

Reaction of Hexamethylene Diisocyanate with Poly(vinyl Alcohol) Films for Biomedical Applications

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Synopsis

The objective of this work was to obtain primary amine groups on the surface of poly(vinyl alcohol) films by means of a reaction with hexamethylene diisocyanate. The reaction was run in such a way as to minimize the internal crosslinking by employing a large excess of hexamethylene diisocyanate in toluene and then hydrolyzing the unreacted isocyanate endgroup to primary amine. After dialyzing out the adsorbed hexamethylenediamine from the aqueous solution of reacted poly(vinyl alcohol), the extent of covalent bonding of hexamethylene diisocyanate onto the polymer was determined by measuring aminohexyl content through a fluorescence assay. This assay is based on the reaction of fluorescamine, 4-phenylspiro[furan-2(3H)-1'-phthalan]-3,3'-dione, with a primary amine to yield a fluorophor which will emit a strong fluorescence at 475-490 nm when excited at 390 nm. Analyses show a range of 1.1×10^{-10} - 3.6×10^{-10} mole of amines per cm^2 of reacted poly(vinyl alcohol) film. When assuming 47% as degree of crystallinity approximated by IR spectroscopy for these polymer films, the availability of hydroxyl groups in amorphous region was estimated to be 3.7×10^{-10} mole/ cm^2 . The extent of reaction based on available hydroxyl groups was then in the range of 31-97%. The primary amine groups attached by this method can now be exploited for binding biomolecules such as heparin (anticoagulant) or fibrinolytic enzymes in an attempt to achieve biocompatible materials.

INTRODUCTION

In the last few years, hydrophilic polymers such as poly(vinyl alcohol) and poly(2-hydroxyethyl methacrylate) have received a great deal of attention as candidates for biocompatible materials. Hydrogels of these hydrophilic polymers suffer from the lack of good mechanical strength and toughness required for biomaterials. Several attempts have achieved an improvement in mechanical properties through methods such as induced crystallinity¹ or radiation grafting onto the inert, strong polymer substrate.²

There were also attempts to achieve antithrombogenic properties on these hydrogels by covalent bonding of heparin,^{2,3} albumin, or streptokinase.² Hoffman and co-workers had reported that the extent of binding increased measurably when the biomolecule was attached through an arm containing six carbon atoms, compared to the direct bonding after cyanogen bromide treatment on poly(2-hydroxyethyl methacrylate).²

Preliminary results from our laboratory indicate that poly(vinyl acetate) is

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one of the least reactive toward blood platelets. Attachment of heparin on poly(vinyl acetate) surface to improve blood compatibility could be achieved by partial hydrolysis to poly(vinyl alcohol) followed by subsequent treatment. In this paper, we report that the successful reaction of hexamethylene diisocyanate with poly(vinyl alcohol) films resulted in grafting of the 6-aminohexyl group. The primary amine group affords the site for permanent bonding of heparin.

EXPERIMENTAL

Poly(vinyl alcohol) was obtained as fully hydrolyzed powder form (du Pont Elvanol, Grade 72-60) with a weight-average molecular weight of 185,000. Films were cast from 10% solution of ethanol-water mixture (30:70) onto the chrome-plated bronze plate with a doctor's blade. Extreme care was taken in drying process to ensure fast and complete drying. After two days of air drying in a dry box, the films were peeled off and kept at 60°C in a high vacuum oven for two more days.

The IR spectrum as shown in Figure 1 after drying two days at 60°C in the vacuum oven showed that the water peak at 1650 cm^{-1} is almost completely eliminated. To ensure complete dryness, the films were left for two more weeks in the vacuum oven at 60°C. Film thickness varied from plate to plate from .002 in. to .005 in.; but was constant ($\pm .0003$ in.) throughout each plate.

Hexamethylene diisocyanate provided by Aldrich Chemical was distilled under 64 mm Hg pressure. The fraction at 170°C was collected. Toluene, reagent grade, was dried over MgSO_4 under a flush of dry nitrogen.

