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Interaction of Blood Proteins with Solid Surfaces

L. E. Smith, R. E. Dehl, W. H. Grant, R. R. Stromberg and B. W. Morrissey

Polymers Division
Institute for Materials Research
National Bureau of Standards
Washington, D. C. 20234

August 16, 1976

Annual Report for Period

November 1, 1975 - August 15, 1976

Issued October 1976

Prepared for

Biomaterials Program
Division of Heart and Vascular Diseases
National Heart and Lung Institute
National Institutes of Health
Bethesda, Maryland 20014

Interagency Reimbursable Agreement

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ABSTRACT

This investigation is designed to help characterize the surface properties of cardiovascular implant materials. Such properties largely determine the success or failure of implants and may therefore be used as the basis for *in vitro* test methods. The focus of this investigation has been on the measurement of protein adsorption on surfaces, emphasizing measurements of the conformational changes which occur upon adsorption. During this reporting period, several materials produced by contractors of the NHLI Biomaterials Program have been examined by ellipsometry and a number have been found suitable for further work. Conformational changes at very short adsorption times are of considerable importance and preliminary results indicate that our techniques are capable of measurements at times as short as two seconds. We have analyzed commercially available radiolabeled human serum albumin and found considerable amounts of dimers and higher aggregates in some commercial preparations. We have begun to assess the errors involved in the use of unfractionated proteins for adsorption measurements by using purified monomer and aggregates. The use of light scattering to measure the conformation of protein adsorbed on small particles has been evaluated in a study of γ-globulin adsorption on polystyrene. The results agree well with similar measurements made by ellipsometry.

SUMMARY

This investigation is designed to aid in the characterization of the surface properties of materials used as implants in the cardiovascular system. Surface properties play a major role in determining the success of an implant and therefore should form the basis of in vitro test methods to select and develop useful materials.

The major emphasis of the work has been on the measurement of conformational change that occurs in the protein upon adsorption. Ellipsometry has proved successful in this regard when applied to several model surfaces. During the current reporting period a number of materials produced by contractors of the NHLI Biomaterials Program and considered of potential clinical importance by the Project Officer have been surveyed. Several materials were found to be suitable for fibrinogen adsorption measurements. In addition, carbon coated samples (ion-beam deposited, ion plated, and vacuum deposited) are suitable if the carbon coating is thicker than 350 nm, which would be the case for coatings of current biomaterial application. Measurements of adsorbed protein conformation on these materials can be compared with in vivo test results and any correlation will guide future work in the development of useful test methods.

The conformation of the protein as it is first adsorbed and the changes in conformation that it undergoes as the surface population increases are important parts in the sequence of events occurring when blood contacts a foreign surface. We have developed techniques for the measurement of such conformational changes at short adsorption times (~2s). Thus far we have been unable to detect conformational changes on this time scale in fibrinogen adsorbed on platinum or silica substrates.

The use of radiolabeled protein for adsorption measurements without careful purification of both the labeled and unlabeled components can lead to confusing and potentially misleading data due to the complex competitive adsorption between the protein monomers, aggregates and polypeptide fragments that are generally present. We have concentrated on this point during this reporting period and have analyzed some commercially available radiolabeled human serum albumin (HSA) samples. There are differences in composition from different manufacturers. Self-radiolysis does not seem to be an important contributor to the production of polypeptide fragments that may be present. We have used purified fractions of HSA-monomer and of HSA-dimer to measure the adsorption and competitive adsorption of the monomer and dimer on a polyethylene surface.

The use of quasielastic light scattering to measure the conformation of protein adsorbed on small particles has been investigated. The method is capable of high precision and the results of γ-globulin adsorption on polystyrene compares favorably with similar measurements made by ellipsometry.

INTERACTION OF BLOOD PROTEINS WITH SOLID SURFACES

INTRODUCTION

The adsorption of plasma proteins is the first in a complex series of events that occurs when a synthetic material is placed in the cardiovascular system. It is known that this protein adsorption can alter the course of subsequent reactions at the implant surface but the mechanism by which this occurs is not well understood. If the detailed behavior of proteins adsorbed at the solid surface were known and capable of control, the fate of artificial materials placed in body could be predicted with greater certainty and more successful materials perhaps could be designed. This investigation is directed toward the identification of factors capable of affecting protein adsorption and the development of a description of the changes that occur in the protein upon interaction with a surface.

A major portion of the research effort this year has been directed toward the examination of potentially useful synthetic biomaterials provided by contractors to the NHLI Biomaterials Program. Where possible, the adsorption of fibrinogen from solution onto these materials has been studied by ellipsometry. It is anticipated that as this data is accumulated, significant differences either in the rate of adsorption, the total quantity adsorbed, or the molecular conformation of fibrinogen on these various surfaces will lead to useful correlations with their blood compatibility as implant materials. This might provide the basis for a rapid in vitro screening test.

Experimental artifacts due to the use of impure radiolabeled proteins have led many workers to erroneous conclusions in the past about the adsorption of these proteins. We have conducted radiotracer studies of labeled HSA on polyethylene and silica surfaces, using carefully fractionated HSA. The adsorption of fractionated HSA monomer and dimer have been separately examined.

EXPERIMENTAL

Fibrinogen Purity

Human fibrinogen was used in these experiments. It was purified by the Batt (1) modification of the Laki (2) method to yield a product which is greater than 96.7% clottable. (The theoretical maximum is 97%). At

the suggestion of Prof. David Waugh of MIT, we are now preparing purified fibrinogen in fairly large quantities, fast-freezing the solution batches in liquid nitrogen, and storing the frozen solutions in dry ice until they are needed. With these precautions, the fibrinogen purity can be assured for relatively long periods of time.

Ellipsometry

The techniques of ellipsometry, methods of sample handling, determination of the optical constants of the substrate, etc., have been described in previous Annual Reports (3,4). In all cases, the fibrinogen adsorption experiments were performed at 37°C. Because many of the NHLI Contractor Biomaterial samples were elastomeric, it was necessary to construct a very heavy Pt weight to hold the sample flat and prevent movement of the sample due to relaxation during the experiment, and still permit light to reach the sample surface.

For the preliminary adsorption experiments with the automatic ellipsometer described in this report, a straightforward method of introducing the protein solutions was adopted. After determining the optical constants of the substrate under buffer solution, the solution was withdrawn from the ellipsometer cell as completely as possible with a syringe, leaving a thin film of solution on top of the (hydrophilic) substrate. A premeasured quantity of filtered protein solution was then quickly poured into the cell. Since the solution is introduced in a highly turbulent manner, it should mix rapidly with any residual buffer solution in the cell. This technique allowed measurement of the ellipsometric parameters in less than 2 seconds after the solution pouring was begun. The resultant changes in the parameters were smooth and monotonic through the total observation times, which ranged up to 3 hours.

NHLI Contractor Biomaterials

The NHLI Contractor Biomaterials which we have received this year were shipped to us in sterile water, in the case of the hydrogels, or in sterilized packages, in the case of uncoated substrates. These precautions should eliminate problems that we have had with some samples in the past, due to bacterial growth in nonsterile aqueous media. For some samples which may be porous as well as hydrophilic, we allow the substrate to soak in buffer solution overnight before the final measurement of its refractive index. This procedure assures that the index will not be changing during the time that protein is being adsorbed, since such changes could lead to serious errors in interpretation of the data.

Radiotracers

A different experimental arrangement for the adsorption of the labeled proteins on surfaces was designed to decrease the volume of solution necessary for an adsorption experiment. Prior to this report an adsorption solution

consisted of non-labeled proteins diluted with labeled proteins and thus large volumes were easily obtainable. In the present case, the solution contains only labeled proteins and has a smaller total volume to compensate for the higher cost of using pure labeled proteins. This modified experimental arrangement is confined to the adsorption vessel. The vessel, which has a total volume of 10 ml is equipped with a plunger that is designed to hold two slides under the solution and also to aid in their removal from the solution. The adsorption vessel is shaken with a mechanical shaker in a thermostatically controlled water bath maintained at 37 ± .1°C. All other experimental procedures are the same as described in previous Annual Reports.

Protein Purity

The commercially labeled proteins obtained from Squibb Pharmaceutical Co. and Amersham/Searle and the non-labeled proteins obtained from Nutritional Biochemicals Corp. were further purified by passing these materials through a Sephadex G-200 gel permeation chromatographic column which separated out the trimer, dimer, monomer and low molecular weight polypeptide.

RESULTS AND DISCUSSION

EXAMINATION OF NHLI CONTRACTOR BIOMATERIALS

Polyether Urethanes (Stanford Research Institute)

We have completed the study of fibrinogen adsorption onto SRI polyether urethanes (A) 3-2000-1-E and (B) 3-1000/425(70/30)-1-X. The results of 3 separate adsorption experiments with each material are shown in Figures 1-4. No significant changes were observed in either the protein film thickness (extension) or adsorbance during the time interval from about 0.5 to 3 or 4 hours. The (B) substrate is mechanically stiffer than the (A) substrate, and unlike the latter it did not absorb water and become translucent after immersion in water for a day or more. Despite these differences, however, the (A) and (B) substrates appear to have a very similar affinity for fibrinogen, adsorbed from solution. The average fibrinogen film thickness calculated from the data of Figure 1 for the (A) material is 30.7 ± 4.6 nm, while the (B) substrate data of Figure 2 yields an average thickness of 32.0 ± 5.7 nm. The average adsorbance on the (A) and (B) substrates, calculated from the data shown in Figures 3 and 4 are, respectively, 4.75 ± 0.23 mg/m^2 and 5.04 ± 0.39 mg/m^2. Thus, within experimental error, there is no difference in the equilibrium film thickness or adsorbance of fibrinogen on the two SRI substrates. However, the rate of protein adsorption during the initial half hour remains as a possible difference between the two substrates.

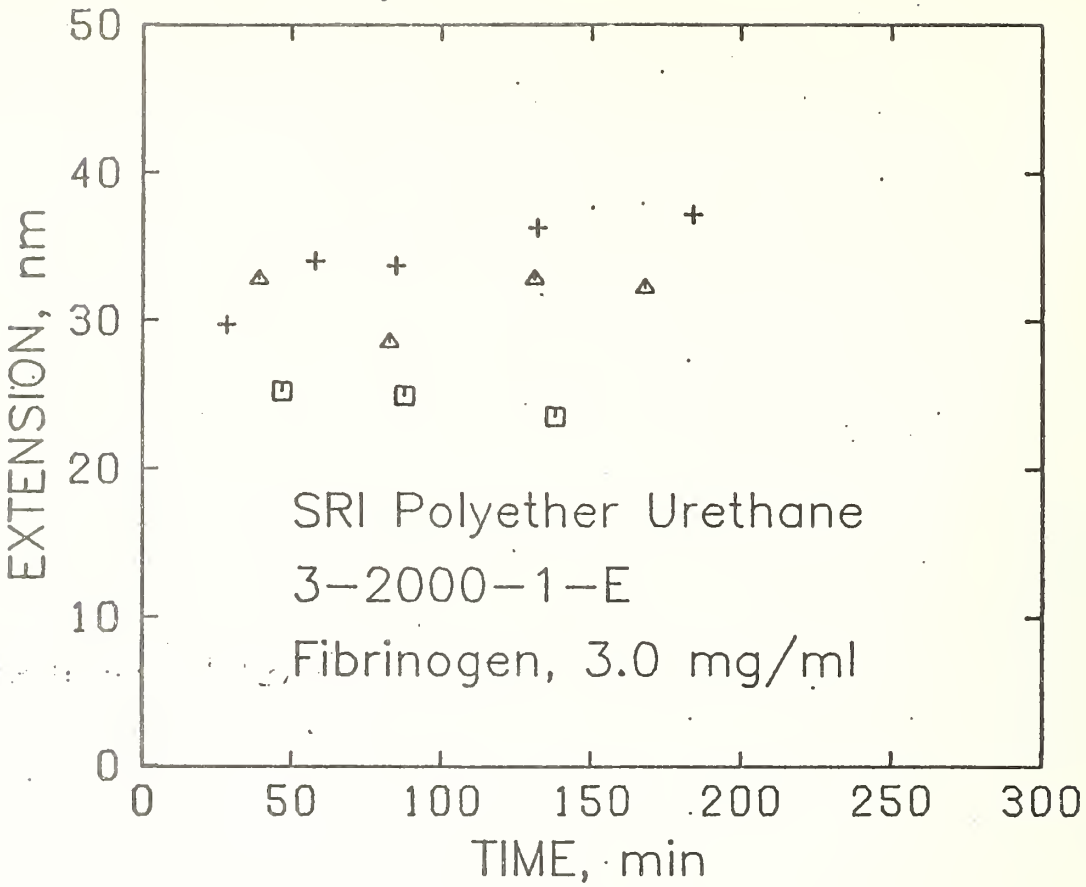

Figure 1: Extension of human fibrinogen on SRI Polyether Urethane 3-2000-1-E. Symbols refer to three different samples.

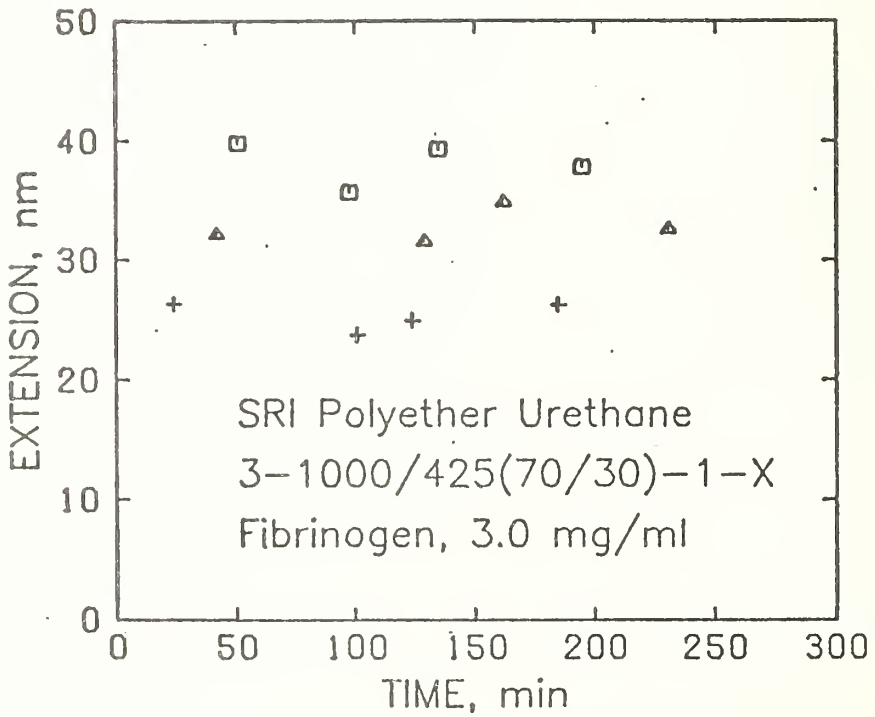

Figure 2: Extension of human fibrinogen on SRI Polyether Urethane 3-1000/425(70/30)-1-X). Symbols refer to three different samples.

