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DIFFUSIONAL EFFECTS DURING ALBUMIN ADSORPTION ON HIGHLY SWOLLEN POLY(VINYL ALCOHOL) HYDROGELS

DONALD R. MILLER* and NIKOLAOS A. PEPPAS†
School of Chemical Engineering, Purdue University, West Lafayette, IN 47907, U.S.A.

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Abstract—^{125}I-labelled albumin adsorption studies on poly(vinyl alcohol) hydrogels are presented. A thorough analysis is presented of the diffusional problems associated with iodide and albumin transport in the hydrogels during adsorption and the methods that can be used to correct for this phenomenon.

INTRODUCTION

Excellent reviews by Baier [1], Leonard and Butrille [2] and Horbett [3, 4] have discussed the adsorption of proteins on hydrogel surfaces and the possible factors affecting protein adsorption and conformation. Correlations have been obtained [5, 6] between several macroscopic properties and blood compatibility but this type of approach has not been satisfactory for all types of materials.

Rapid evaluation of a candidate material's potential "blood-compatibility" can be obtained *in vitro* using several methods. One such method measures the degree of retention of thrombocytes. An alternative method consists of determination of the level of blood protein (mono or competitive) adsorption. It has been shown qualitatively that high albumin adsorption levels (relative to other materials) indicate comparatively improved blood compatibility.

This communication discusses the problems encountered with ^{125}I-labelled albumin adsorption into hydrogels. The hydrogel employed was a lightly crosslinked poly(vinyl alcohol) (PVA) network.

EXPERIMENTAL

Preparation of polymer

Vinyl acetate (VAc; Aldrich Chemical Co., Milwaukee, Wis.) was purified by atmospheric distillation at 64°. The first and last 25 ml of each distillation were discarded to maintain purity. To remove dissolved oxygen, VAc was degassed in separate round-bottom flasks at 10 mmHg total pressure. Polymerization was initiated by using γ-rays (^{60}Co unit) at a constant temperature of 35°. For approx. 10–15% conversion of VAc, the irradiation dose employed was 0.102 Mrad. The VAc sample autoaccelerated with higher degrees of conversion requiring a low irradiation dose. The irradiation dose rate was 5591 rad/min. The excess VAc monomer was removed from the irradiated solution by distillation with methanol (spectroscopic grade; Aldrich Chemical Co., Milwaukee, Wis.) at 64°.

The PVA homopolymer was synthesized by dissolving pure PVAc in methanol to produce a 2.5 wt% polymer solution. The solution was made approx. 0.01 M in NaOH by adding an aq. 1.0 M NaOH solution, mixed briefly, and allowed to react with agitation. Solid PVA rapidly precipitated from the solution and the reaction was terminated after 30 min reaction time. The degree of hydrolysis was higher than 99.3%.

PVA networks were synthesized by irradiating 5 wt% aq. solutions under N_2 at 35° in glass Petri dishes. Equilibrium swollen hydrogels were obtained by soaking the samples in deionized water at 35° for several days. The volume swelling ratio was evaluated using the buoyancy principle [7] in cyclohexane before (swollen state) and after drying.

Radioisotope labelling/diffusion studies

Radio-iodination of albumin was performed according to the methods of McFarlane [8, 9] and Helmkamp *et al.* [10]. A quantity of 60 mg albumin (bovine albumin, crystallized; Miles Chemical Co., Elkhart, Ind.; used as received) was dissolved in 3 ml of pH = 10.3 glycine buffer (Sigma Chemical Co., St Louis, Mo.), and 2 ml filtered through a 0.45 μm Millipore filter and placed in a new test tube. The ^{125}I was obtained as Na^{125}I in 0.1 N NaOH (New England Nuclear, Boston, Mass). With a syringe, 0.5 ml of fresh glycine buffer was added to the Na^{125}I vial, removed and shaken for approx. 30 sec. Iodine monochloride (0.00588 M ICl, 2 N NaCl, 0.1 N HCl) was used to exchange the iodine with the radioactive iodide ion. Within 10 sec, 0.5 ml of the ICl solution was added to the ^{125}I vial and this was mixed with the 2 ml of filtered albumin in glycine buffer; disproportionation of the hypoiodide produced HI and HIO_3, which drastically reduced the labelling efficiency.