Two square Teflon frames of dimensions 2 in. \times 2 in. \times $\frac{1}{16}$ in. (with center of $1\frac{1}{2}$ in. \times $1\frac{1}{2}$ in. cut out) were used to support each film, and the edges of these frames were clamped together with small Teflon clips. Each frame was fitted in one of five slots on a small removable Teflon platform, with a carrying handle, within the reactor. The platform was mounted on four $1\frac{1}{2}$ in.-long Teflon pegs to permit magnetic stirring underneath. Most of the remaining surface of the platform was perforated to facilitate circulation, as shown in Figure 2. The entire supporting device was designed to fit inside a 1000-ml resin reactor kettle, as shown in Figure 3. Five milliliters distilled hexamethylene diisocyanate were dissolved in 600 ml benzene, and the solution was introduced to the reactor

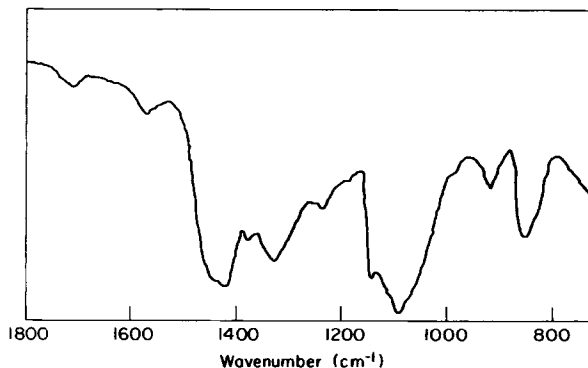


Fig. 1. IR Spectrum of dry poly(vinyl alcohol) film.

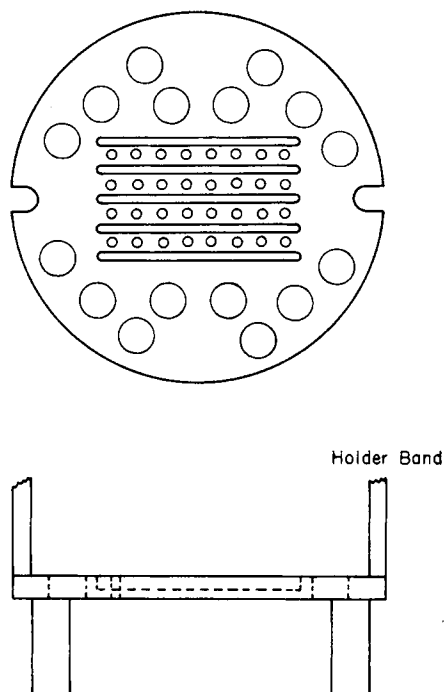


Fig. 2. Teflon platform for holding poly(vinyl alcohol) films.

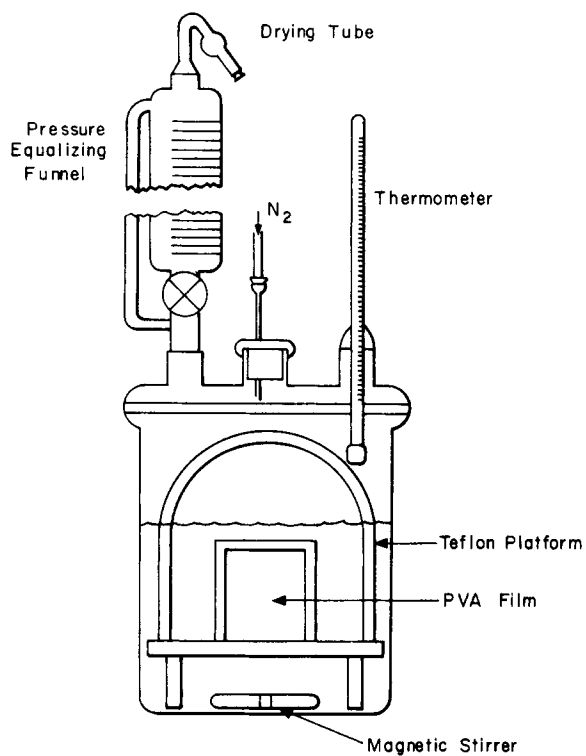


Fig. 3. Reaction apparatus for surface modification of poly(vinyl alcohol) films.

through a pressure-equalizing funnel. The reactions with poly(vinyl alcohol) films were carried out for 4 hr at 24°C under a slight positive pressure of dry nitrogen.