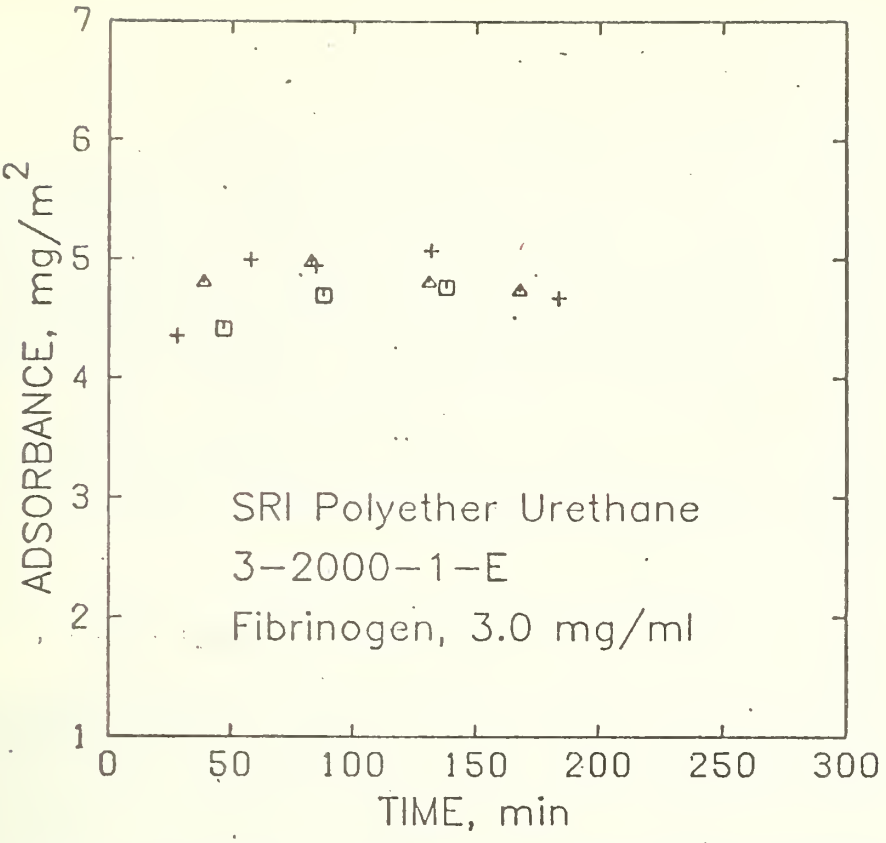

Figure 3: Adsorbance of human fibrinogen on SRI Polyether Urethane 3-2000-1-E. Symbols refer to three different samples.

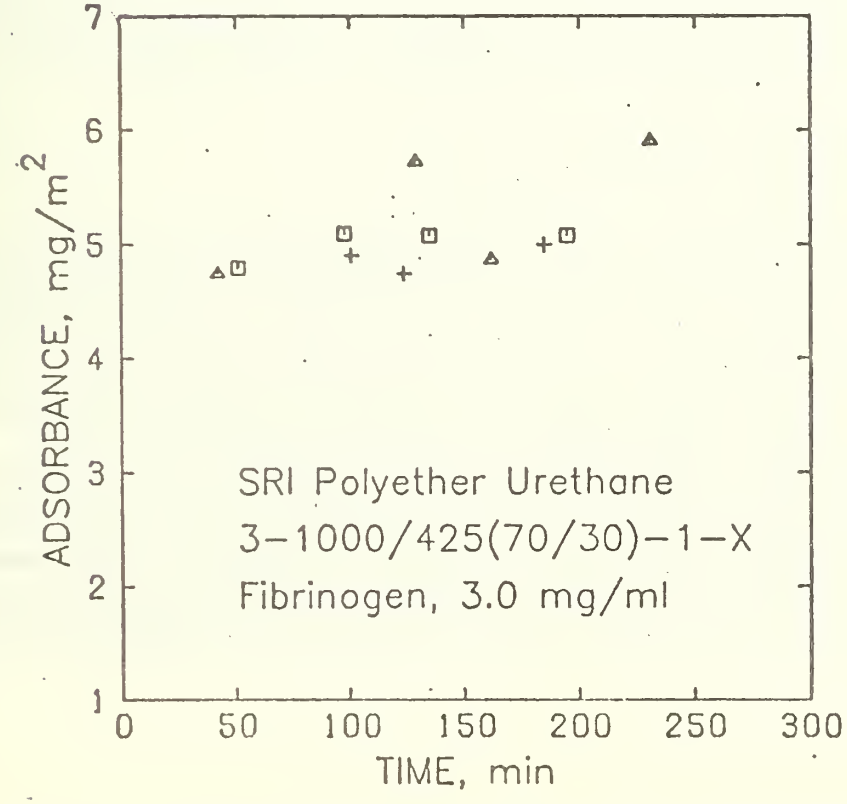

Figure 4: Adsorbance of human fibrinogen on SRI Polyether Urethane 3-1000/425 (70/30)-1-X. Symbols refer to three different samples.

Ethylcellulose Perfluorobutyrate (Midwest Research Institute)

A one mm thick sheet of sterile ethylcellulose perfluorobutyrate membrane material was prepared for our ellipsometry studies by Dr. R. J. Peterson (MRI), and received by us on April 27. Although the surface was marred by rippling, due to the effects of casting such a thick film, sufficient flat portions were found for use as ellipsometer samples. Preliminary measurements of fibrinogen adsorption on this material indicate (1) a typical film thickness of about 30-40 nm and (2) a much greater adsorbance after 2-3 hours than we have observed on any other substrate thus far (20-30 mg/m^2). There is some evidence that the optical constants of the substrate itself might have been changing during the course of a given experiment, and further work is in progress to determine whether the unusually high apparent adsorbance is real or due to an experimental artifact.

Polyacrylamide Hydrogel (Franklin Institute Research Laboratories)

Eleven individually-prepared sterilized samples of polyacrylamide hydrogel, grafted with active hydrogen to SRI polyether urethane substrate (3-2000-1-E) were received from Dr. P. L. Kronick (FIRL) in December 1975. To date, four samples of this material have been examined by ellipsometry and only one was found to give the specular reflection which is required to make the optical measurements. Results from this one sample are ambiguous and cannot be confirmed without another suitable sample. There are apparently other differences between the individual samples, as evidenced by (1) the cracking of the surface on some samples but not others and (2) the ability to obtain a specular reflection on the "uncoated" side of some samples, but not others. Given the sample-to-sample variability, we do not feel that it is meaningful to perform fibrinogen adsorption experiments on these materials, since the results for any given sample are not necessarily an accurate representation of the adsorption characteristics of all samples.

Polyacrylamide Hydrogel (Polysciences, Inc.)

Six sterile samples of solution-grafted polyacrylamide hydrogel on SRI polyether urethane (3-2000-1-E) were received from Dr. O. Solomon on May 25. We were unable to obtain a coherent reflection from the surface of these samples, and therefore could not perform ellipsometric measurements on them. Examination of a dried sample of this material under the microscope (100X) revealed that the surface was completely covered with bumps sufficiently large to scatter light. There were no flat spots to give a specular reflection. While these results do not directly imply anything about the biocompatibility of this material, it is clear that protein adsorption experiments will have to be made by some other means, such as with radio-tracer-labeled proteins. The coated substrate also appears to be somewhat stiffer than the uncoated SRI material, suggesting that some modification of the bulk mechanical properties of this material has resulted from the coating process.

CURRENT STATUS OF OTHER NHLI CONTRACTOR BIOMATERIALS

Glow Discharge Coatings (Research Triangle Institute)

On June 25, we received from Dr. N. Morosoff (RTI) seven sterile samples of vacuum-cast polyethylene film, supplied by us, coated by RTI with an 800 Å film of polymer deposited from a glow discharge of tetramethyl disiloxane. We have not as yet examined these samples by ellipsometry.

Polyalkylsulfone (A. D. Little)

By agreement during a telephone conversation on March 16, 1976, Dr. J. D. Birkett (A. D. Little) agreed to attempt to cast a sheet of polyalkylsulfone membrane material suitable for ellipsometric studies, i.e., at least 1.5 mm thick. We received such a sample on August 12.

Ion-plated Carbon (General Atomic Corp.)

As we reported in our Annual Report for 1975 (5), we have measured the refractive index of several samples of "ion-plated" (manufacturer's notation) carbon on stainless steel and on glass. Significant differences were found in the indices of different thicknesses of carbon on the same substrate (stainless steel), and in the index of a nominally identical thickness of carbon on two different substrates (stainless steel and glass). Because carbon is an absorbing film, we did find that its refractive index, calculated from its reflection coefficients, becomes independent of the underlying substrate when the film thickness exceeds about 350 nm. This means that quantitative ellipsometric measurements of protein adsorption on any carbon-coated substrate should be possible, providing the film is at least 350 nm thick. According to a letter which we received from Dr. A. Haubold of General Atomic on June 29, 1976, the film thicknesses which will be used on biomaterials will probably be on the order of 500 nm. Thus, there should be no problems due to the uncertain index of carbon on these materials, since the index can be measured independently of the underlying substrate. We already have a number of 400 nm carbon-coated steel coupons prepared by General Atomic, and these will be used in future protein adsorption experiments.

Heparin Bonded to Silastic-coated Glass (Carnegie-Mellon University)

On June 11, we received from Dr. M. S. Morgan (Carnegie-Mellon University) a number of samples which should provide information about the effect of heparin on the adsorption of blood proteins. Because of the complexity of the film on these coated slides, it will be necessary to perform a number of measurements on partially coated slides, in order to establish the optical constants of each layer in the film. Dr. Morgan has supplied us with 3 samples each of (1) plain glass slides, (2) silastic-coated glass slides,

(3) slides coated with silastic + the bonding reagent, APTES (γ-aminopropyl triethoxysilane) and (4) all of the above + heparin. Because of the number of intermediate measurements required by such a complex surface, there may be considerable uncertainty in the fibrinogen adsorption measurements. However, we expect to perform such studies on these surfaces and hope that at least semiquantitative information can be derived.

RADIOTRACER ADSORPTION STUDIES

Effect of Molecular Impurities on Adsorption of HSA

We have been concerned with the use of unpurified proteins for the measurement of the adsorption of proteins on surfaces. These adsorption measurements are commonly made using labeled and unlabeled proteins which may contain varying amounts of dimers and higher aggregates as well as low molecular weight fragments. Depending upon the relative amounts of these components and their competitive adsorption properties, the rates and amounts measured from such mixtures will be different from the desired values of the monomer alone.

We have analyzed and separated commercially available human serum albumin (HSA), both labeled and unlabeled, and measured the adsorption of these fractions on two different substrates, polyethylene and silica. These fractions were separated on a Sephadex G-200 gel permeation chromatographic column. Figure 5 shows the results for ^{131}I HSA. Starting from low molecular weight to high, 31% of the sample is low molecular polypeptide, 49% monomer and 20% dimer and higher aggregates. In Figure 6 the same separation is shown for ^{125}I labeled HSA obtained from the same commercial source which gave a similar distribution of products. The relative amounts are also similar to ^{131}I labeled material with 40% polypeptide, 40% monomer and 20% dimer and higher aggregates. There is no significant difference between the ^{131}I and ^{125}I sample.

Because of self-radiolysis caused by the more energetic labeling atom, one might expect that the ^{131}I sample would have more low molecular weight fragments than the ^{125}I. This analysis does not support that contention. The ^{131}I HSA was more than four months old, ample time for maximum radiolysis, but its elution pattern shows no more fragments than the ^{125}I HSA, a fairly new sample. The distribution of the radioactivity in the ^{125}I sample is shown in Figure 7. There is no activity in the polypeptide peak, indicating that it is probably not a radiolysis product, but most likely formed during the labeling of the material. Therefore, self-radiolysis does not appear especially significant in determining sample composition.

Following the work presented above, we examined by gel permeation chromatography a sample of HSA ^{125}I supplied by another commercial source. As shown in Figure 8, this material is clearly much less contaminated with dimer and higher aggregates than that from the other commercial source.

