is solubilized in swollen micelles. In the w/o microemulsion this situation is reversed. Copolymerization of acrylic acid and styrene in o/w microemulsion made with dodecyltrimethylammonium bromide produces very stable monodisperse latices with particle sizes ranging from 20 to 40 nm. The copolymer consists of isolated acrylic acid units randomly distributed among polystyrene blocks. Inverse microemulsion copolymerization of these monomers also yields stable, monodisperse latices. Aerosol OT or dodecyldimethylammonium bromide (DDAB) was used to form the water-in-oil microemulsions.

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(To be filled in by Division)

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Ab-Zhang-1

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Y.Q. Zhang J.D. Andrade A.P. Wei	Univ of Utah College of Engineering SLC UT 84112	☑ Yes ☐ No	☑ Yes ☐ No	☐ Chemist ☐ Chemical Engineer Other _____

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Metastable Conformational States of Proteins at Interfaces. Y.Q. Zhang, J.D. Andrade, A-P Wei, Department of Bioengineering, University of Utah, Salt Lake City, UT84112.

A general theory of conformational change of proteins at the protein solution-air interface is proposed. The relationship between surface tension and time is obtained. Two kind of middle metastable states, backbone middle metastable state and secondary structural middle metastable state are suggested. Processes with few-middle-backbone-metastable-conformational states are analysed in detail. A conjecture about the relation between the number of the extreme points of the surface tension-time diagram and the number of the backbone middle metastable states is given. The theory is fitted with the experimental data obtained from Lysozyme, Ribonuclease-A, Myoglobin, Cytochrome-c and Superoxide dismutase solutions-air interfaces. We find that Lysozyme and Superoxide dismutase could have backbone middle metastable conformational states. Ribonuclease-A, Myoglobin and Cytochrome-c could have middle secondary structural metastable conformational states. The decay constants of the conformations of model proteins are not constants due to the time-dependent microenvironmental changes of proteins at the interfaces. A semiempirical formula is proposed to describe the experimental data.

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