Reacted film of size 2 in. × 1 in. was dissolved in 25 ml water in order to determine the extent of reaction. By dissolution in water, the end-isocyanate group attached by the hexamethylene urethane group to the polymer chain was hydrolyzed to free amine group. The unreacted hexamethylene diisocyanate was hydrolyzed to hexamethylenediamine, and this was removed by dialysis for 48 hr from the aqueous solution of reacted poly(vinyl alcohol).

The extent of reaction was determined by measuring the concentration of aminoethyl endgroup attached on poly(vinyl alcohol) chain through fluorescence assay. This assay is based on the reaction of fluorescamine, i.e., 4-phenylspiro[furan-2(3H)-1'-phthalan]-3,3'-dione with aminoethyl residue to yield a fluorophor which emits a strong fluorescence at 475–490 nm when excited at 390 nm.⁴

One milliliter of the solution to be analyzed was diluted with 9 ml 0.2M Na₂HPO₄ buffer (pH ~9.1). Four milliliters of this buffered solution was mixed with 0.5 ml fluorescamine solution in acetone (concn. 30 mg/100 ml) with vigorous stirring. Fluorescence spectra were taken with a Perkin-Elmer spectrophotometer and corrected for blank.

RESULTS AND DISCUSSION

Estimation of Hydroxyl Groups Available on the Film Surface

Since it can be assumed that only the hydroxyl groups in the amorphous region of poly(vinyl alcohol) film will be accessible to the reagent under the reaction conditions, it is extremely important to determine the degree of crystallinity in order to determine the extent of the reaction. The degree of crystallinity was derived from the density value according to the Sakurada relationship:⁵

$$\rho = 8.4 \times 10^{-4}(X) + 1.265$$

where ρ is density (g/cm³) and X is per cent crystallinity. The density of the film was estimated from a ratio of two IR absorption bands,

$$D_{916} \text{ cm}^{-1}/D_{850} \text{ cm}^{-1} (= 0.44 \pm 0.001)$$

based on the Toyoshima curve,⁶ which provides the density of 1.306. Sakurada's relation then yields an approximate degree of crystallinity of 47%.

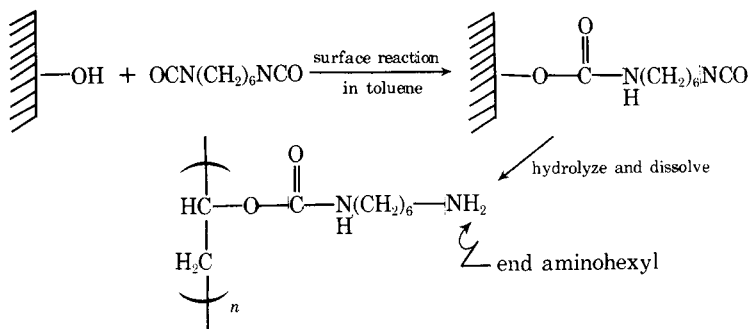
In trying to estimate the hydroxyl groups on the surface of the polymer film available for reagents, one is faced with several difficult problems: (1) The bulk crystallinity of the film may not necessarily represent the true situation in surface crystallinity, since boundary conditions at the solid-vapor interphase and evaporation diffusion of the solvent through the surface layer may cause variations in the final surface degree of crystallinity as compared with that of the bulk. (2) One cannot predict the distribution and the conformation of the hydroxyl groups in the amorphous region. (3) Poly(vinyl alcohol) films may be abundant in surface irregularities as can be observed with the electron microscope. (4) The effect of swelling by the reagent through the glassy amorphous region of the film surface, reacting with hydroxyls was not accounted for in surface calculation.