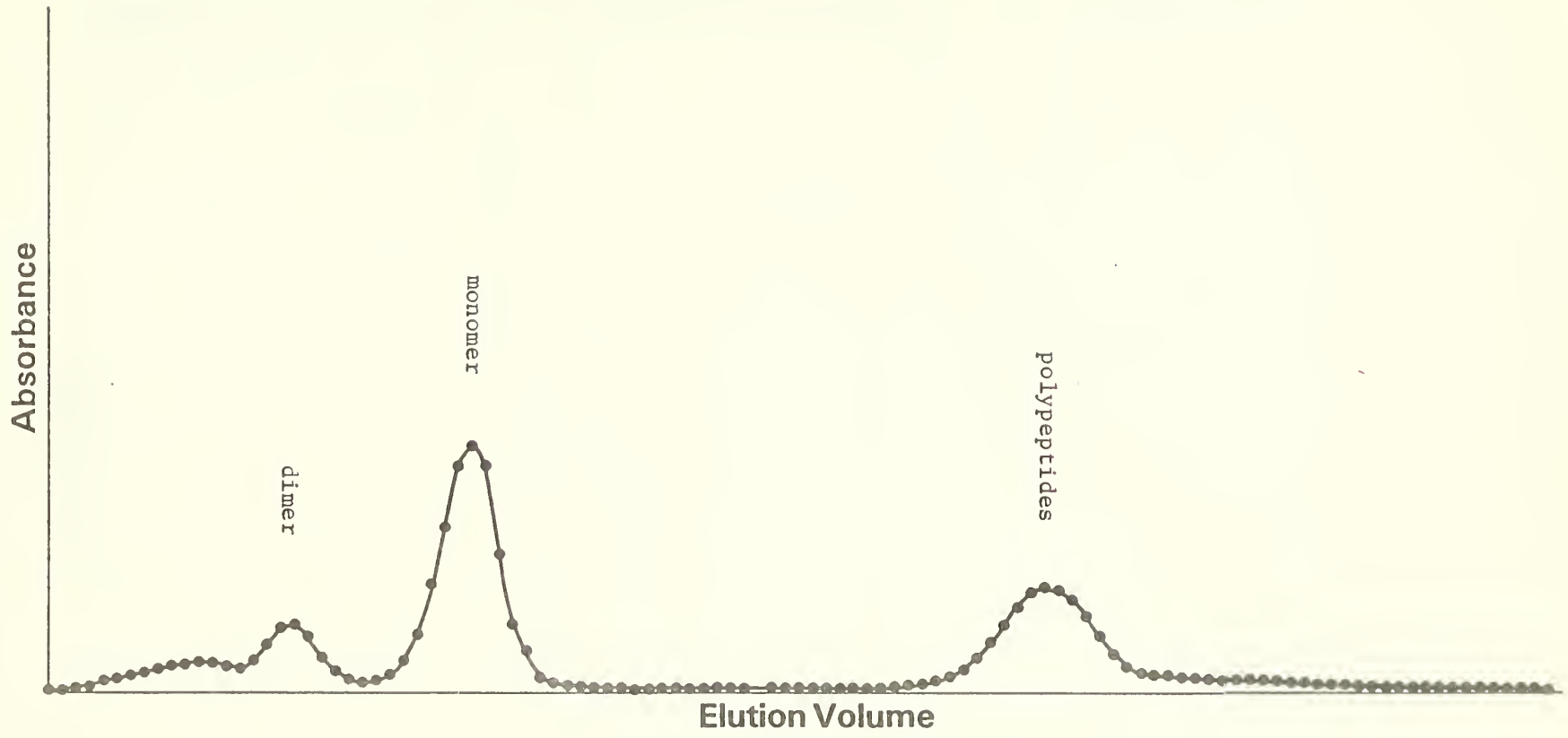

Figure 5: Gel Permeation Chromatogram of ^{131}I-HSA

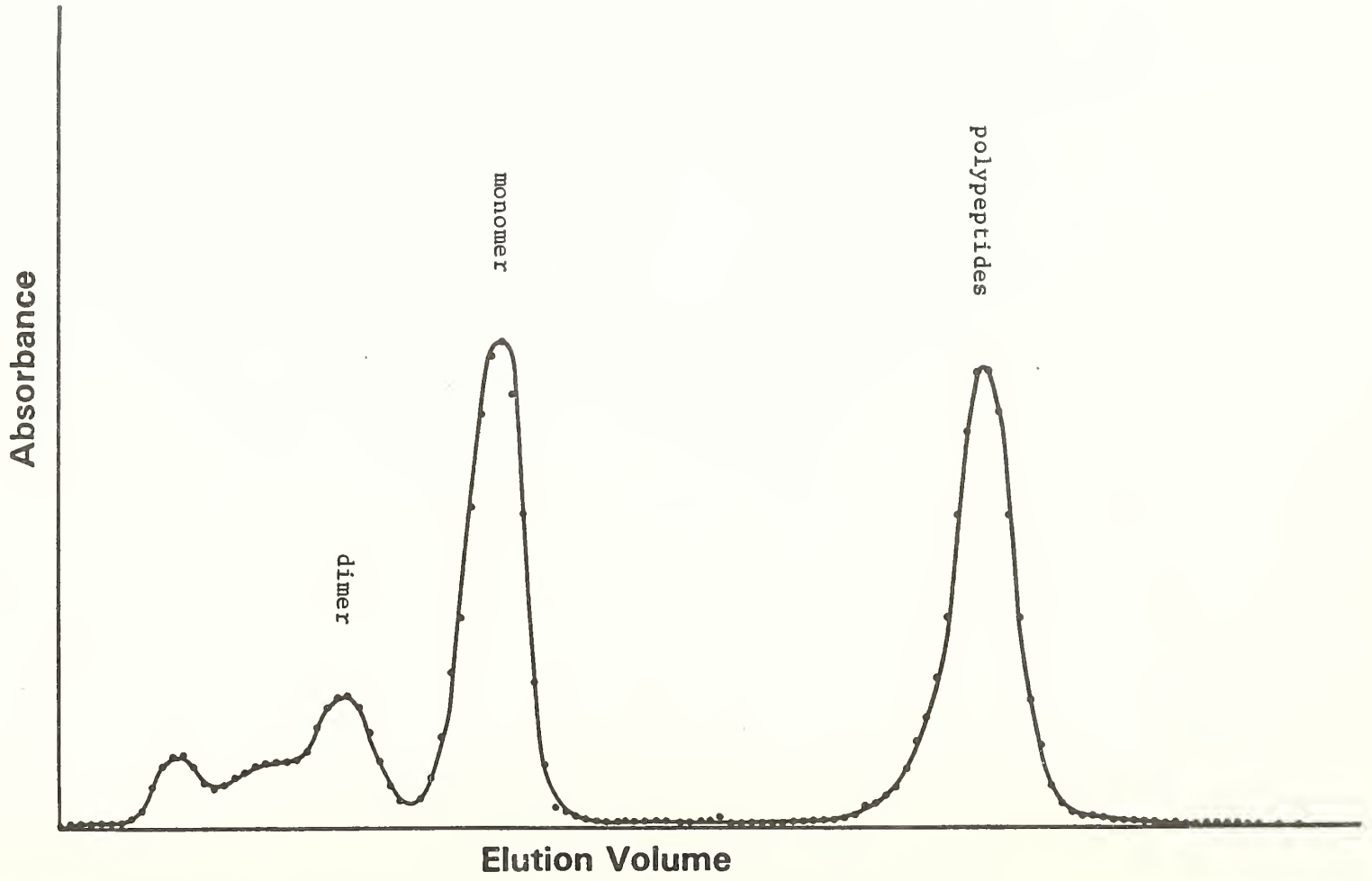

Figure 6: Gel Permeation Chromatogram of ^{125}I-HSA

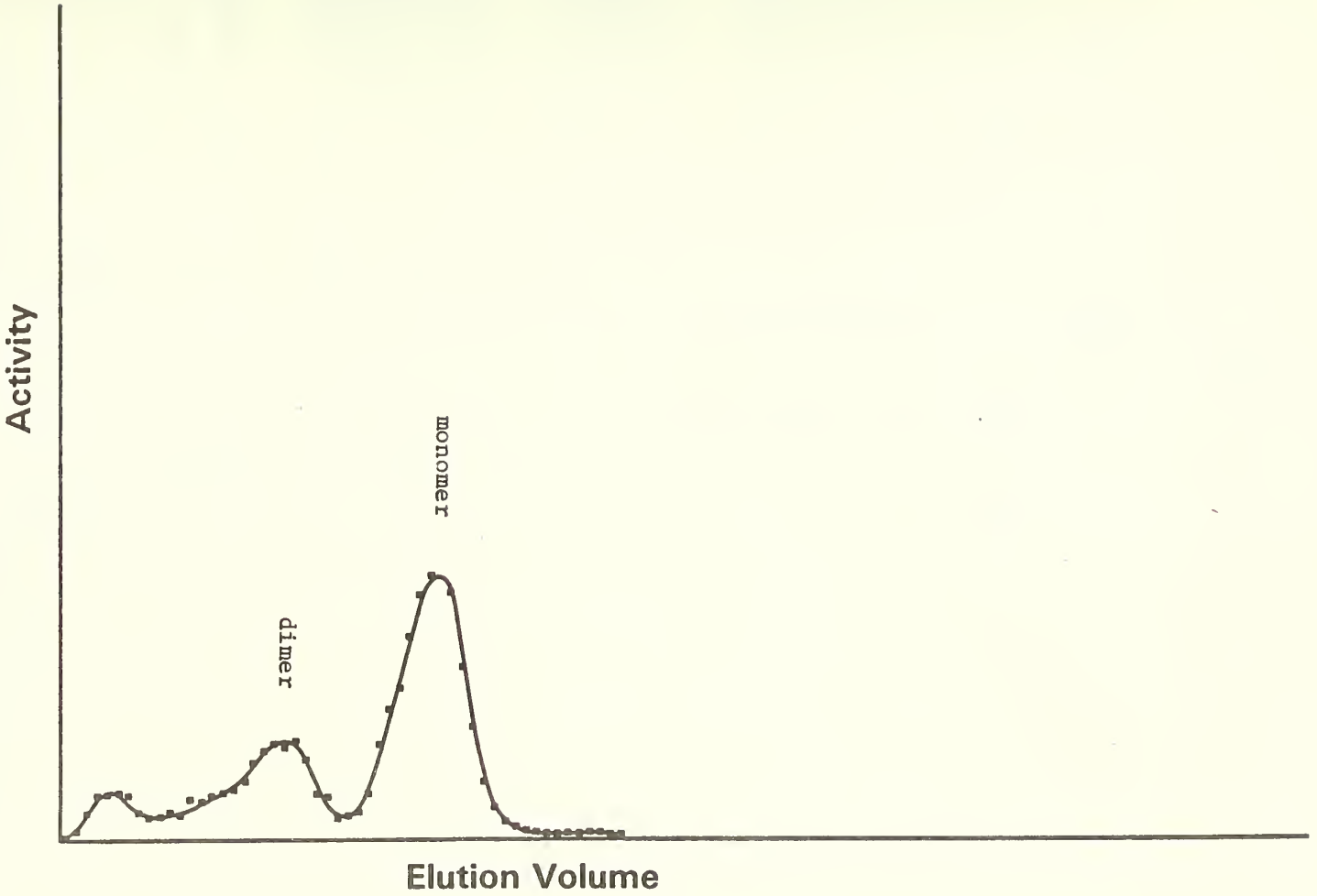

Figure 7: Relative Radioactivity of the ^{125}I-HSA Chromatogram Shown in Figure 6.

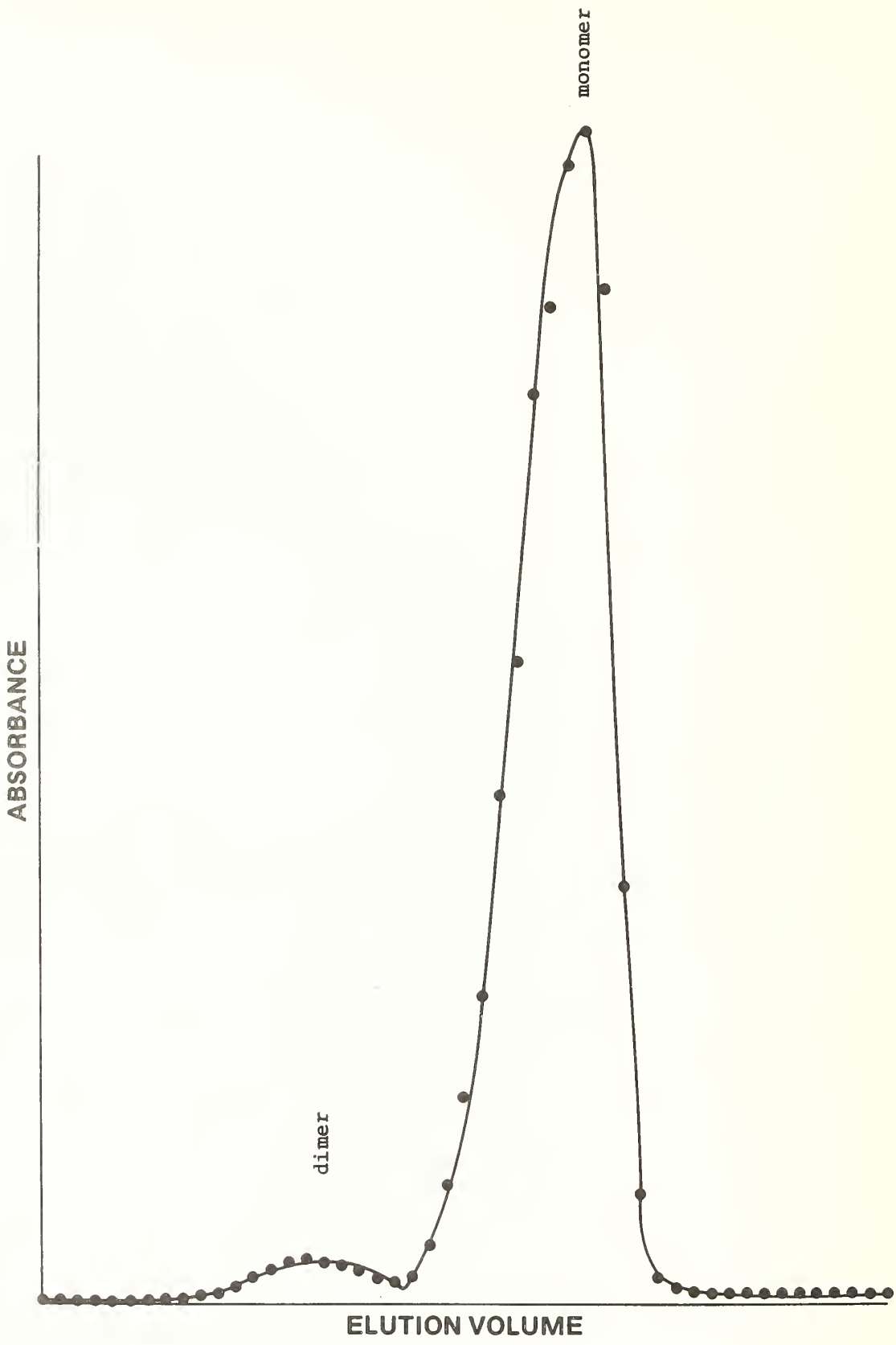

Figure 8: Gel Permeation Chromatogram of ^{125}I-HSA

In Figure 9, the elution curve for the unlabeled HSA is presented. This sample has less than 0.5% low molecular weight polypeptide, 72% monomer and 28% dimer and higher aggregates. For the adsorption measurements, the ^{125}I labeled material shown in Figure 6 was used and fractions were collected from each peak.