Labelling efficiency was determined by taking 10 μl of the labelled solution and diluting it to 10 ml in a volumetric flask with buffer (0.01 M NaH_2PO_4 and 0.14 N NaCl, pH = 7.4). Five 100 μl samples of this solution were placed in separate centrifuge tubes, 1.0 ml of 10 vol% trichloroacetic acid added and 0.02 ml of 6 wt% albumin solution added to each tube and shaken. The tubes were centrifuged for 30 min at 3000 rpm and 4° to precipitate the albumin. Five 500 μl samples of the supernatant of each tube were placed in counting vials. The counts per minute (cpm) of the five samples were determined and averaged (C_2). The counts per minute of 0.1 ml of the 10 ml labelled albumin solution (C_1) were evaluated to obtain the initial count rate before albumin precipitation. The labelling efficiency was calculated using equation (1):

$$\text{efficiency} = 1 - 2.24\frac{C_2}{C_1}. \quad (1)$$

The free iodide was removed from the labelled albumin mixture by dialysis in buffer. Dialysis was continued for approx. 48 hr with fresh buffer added every 5–6 hr. The final

*Present address: Medical Research Division, Lederle Laboratories, Pearl River, NY 10965, U.S.A.
†To whom correspondence should be addressed.

percentage of iodide attached to albumin was evaluated as discussed previously before dialysis was terminated.

The integrity of the ^{125}I-albumin was evaluated by protein electrophoresis using Sepraphore® III cellullose acetate membranes as the substate. Visualization was done with Ponceau S stain.

Preferential adsorption of labelled albumin relative to native albumin was studied with adsorption onto a glass surface. The adsorption was done at 37° without agitation with three different labelled albumin concentrations while the total albumin concentration remained constant, with the adsorption terminated after 2 hr. To avoid artifacts from the denatured protein layer at air/solution interfaces, the glass surfaces were covered with pure buffer and an equal amount of protein solution (twice as concentrated as desired for the final concentration) was added and briefly mixed. Adsorption was terminated by a dilution rinse where fresh buffer was passed through the adsorption cell at approx. 350 ml/min for 1 min. A sample of labelled protein solution, where the fraction of ^{125}I-labelled albumin was known, was counted to relate the cpm to the quantity of albumin on the sample surface.

The kinetics of ^{125}I-labelled albumin adsorption was measured on PVA networks formed by ^{60}Co-irradiation at 2.28 Mrad. The PVA samples were equilibrated in buffer one day before the adsorption studies. The hydrogels were cut into circular discs 1.0 cm dia and 2.0 mm thick. The samples were then placed in holders which exposed only one of the faces. Each sample holder was then placed in an adsorption cell which had a volume (excluding the sample holder) of 15 ml.

To start an adsorption test, a sample was suspended in 5 ml of buffer at 37° and 5 ml of 64 mg/ml albumin solution (0.544 mol% labelled albumin) was added and briefly mixed. The mixing was done for 10 sec using a Pasteur pipet. For the remaining adsorption time period, the solution was maintained in the quiescent state. The adsorption studies were terminated by the dilution method discussed above and the cpm of the solid sample evaluated in the γ-counter.

RESULTS AND DISCUSSION

The physical properties of PVA samples employed in these studies are presented in Table 1. Complete details of the determination of these hydrogel properties can be obtained from Ref. 11.

The dynamic adsorption of ^{125}I-labelled albumin onto PVA hydrogels was studied. The technique of labelling proteins for tracer studies is prone to artifacts due to alterations in protein properties (adsorption, diffusion etc.) by the label or labelling reaction [12]. Two experiments were done in this study to evaluate possible radioactive label effects on albumin properties, *viz.* electrophoretic mobility and preferential adsorption. The goal of the first experiment was to examine the conformation of the labelled proteins; the electrophoretic mobility of the labelled material was compared to the native protein and was found to be unaltered.

The adsorption characteristics of the native and labelled albumin molecules were studied by equilibrium adsorption experiments onto glass. The fraction of the adsorption solution which was ^{125}I-labelled albumin was varied and the total uptake of protein on the glass surface was measured by the radioactivity present. The results of Table 2 shows some scatter but indicate that the amount of albumin adsorbed onto glass does not correlate with the tracer concentration. Thus, preferential adsorption of labelled or native albumin, which would result in respectively, an increase or decrease in the calculated adsorbed amounts, does not occur for the samples studied in this work.

Table 2. Preferential ^{125}I-labelled albumin adsorption on glass substrate

^{125}I-labelled albumin fraction	Total albumin (μg/cm^2)
0.007	6.7
0.010	9.9
0.020	9.8
0.050	4.5

The adsorption of albumin onto PVA hydrogels was studied as a function of adsorption time and the results are presented in Fig. 1. Blank samples were employed to calibrate the count rate with the amount of protein contained by the sample. Figure 1 shows the apparent specific adsorption of albumin onto the PVA hydrogel as a function of adsorption time.