However, for a first approximation, we assumed the following to hold: (1) The bulk degree of crystallinity will represent the degree of crystallinity on the surface. (2) Surface irregularities on the film is negligible. (3) The effect of swelling by hydrolyzed reagent, hexamethylenediamine, is negligible.

Then a surface concentration of hydroxyls in the amorphous region of density 1.265 g/cm^3 was estimated to be 7.0×10^{-10} mole of OH/cm^2 by comparing with the value of 7.5×10^{-10} mole of OH/cm^2 for a perfect crystalline region of density,⁷ 1.349 g/cm^3 . The amorphous region of each 2 in. \times 2 in. face of the poly(vinyl alcohol) films which showed 47% degree of crystallinity was assigned an approximate area of 13.7 cm^2 . The available hydroxyl groups on each face were then estimated as 3.7×10^{-10} mole/ cm^2 of film.

Extent of Surface Reaction with Hexamethylene Diisocyanate

The following scheme represents the course of reactions:



Reacted films of size 2 in. \times 1 in. were dissolved in 25 ml double-distilled water and dialyzed to remove hexamethylenediamine for 48 hr. The concentration

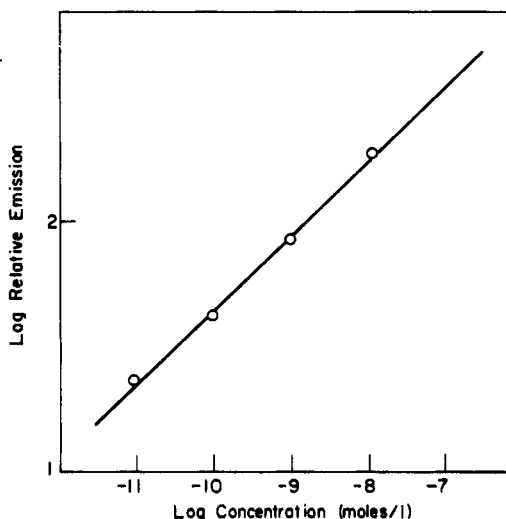


Fig. 4. Fluorescence intensity as a function of hexamethylenediamine concentration when reacted with fluorescamine at pH 9.1. Fluorescamine concn., $0.5 \times 10^{-6} M$; λ excit. = 390 nm; λ emission max. = 480 nm. Intensity corrected for blank.

of aminoethyl residues attached on poly(vinyl alcohol) was then determined by fluorescence assay employing fluorescamine. As a calibration, hexamethylenediamine was used in the concentration range of 10^{-11} to $10^{-7}M$ to give a linear relationship of fluorescent intensity with the concentration as shown in Figure 4.

The fluorescence assay showed the presence of aminoethyl groups attached to poly(vinyl alcohol) in the range of $(1.2-3.7) \times 10^{-7}$ mole of amine/l. in the aqueous solution. This range corresponds to $(1.1-3.6) \times 10^{-10}$ mole of amines/cm² of the film since the film of 2 in. \times 1 in. was dissolved in 25 ml water.

When considering the concentration of hydroxyls available for the reaction to be 3.7×10^{-10} mole/cm² of the film as estimated by the degree of crystallinity, we obtain the extent of reaction in the range of 31-97%. Despite the fluctuations of the extent of reaction, this result strongly indicates that the reaction indeed proceeded, thus providing primary amine groups on the surface. The fluctuations in the extent of reaction could have resulted from any deviation from the assumptions made to simplify the situation. The surface irregularities in crystallinity, chain conformation, and swelling might have caused the fluctuations in the result.

CONCLUSIONS

Even though it is difficult to obtain reproducibility in the extent of a heterogeneous reaction such as that of hexamethylene diisocyanate with poly(vinyl alcohol) films in toluene, the following conclusions can be made:

1. This surface reaction does indeed occur to a substantial extent.
2. Pendent isocyanates can be obtained and hydrolyzed to yield pendent primary amines with a six-carbon arm through urethane linkage to the PVA surface.
3. Fluorescamine analysis can be used quantitatively to determine the amount of grafted primary amines per unit area of PVA film exposed to the reaction media.

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