Table I shows some preliminary results for the adsorption of selected fractions on polyethylene. The second and third columns are really a test of internal consistency, experimental reproducibility, and the effect of the labeling atom. There is good agreement between these two columns in both amounts and rates. The third column represents an approximation of the usual practice in adsorption measurements, with only the elimination of the labeled aggregates. In this case, measurement is made of only the monomer and the degree to which it is affected by the presence of aggregates. The adsorbance of the monomer is apparently unaffected by the aggregates at short times but the values at longer times are distinctly different. The last column shows the adsorption of the aggregates at two different concentrations. First, at the same concentration (2 mg/ml) as the monomer in the preceding columns and secondly at a lower concentration (0.2 mg/ml) corresponding to the concentration of the aggregates in the experiment reported in the third column. Solutions of the labeled and unlabeled were made up by weight in pH 7.4 phosphate buffer.

These results for the adsorption of a mixture of monomer and aggregates (dimer) are shown graphically in Figure 10. In mixtures containing 10% aggregate, the adsorption of the monomer is shown in Figure 11. The maximum adsorbance here is lower than that shown in Figure 10 for the monomer alone indicating that the aggregates can compete effectively with the monomer.

In the second half of Table I, results of similar experiments are shown for the adsorption of HSA on silica at a solution concentration of 2 mg/ml for monomer and 0.2 mg/ml for aggregates. While the total adsorbance on this substrate is several times lower than that on polyethylene, the general behavior is quite similar to that observed for the adsorption on polyethylene and the same conclusions would apply. Further experiments are underway to understand and interpret these results more fully.

Adsorption Isotherms of HSA ^{131}I Monomer and Dimer on Polyethylene

Figures 12-16 show the adsorbance, from different concentrations of HSA ^{131}I monomer on polyethylene, with readings taken at 0.5, 1, and 3 hour intervals. Figure 17 is the adsorption isotherm of the monomer, resulting from the average values of the data presented in Figures 12-16. Similarly, Figures 18-21 represent the rate of adsorption of the HSA ^{131}I dimer on polyethylene and Figure 22 is the adsorption isotherm for the dimer. As shown in Figures 17 and 22, there seems to be no significant difference in the total adsorbance between the monomer and dimer.

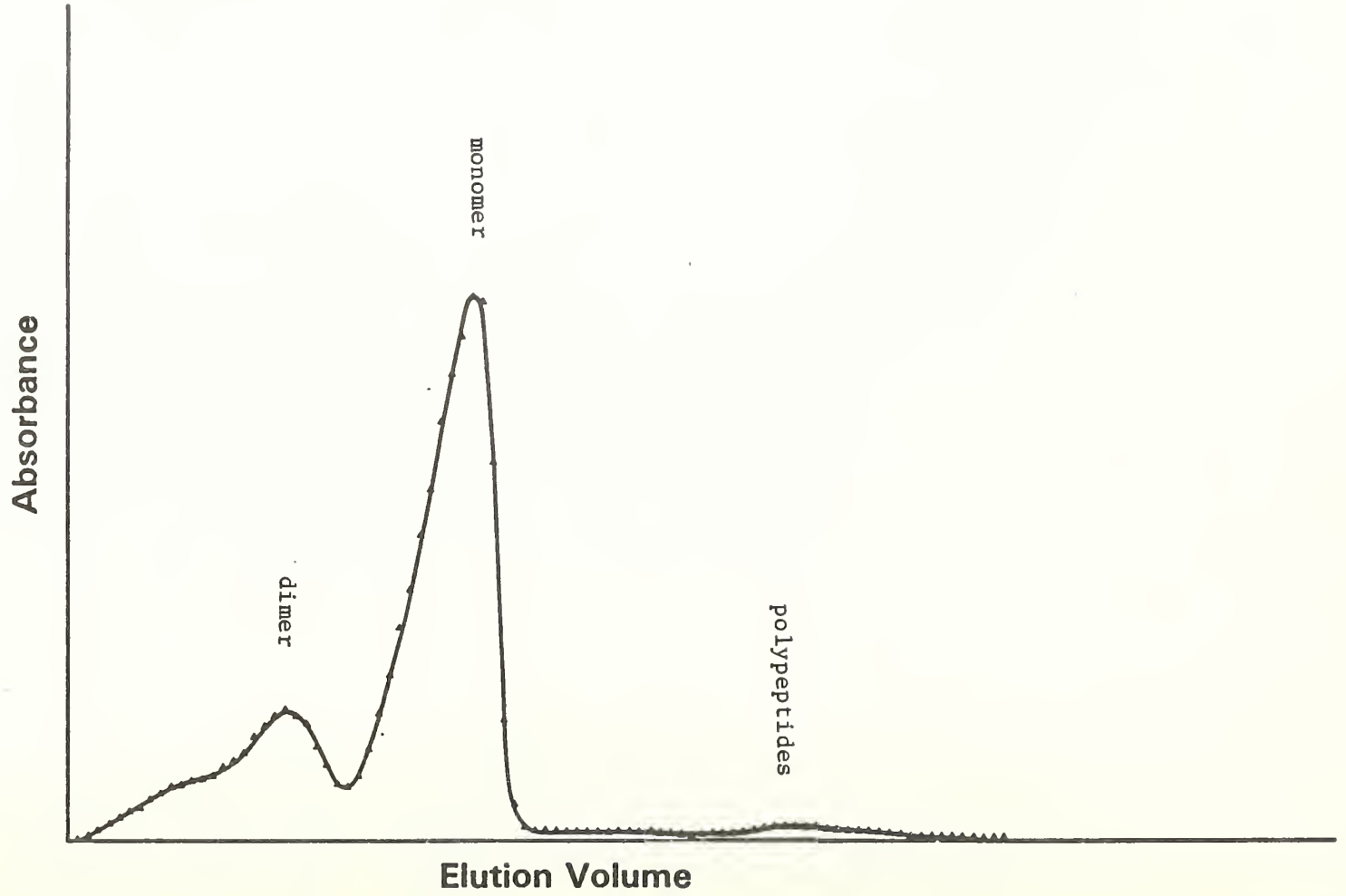

Figure 9: Gel Permeation Chromatogram of Unlabeled HSA

TABLE I

ADSORBANCE OF SELECTED FRACTIONS OF ^{125}I LABELED AND UNLABELED HSA ON POLYETHYLENE AND SILICA

POLYETHYLENE

Time of ADS. Hours	Fractionated Labeled Monomer mg/m^2	Fractionated Labeled Monomer and Fractionated Un-Labeled Monomer mg/m^2	Fractionated Labeled Monomer and Un-Fractionated Un-Labeled Monomer mg/m^2	Fractionated Aggregates mg/m^2	
.5	9.40	8.66	10.28	24.91	8.98
1.0	9.86	10.05	11.69	27.89	10.23
3.0	15.53	15.38	10.96	26.76	9.84

SILICA

.5		4.23		7.66	4.37
1.0		3.91		7.38	3.73
3.0		3.95		7.47	4.04

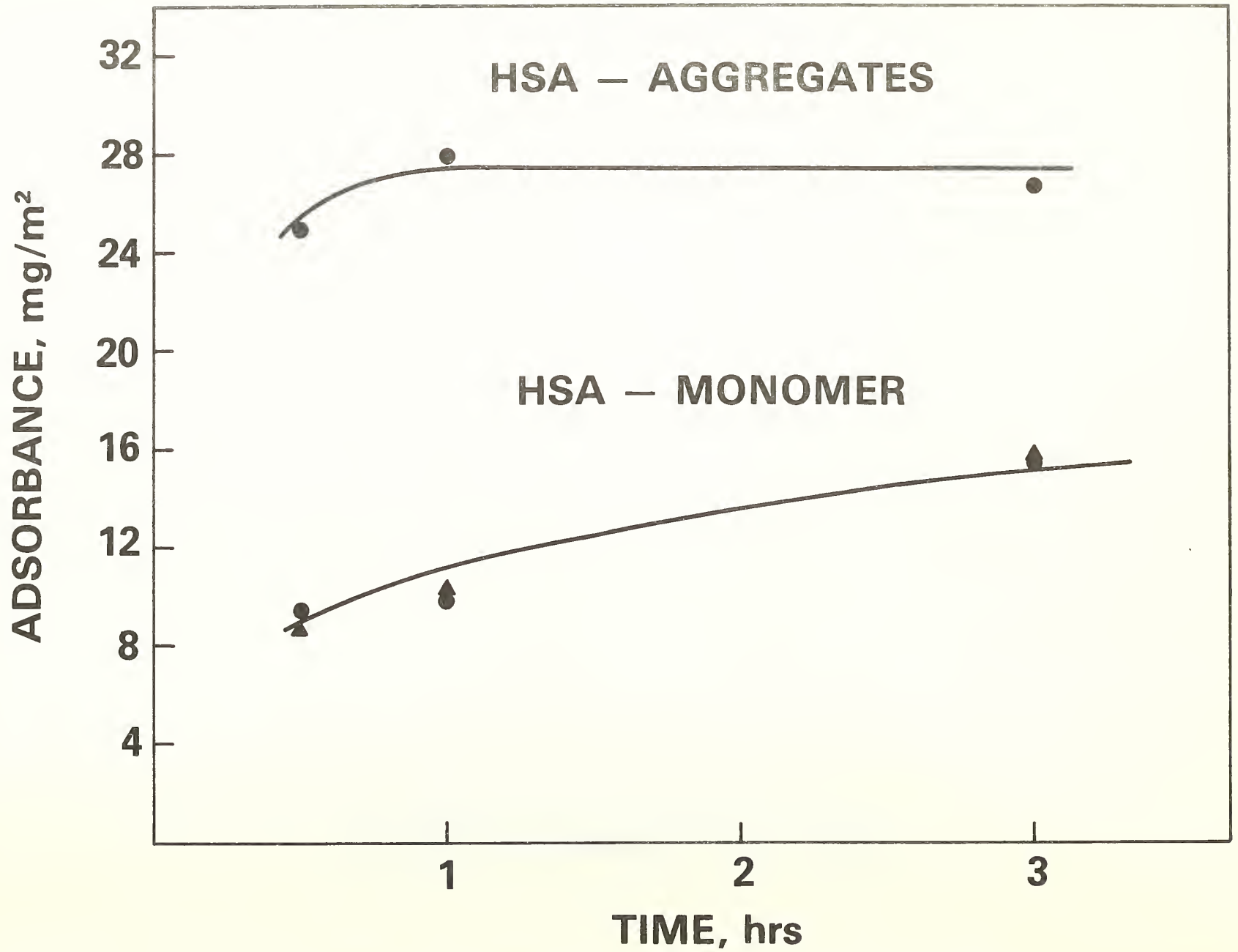

Figure 10: Adsorption of ^{125}I HSA Fractions on Polyethylene.

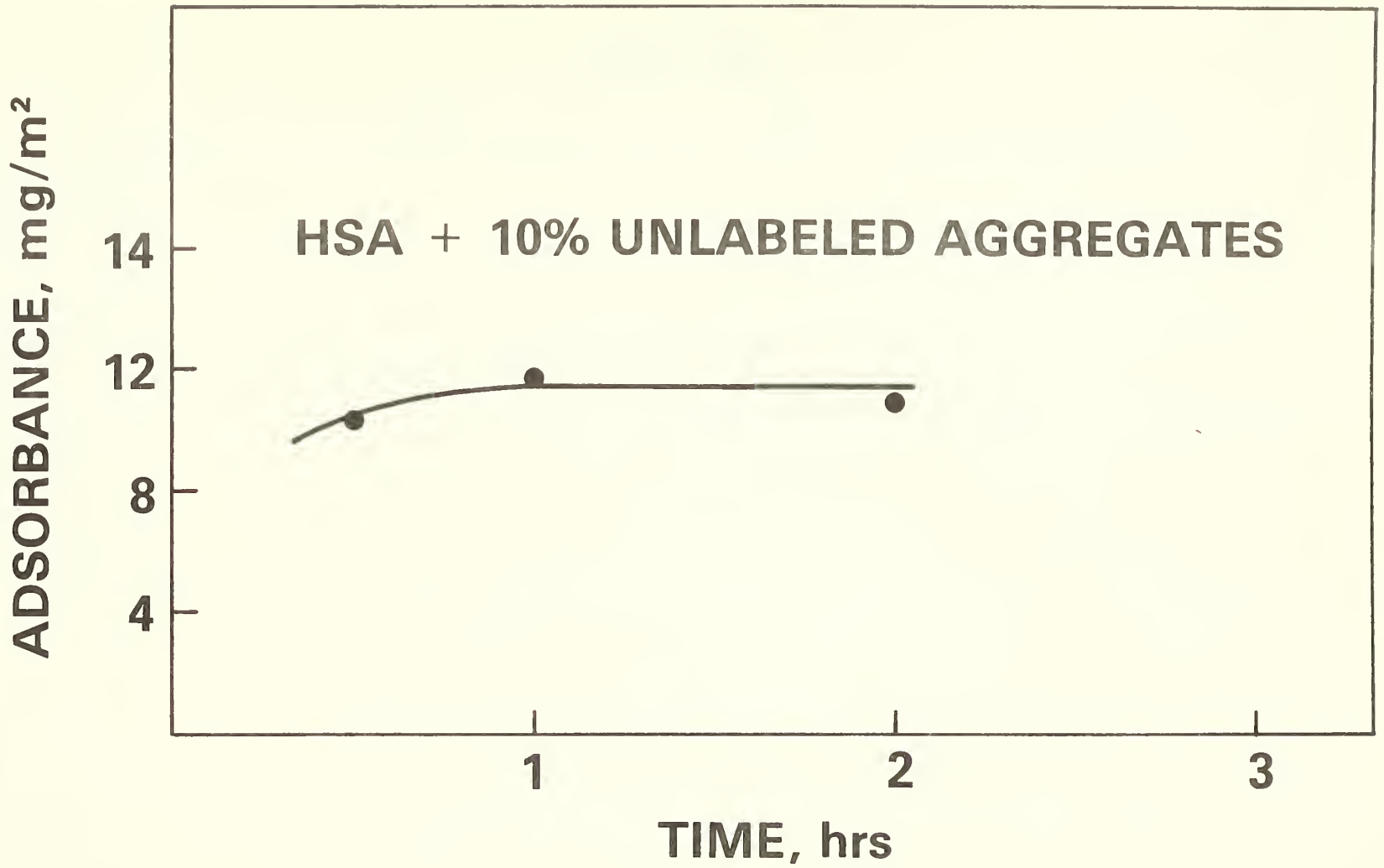

Figure 11: Adsorption of ^{125}I HSA Monomer on Polyethylene in Presence of Unlabeled Aggregates.