The results in Fig. 1 do not represent the exact amount of protein on the hydrogel surface because of free iodide and protein diffusion. This problem is more important with PVA hydrogels than with other hydrogels reported before, because PVA hydrogels are highly swollen, and thus diffusional problems during adsorption become significant. In fact, Table 1 gives the characteristic mesh size of the networks studied. The radioisotope labelled proteins used for this study contained 98.4% of the solution counts per unit volume. The remaining fraction was the result of either free iodide or protein fragments containing ^{125}I. For quantitative analysis, the free solution counts were assumed to be the result of only free iodide. Due to the small size of the iodide and the high degree of swelling of the hydrogels employed, the free iodide resulted in substantial counts which produced an excess in the calculated protein content in the hydrogel. The iodide content of the hydrogel was estimated by 1-D diffusion calculations. The diffusion coefficient of the iodide in PVA membranes was estimated theoretically and experimentally. For a theoretical estimate, the infinite dilution diffusion coefficient was estimated using Einstein's equation:

$$D_\infty = \frac{kT}{6\pi\mu R_a} \tag{2}$$

where μ is the solvent viscosity and R_a is the solute

Table 1. Characterization of PVA hydrogels used in this study

Irradiation dose (Mrad)	Equil. degree of swelling, Q	Molecular weight between crosslinks, $\bar{M}_c$	Mesh size of network ξ (Å)
2.28	31.7	9800	275
6.00	18.5	7200	230
10.00	14.9	5600	190

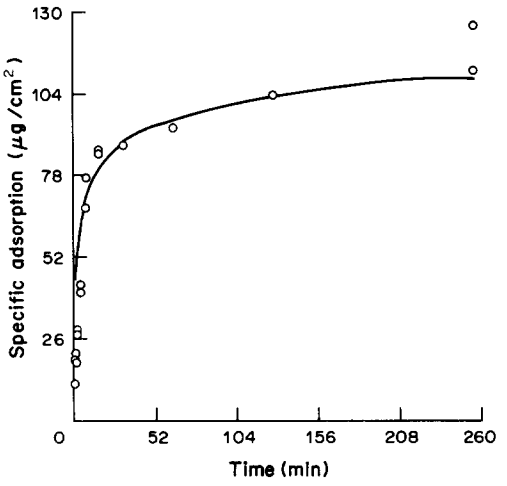

Fig. 1. Specific adsorption of albumin on PVA hydrogels (irradiated with 2.28 Mrad) as a function of adsorption time.

radius. For the diffusion studies in this work, the solvent employed was water with a viscosity at 37° of $\mu = 6.92 \times 10^{-3}$ g/cm sec. For a hydrated iodide anion with $R_a = 2.3$ Å, it was found that $D_\infty = 1.43 \times 10^{-5}$ cm²/sec.

The experimental estimation of D_∞ of iodide was done by monitoring the iodide concentration in solution as KI was released from a PVA hydrogel. The concentration profile in the PVA membrane was assumed to follow a finite Fickian release curve [13], presented in equation (3). This equation represents the concentration profile in half of a slab of thickness $2l$ where $l = 0$ is defined as the center of the slab:

$$C_i = C_{0i}\left[1 - \frac{4}{\pi}\sum_{n=0}^{\infty}\frac{(-1)^n}{2n+1} \times \exp(-D_1(2n+1)^2\pi^2 t/4l^2)\cos\left(\frac{(2n+1)\pi x}{2l}\right)\right]. \quad (3)$$

For the amount of iodide released by the hydrogel, the concentration profile given in equation (3) was employed and integrated over the sample length and multiplied by the sample area. The result is the total amount of iodide diffused from the sample as a function of time:

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2} \times \exp(-D_1(2n+1)^2\pi^2 t/rl^2). \quad (4)$$

The fractional release curve for I^-, M_t/M_∞, from a PVA hydrogel is presented in Fig. 2. The solid line in Fig. 2 is the nonlinear estimation of D_1 using equation (4). This estimation was done on a computer employing a nonlinear parameter estimation routine based on a modified Marquart method. The theoretical curve is slightly below the experimental curve at low times and above for larger release times. This was due to swelling alterations of the hydrogels with removal of the electrolyte (volume swelling ratio of the PVA hydrogel swollen in KI solution was approx. 3.6% higher than for membranes swollen in pure water). The value for the iodide diffusion coefficient obtained by analysis of the release data was $D_1 = 1.51 \times 10^{-5}$ cm²/sec. This compares very well with the value estimated employing equation (2).

The diffusion of iodide into the hydrogels during ^{125}I-labelled albumin adsorption was modelled using the experimentally determined iodide diffusion coefficient and equation (4). This equation can also be employed for albumin diffusion in the hydrogel by substituting D_a for D_1. The diffusion coefficient of albumin in the PVA sample [14] was initially assumed to be $D_a = 3.0 \times 10^{-8}$ cm²/sec.

The iodide content of the hydrogel can be looked at as a specific quantity of equivalent albumin (using the specific activity, cpm/mg labelled albumin and isotope label fraction) which is subtracted from the total amount of albumin adsorbed. When the equivalent albumin from free iodide is substrated from the results in Fig. 1, the actual specific adsorption values for albumin onto PVA are obtained which are presented in Fig. 3. The magnitude of adsorption was slightly diminished but the general shape and magnitude of the curves was not altered.

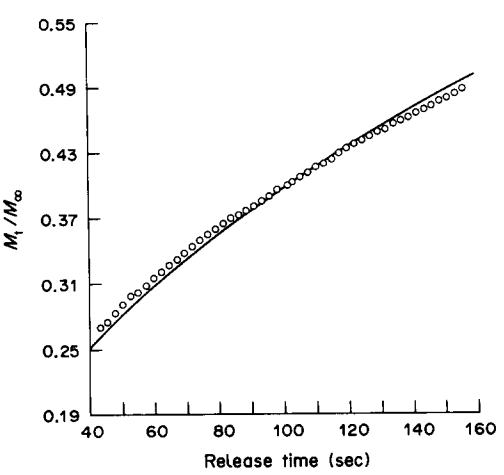

Fig. 2. Comparison of fractional release of iodide from PVA hydrogels (irradiated with 2.28 Mrad) as a function of time with the theoretical curve using $D_1 = 1.43 \times 10^{-5}$ cm²/sec.

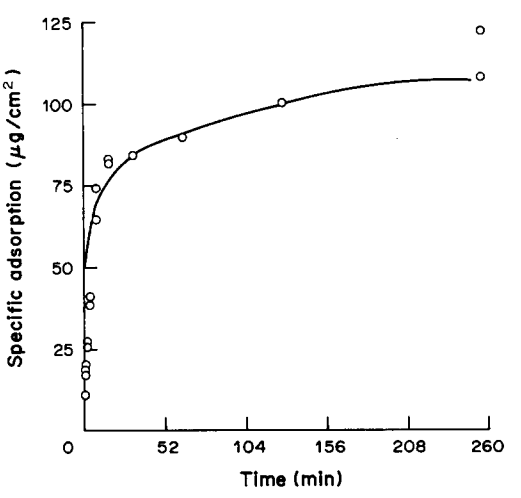

Fig. 3. Specific adsorption of albumin on PVA hydrogels (irradiated with 2.28 Mrad) as a function of time after free iodine correction.

The adsorption phenomenon on these hydrogels can be explained by four separate events: diffusion of albumin to the hydrogel/solution interface; adsorption into the interface; growth of a gel layer at the interface; and diffusion of the protein into the hydrogel bulk decreasing the gel layer thickness. The diffusion coefficient for albumin in solution is so much higher than the value in the hydrogel that the rate of solution diffusion can be considered inifinite. With this assumption, the gel layer thickness (specific adsorption) was determined by evaluating the difference between the experimentally obtained albumin concentration (Fig. 3) and the calculated albumin diffusion level. Results were considered consistent if the gel layer level was zero or positive. The rate of adsorption was simply the difference between the experimental albumin adsorption rate (this is total rate onto the hydrogel and diffusion into the polymer) and the albumin diffusion rate into the hydrogel.

The effect of albumin diffusion into the hydrogel was to decrease the surface layer. For modelling purposes, equation (4) was used with the albumin diffusion coefficient obtained by Meadows and Peppas [14]. The result for a bulk albumin concentration of 32 mg/ml indicated that the sample contained less albumin than predicted by Fickian diffusion (equation 4). The albumin diffusion coefficient was treated as an adjustable parameter and the results of several values of D_a are presented in Fig. 4. The highest value of D_a that produced consistent results was approximately $D_a = 5 \times 10^{-10}$ cm^2/sec. It is interesting to note that, at this value of D_a, the specific adsorption exhibited a maximum indicating that under these conditions the diffusion rate exceeded the adsorption rate.