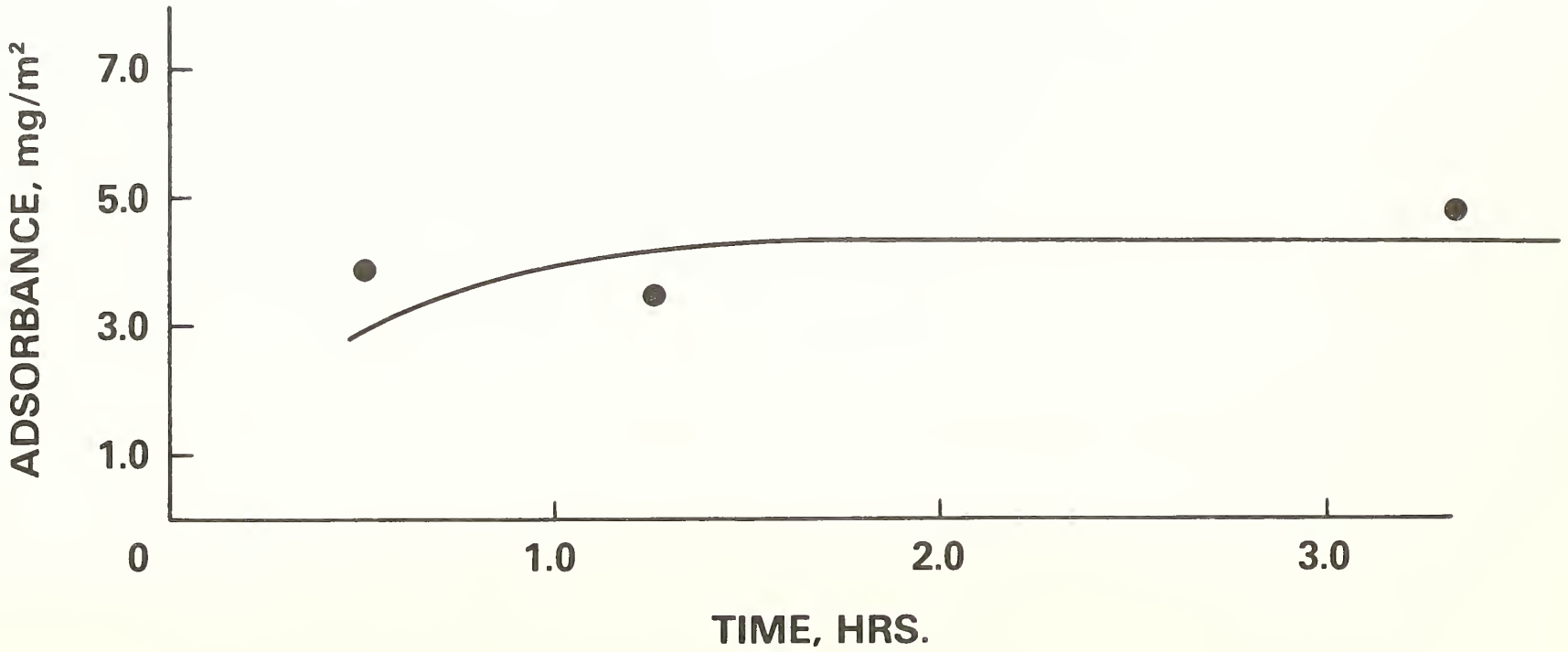

Figure 12: Adsorption of the Monomer of ^{131}I HSA on Polyethylene. Solution concentration 0.309 mg/ml.

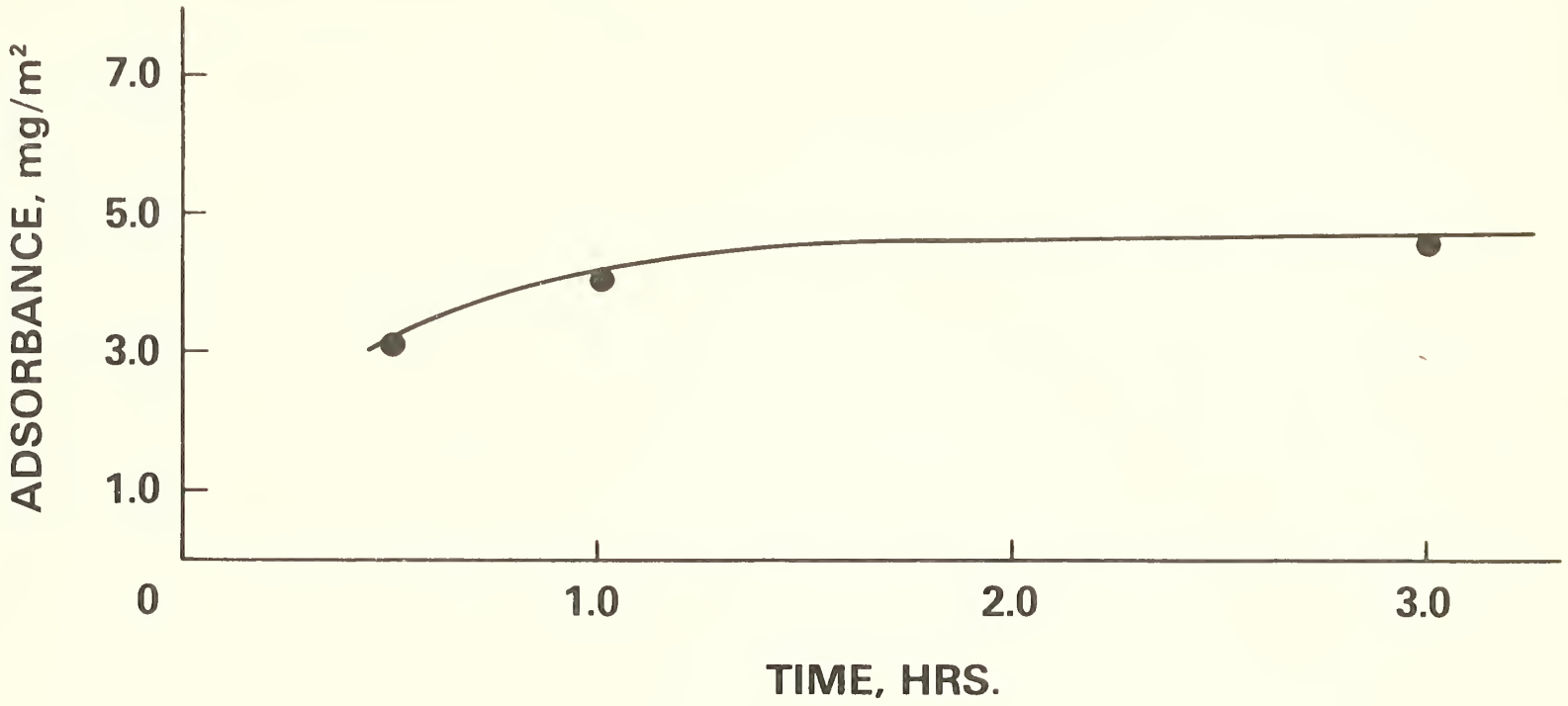

Figure 13: Adsorption of the Monomer of ^{131}I-HSA on Polyethylene. Solution concentration 0.50 mg/ml.

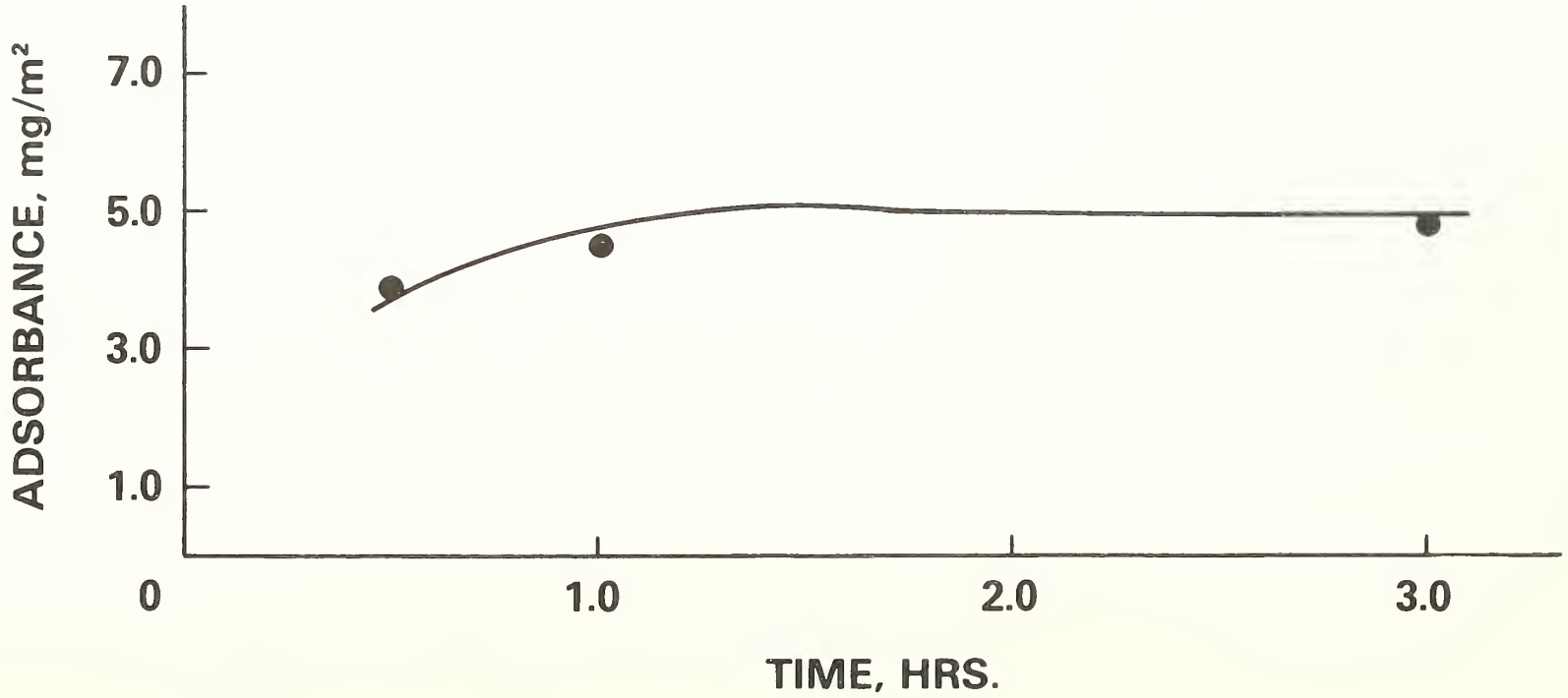

Figure 14: Adsorption of the Monomer of ^{131}I HSA on Polyethylene. Solution concentration 1.30 mg/ml.

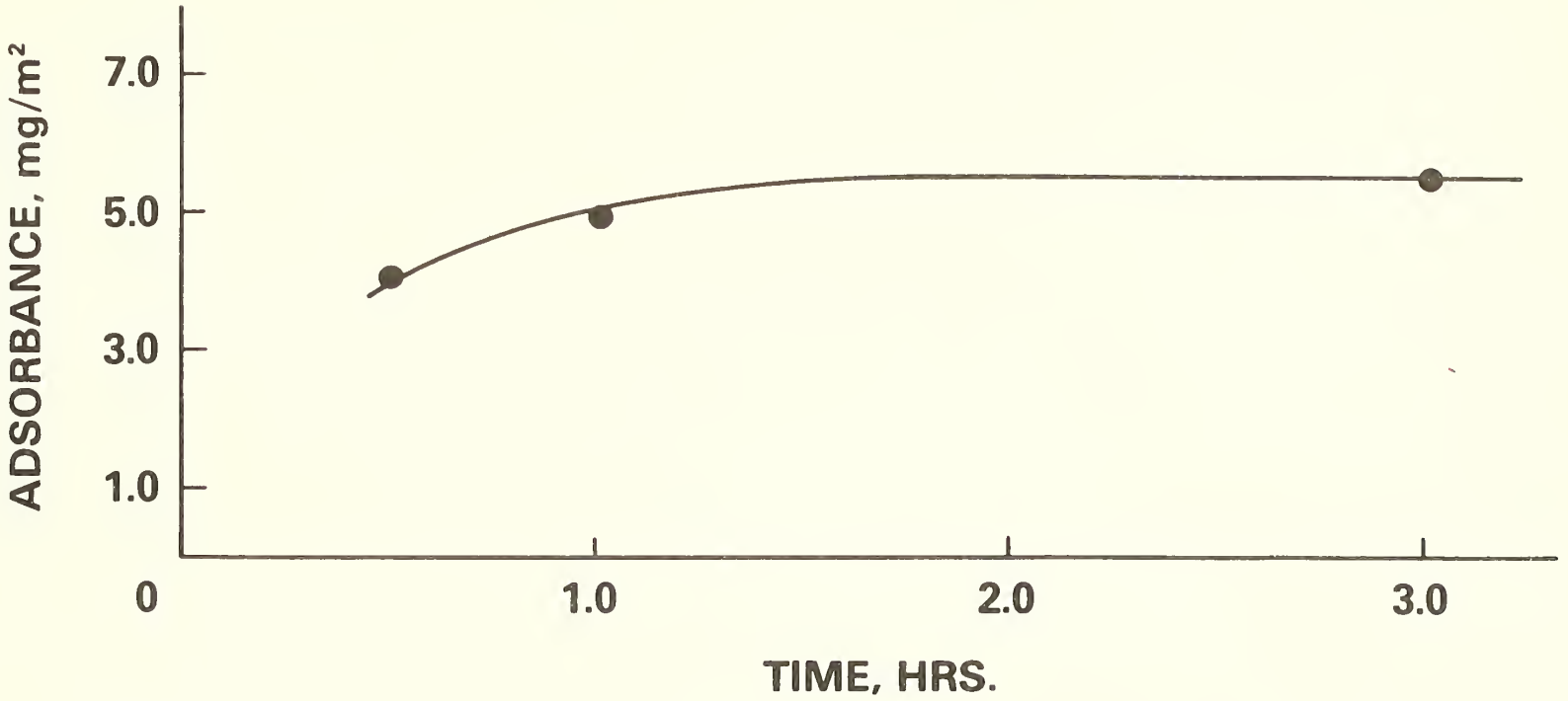

Figure 15: Adsorption of the Monomer of ^{131}I HSA on Polyethylene. Solution concentration 2.23 mg/ml.

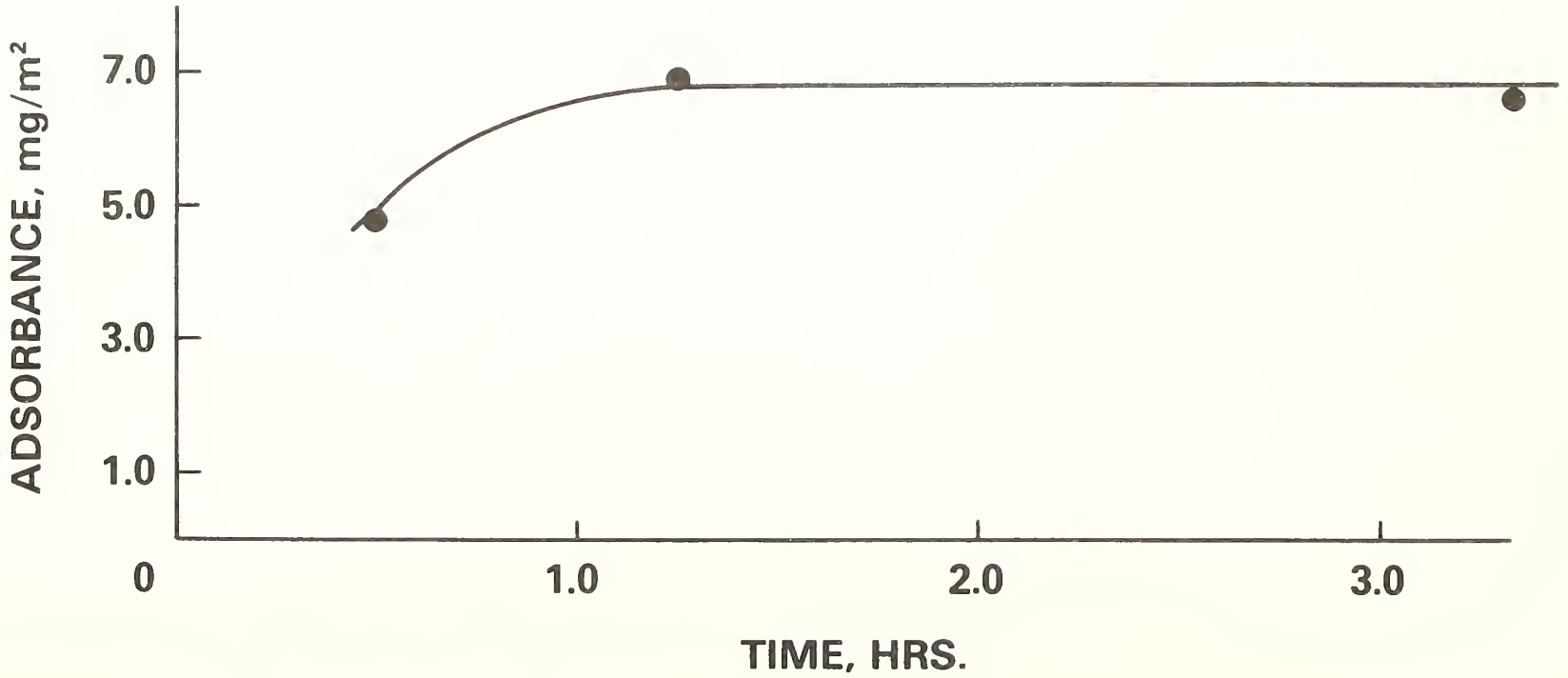

Figure 16: Adsorption of the Monomer of ^{131}I HSA on Polyethylene. Solution concentration 3.40 mg/ml.

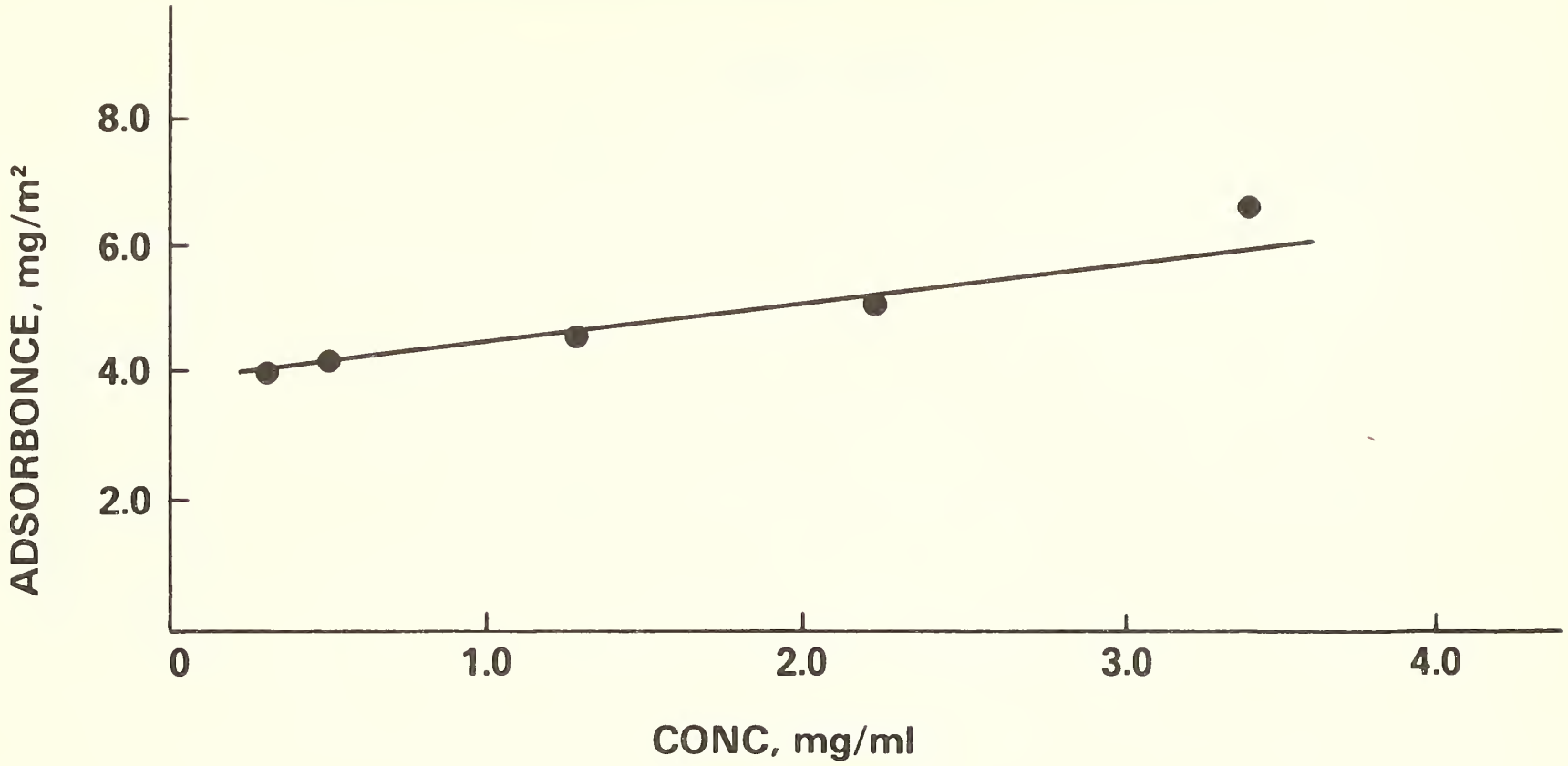

Figure 17: Adsorption Isotherm for the Monomer of ^{131}I HSA on Polyethylene.

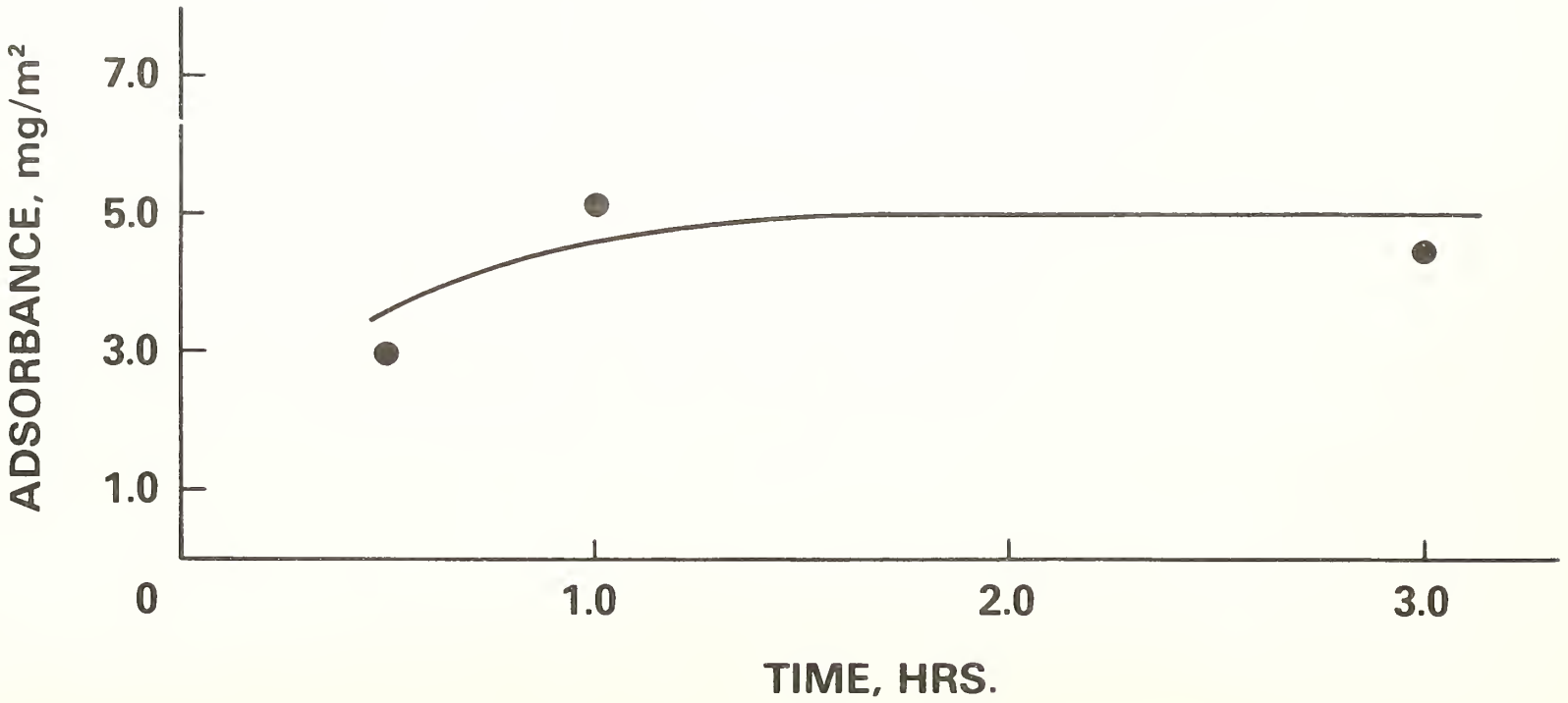

Figure 18: Adsorption of the Dimer of ^{131}I HSA on Polyethylene. Solution concentration 0.36 mg/ml.

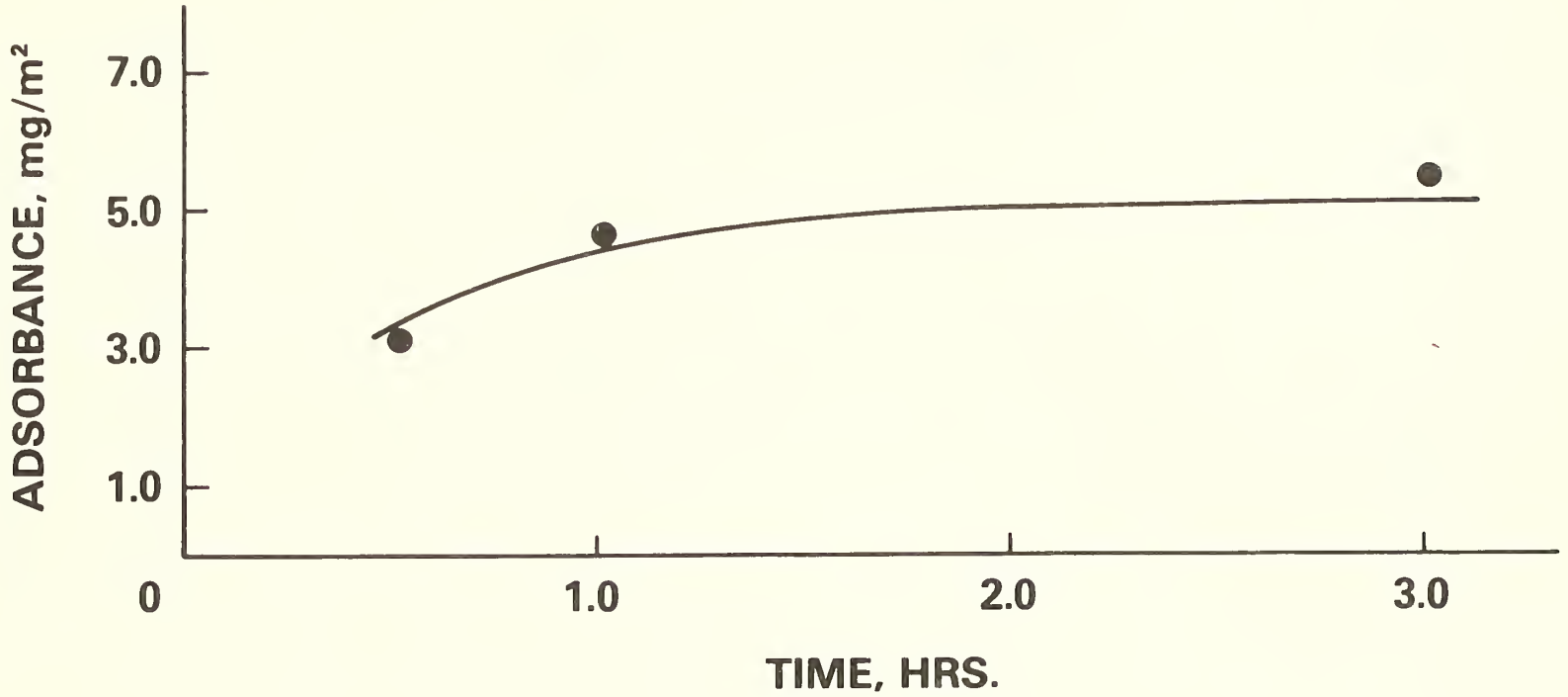

Figure 19: Adsorption of the Dimer of ^{131}I HSA on Polyethylene. Solution concentration 0.42 mg/ml.