The very low value of D_a obtained from the PVA data is consistent with self diffusion coefficients in gels. Studies by Amis *et al.* [15] on gelatin solutions produced self diffusion coefficients for a species with $\bar{M}_N = 35{,}000$ of 1.6×10^{-9} cm^2/sec (for a 15% gel phase). The studies by Vilker *et al.* [16] on albumin

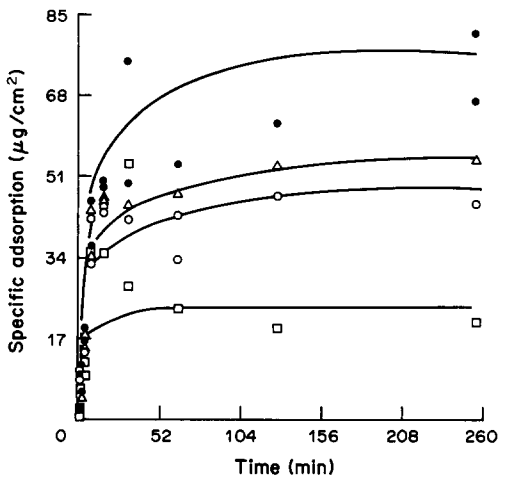

Fig. 4. Surface specific adsorption of albumin on PVA hydrogels (irradiated with 2.28 Mrad) after free iodine correction and albumin diffusion correction for $D_a = 5.0 \times 10^{-10}$ (□), 1.0×10^{-10} (○), 5.0×10^{-11} (△), and 1.0×10^{-11} cm^2/sec (●).

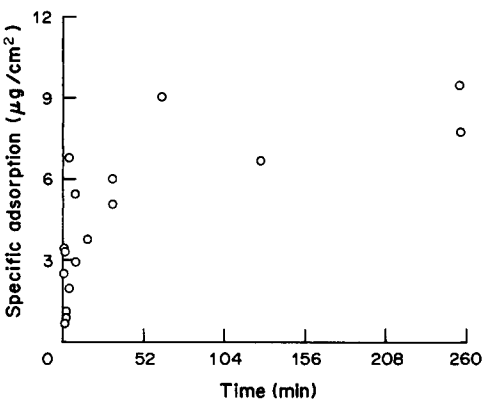

Fig. 5. Specific adsorption of albumin on PVA hydrogels (irradiated with 2.28 Mrad) as a function of time, after soaking.

concentration polarization estimated an albumin gel phase with $v_2 = 0.45$. Employing this volume fraction with the plot of normalized diffusion coefficient [7], the albumin diffusion coefficient would be in the 10^{-10} range if a gel phase formed on the hydrogel/protein solution interface ($D_\infty = 9 \times 10^{-7}$ cm^2/sec). As inferred from Fig. 4, the gel layer thickness could vary with time depending on the value of the albumin diffusion coefficient. The extent of the protein gel layer on the polymer/protein solution interface was determined by the inequality of the adsorption vs the diffusion rates.

After the initial count the PVA samples were soaked in 2 ml of fresh buffer overnight to evalute the reversibility of the adsorption process. The specific adsorption after soaking, M_{pt}, is presented in Fig. 5. If the fraction of the irreversibly bound albumin is given by f, then the comparison of the first and second (after soaking) counting allows calculation of f after correction for free iodine.

Within the accuracy of the experiment, the fraction of irreversibly bound albumin was either too low to measure or negligible. Thus, the protein formed a gel phase initially which could easily be solvated in a good solvent. When this result is compared to similar studies by Horbett [17], the conclusion is that the more hydrated material studied in this work [Horbett examined poly(2-hydroxyethylmethacrylate-co-ethylene) and poly(ethyl methacrylate-co-ethylene) copolymers] produces fewer protein conformational changes compared to lower water content hydrogels. The fraction of protein permanently bound also did not increase with residence time of the protein on the polymer as expected by the work of Sonderquist and Walton [18], indicating that highly swollen PVA acts as an unobtrusive surface to the proteins.

The results indicate that a highly swollen network absorbs higher amounts of protein than a slightly swollen material (comparing present data with literature results [17]). This is due to the more intimate mixing of the protein with the polymer chains by diffusion. The adsorption was entirely reversible within the accuracy of the experiment, indicating a low degree of protein denaturation in the material. Thus for albumin, a highly swollen PVA network

provides a very compatible environment for the protein solution.

CONCLUSIONS

Albumin adsorption onto PVA hydrogels demonstrated that the diffusion of the protein into the network can be of great importance. The albumin content of the samples was below normal diffusion values indicating that other factors were controlling the protein mass transfer. It was suggested by approximate estimation of albumin diffusion values that an albumin gel phase was formed on the hydrogel producing reduced adsorption. Adsorption was essentially completely reversible. This result indicates that highly swollen networks are much less disruptive to proteins than many standard biomaterials.

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