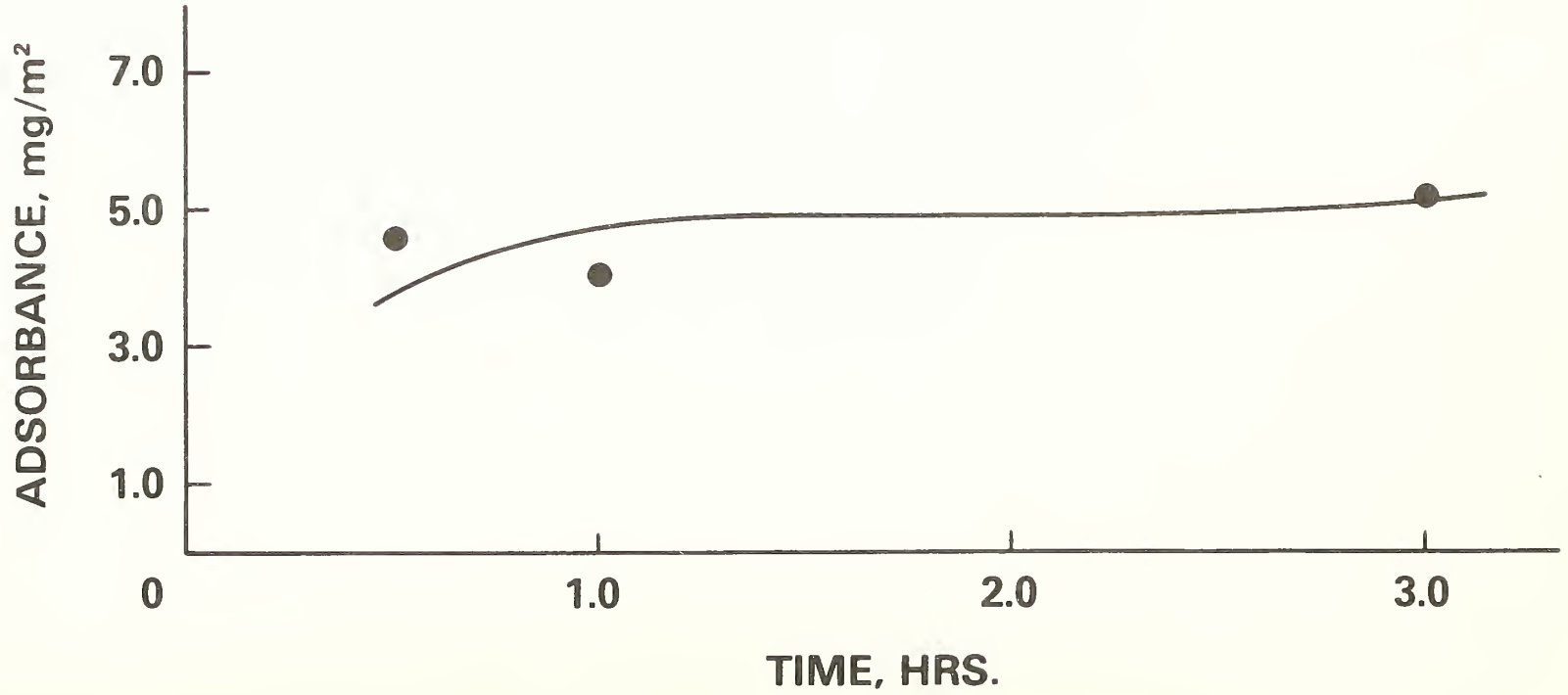

Figure 20: Adsorption of the Dimer of ^{131}I HSA on Polyethylene. Solution concentration 0.66 mg/ml.

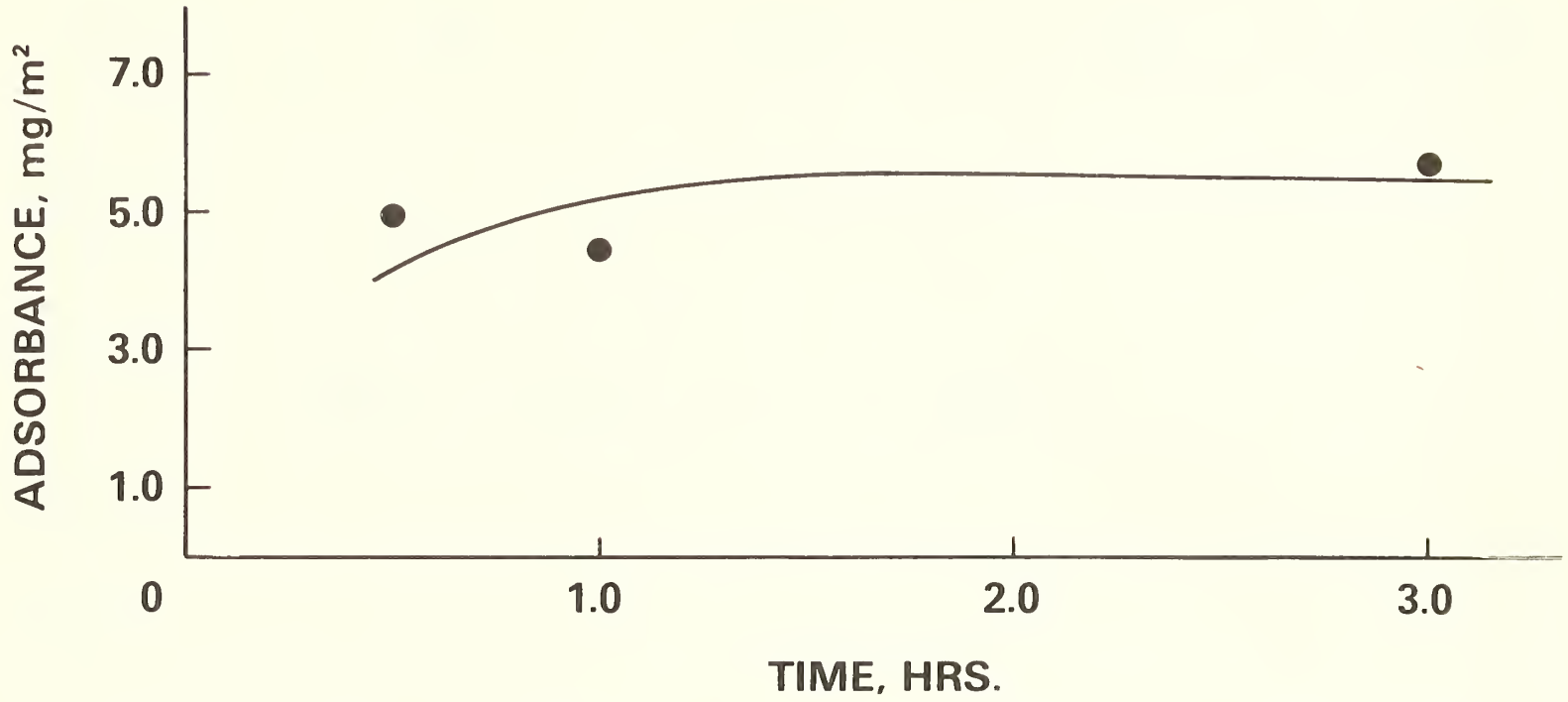

Figure 21: Adsorption of the Dimer of ^{131}I HSA on Polyethylene. Solution concentration 1.30 mg/ml.

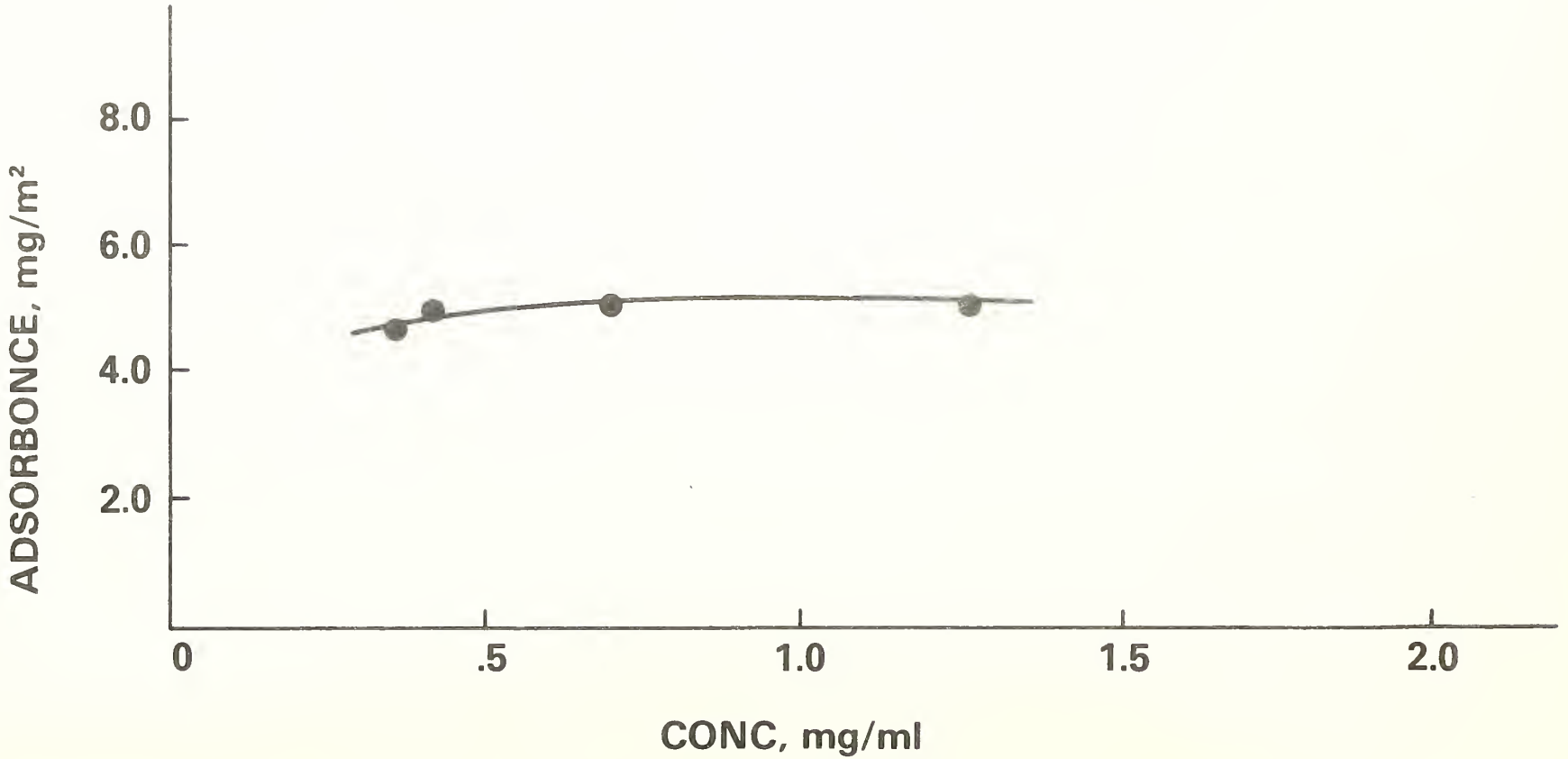

Figure 22: Adsorption Isotherm for the Dimer of ^{131}I HSA on Polyethylene.

FIBRINOGEN ADSORPTION STUDIED BY AUTOMATIC ELLIPSOMETRY

In our Annual Report for 1975, we reported the results of several adsorption experiments of proteins on platinum, using the automatic recording ellipsometer. We noted that one of the protein solutions studied, namely 0.42 mg fibrinogen/ml, showed a large increase in the film thickness (molecular extension) with time. We have recently repeated this experiment and found the initial thickness of the adsorbed film to be much greater than that found in the previous experiment. The extension also remained approximately constant over a time period from two seconds to about two hours after the initial contact between the solution and the substrate. The adsorbance increased smoothly and monotonically from 3 to 5 mg/m^2 during the first minute of observation, and from 5 to 7 mg/m^2 during the next two hours.

Subsequent adsorption experiments were performed from more dilute solutions (0.043 mg/ml). In one case, the calculated thickness decreased within the first two minutes. The two hour (equilibrium) value was about 40 nm, which is comparable to values obtained from the more concentrated solution. The initial adsorbance was lower than that from the more concentrated solution but increased by more than 100% during the first two minutes and by another factor of two during the next two hours. The final adsorbance value was also comparable to that obtained from the more concentrated solution. A second experiment performed at this dilute concentration proceeded somewhat differently during the first two minutes, but both the film thickness and adsorbance curves closely paralleled those of the other experiment during the rest of the 2 hour run. For the first 2 minutes, the calculated film thickness remained constant at about 50 nm, instead of apparently decreasing from 120 to 50 nm as in the earlier experiment. Another adsorption experiment using a very dilute solution of fibrinogen (0.003 mg/ml) onto a silicon oxide surface proceeded sufficiently slowly that we could observe all of the adsorption process with the automatic instrument, beginning about two seconds from the initial contact of the solution with the surface. The results of this experiment did not indicate a change in conformation (molecular extension) of the adsorbed protein as the surface concentration increased from 0.05 to 0.75 mg/m^2. However, other protein-substrate combinations may well produce different results.

All of these results are highly tentative as the reproducibility of the mixing techniques and the accuracy of the instrument have yet to be determined, but it is clear that the adsorbance and molecular extension can be observed and followed, beginning about two seconds from the first contact of the protein solution with the substrate. We believe that the instrument is capable of providing valuable information about the rate of adsorption and conformational changes of adsorbed proteins during the first critical few minutes after the exposure of a foreign surface to a protein solution.

ADSORBED PROTEIN CONFORMATION BY LIGHT SCATTERING

Quasielastic light scattering techniques have been applied *in situ* to study the conformation of γ-globulin adsorbed on polystyrene latices. In collaboration with Dr. Charles Han of the Polymers Division, we have measured the frequency distribution of scattered light to determine the radius of bare and protein coated latex particles. Using the basic light scattering equation

$$\Delta\nu_{1/2} = (q^2/\pi)D \qquad (1)$$

where $\Delta\nu_{1/2}$ is the half width of the scattered frequency distribution, q the scattering vector given by

$$q = \frac{4\pi n}{\lambda o} \sin\frac{\theta}{2} \qquad (2)$$

where n is the index of refraction, λo the wavelength of light and θ the scattering angle, one can determine D, the translational diffusion coefficient. Combining the Einstein equation for the diffusion coefficient

$$D = kT/f \qquad (3)$$

where k is Boltzmann's constant, T the temperature, and f the frictional coefficient, with the Stoke's equation describing the hydrodynamic properties of a sphere

$$f = 6\pi\eta R \qquad (4)$$

where η is the viscosity and R the radius of the sphere, one obtains an equation

$$R = \frac{kT}{6\pi\eta}\ \frac{1}{D} \qquad (5)$$

which can be used to calculate the radius of a particle from its measured translational diffusion coefficient..

As shown in Figure 23 for the bare polystyrene latex, a plot of HWD($\Delta\nu_{1/2}$) vs q^2/π results in a linear relation which corresponds to a particle radius of 44 nm. The adsorption of human γ-globulin at 1.04 mg/ml and 0.004 mg/ml concentration on the latex has been measured. At the higher concentration where the surface is saturated, the increased particle radius calculated from

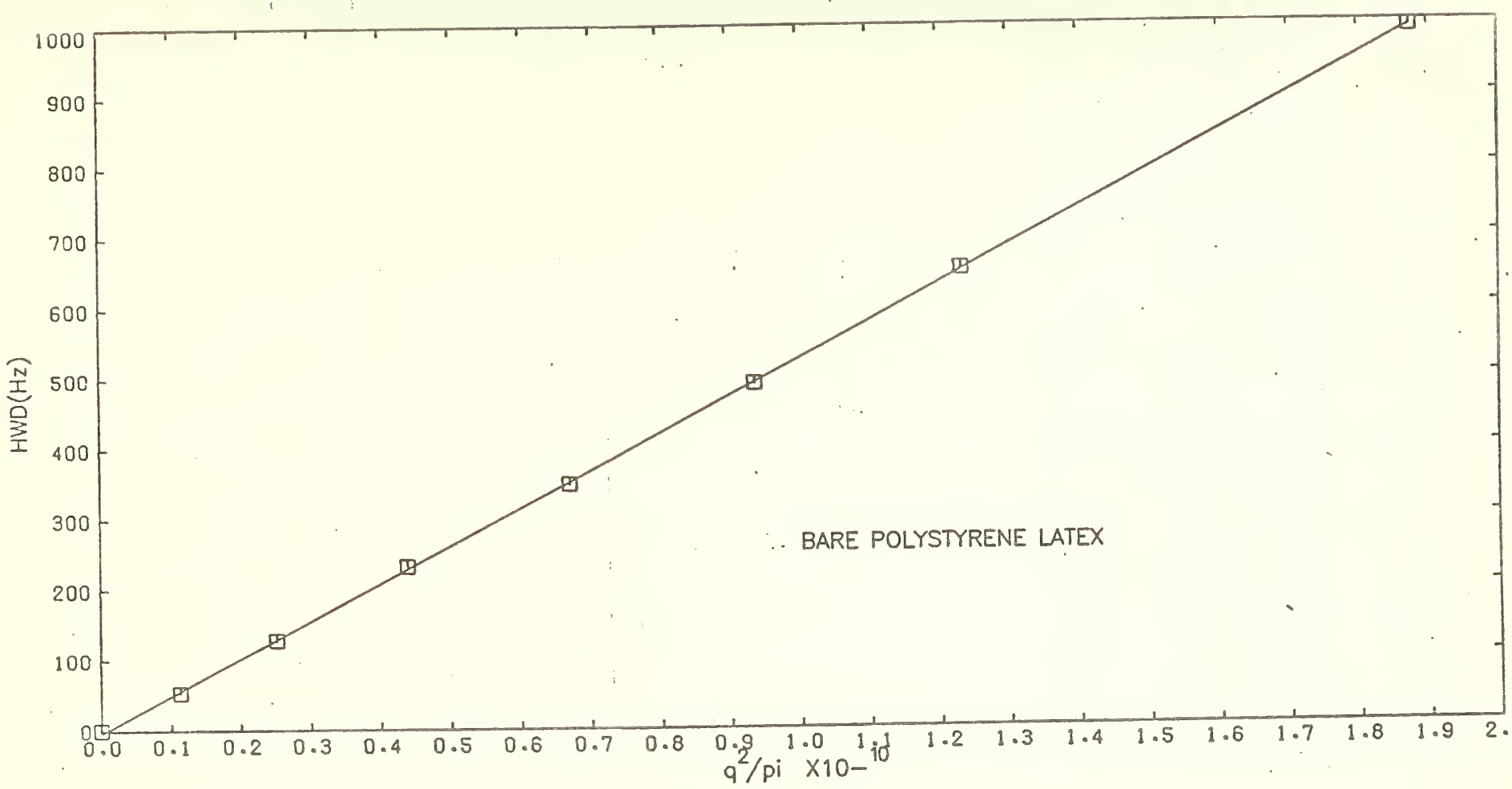

Figure 23: Width at half height (HWD) of Scattered Frequency Spectrum vs. q^2/Pi (q = scattering wave vector) for Surfactant Free Polystyrene Latex.

equation (5) is 20 nm, very similar to the ellipsometric extension of γ-globulin adsorbed on polyethylene when a correction for the distribution of protein segments is made. At the lower concentration where we estimate about 20% coverage, the increased radius is 15 nm as illustrated in Figure 24.

In order to determine if the observed difference in extension represents a change in conformation (which would be consistent with our bound fraction and ellipsometric measurements), further analysis of the hydrodynamic properties of partially coated spheres is required. The hydrodynamic measurements in this case result in an averaging of molecules and "holes" and an underestimation of the extension. Recent analytical solutions to this problem are being utilized to assess the magnitude of the effect, and it should be possible to determine if the measured differences represent conformational changes.

The technique of quasielastic light scattering is applicable to all materials for which uniform particulate suspensions can be prepared. It has great precision and can provide additional measures of adsorbed protein conformations.

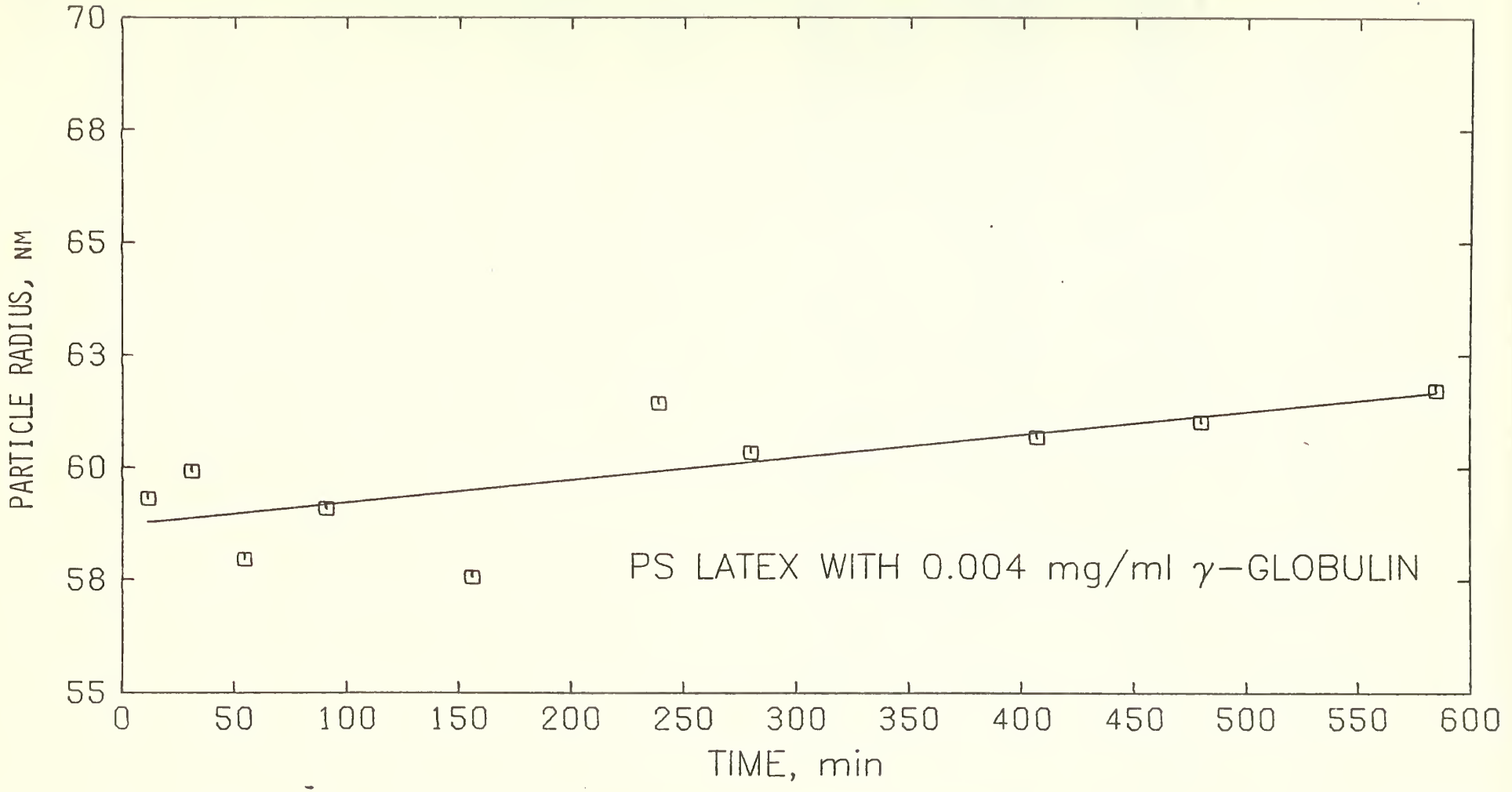

Figure 24: Radius of Polystyrene Particle with Adsorbed Human γ-Globulin vs. Time.

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4. Stromberg, R. R., Morrissey, B. W., Smith, L. E., Grant, W. H., and Fenstermaker, C. A., "Interaction of Blood Proteins with Solid Surfaces," PB 241 267/4SL (Available from the National Technical Information Service), Annual Report prepared for the Biomaterials Program, National Heart and Lung Institute, NIH, Bethesda, Md., January 1975.

5. Stromberg, R. R., Morrissey, B. W., Smith, L. E., Grant, W. H., and Dehl, R. E., "Interaction of Blood Proteins with Solid Surfaces," NBSIR 76-1017, Annual Report prepared for the Biomaterials Program, National Heart and Lung Institute, NIH, Bethesda, Md., March 1976.

APPENDIX

PUBLICATIONS

1. L. E. Smith, R. R. Stromberg, C. A. Fenstermaker and W. H. Grant, "Conformation of Adsorbed Blood Proteins: Extension and Rate Studies," Colloid and Surface Chemistry Abstracts, Chicago Meeting (1973).

2. B. W. Morrissey and R. R. Stromberg, "Conformation of Adsorbed Blood Proteins," ACS Organic Coatings and Plastics Preprint, 33 (2), 333 (1973).

3. B. W. Morrissey, L. E. Smith, C. A. Fenstermaker, R. R. Stromberg and W. H. Grant, "Conformation of Adsorbed Blood Proteins," Proc. of Symposium of AAMI, New Orleans, La (1974) NBS Spec. Publ. 415.

4. C. A. Fenstermaker, W. H. Grant, B. W. Morrissey, L. E. Smith and R. R. Stromberg, "Interaction of Plasma Proteins with Surfaces," PB 232 629/6 (Available from the National Technical Information Service), Annual Report prepared for the Biomaterials Program, National Heart and Lung Institute, NIH, Bethesda, Md., March 1974.

5. B. W. Morrissey and R. R. Stromberg, "The Conformation of Adsorbed Blood Proteins by Infrared Bound Fraction Measurements," J. Colloid and Interface Sci., 46, 1 (1974).

6. R. R. Stromberg, B. W. Morrissey, L. E. Smith, W. H. Grant and C. A. Fenstermaker, "Interaction of Blood Proteins with Solid Surfaces," PB 241 267/4SL (Available from the National Technical Information Service), Annual Report prepared for the Biomaterials Program, National Heart and Lung Institute, NIH, Bethesda, Md., January 1975.

7. B. W. Morrissey, L. E. Smith, R. R. Stromberg, C. A. Fenstermaker, "Ellipsometric Investigation of the Effect of Potential on Blood Protein Conformation and Adsorbance," J. Colloid and Interface Sci. (in press).

8. B. W. Morrissey, C. A. Fenstermaker, "Conformation of Adsorbed γ-Globulin and β-Lactoglobulin. Effect of Surface Concentration," Trans. Amer. Soc. Artif. Int. Organs (in press).

9. W. H. Grant, L. E. Smith, R. R. Stromberg, "Radiotracer Techniques for Protein Adsorption Measurements," J. Biomedical Materials Res. (in press).

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11. B. W. Morrissey, "The Adsorption and Conformation of Plasma Proteins-Physical Approach," Annals of the New York Academy of Sciences (in press).

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