Permselectivity of Nonporous Polyurethane Membranes for Immunoisolation. I. The Influence of Hydrogen Bonding

SHEELA GEORGE, PRABHA D. NAIR

Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvanathapuram 695 012, Kerala, India

Received 3 June 1998; accepted 22 January 1999

ABSTRACT: Nonporous polyurethane membranes were synthesized using toluene 2,4-diisocyanate (TDI), polypropylene glycol (PPG), or polytetramethylene glycol (PTMG) as polyols and 1,4-butanediol as chain extender. The percentage of hard segments was varied keeping the NCO/OH ratio constant as 1. 1. The permeation of glucose, albumin, and insulin through these membranes was studied using spectrophotometric assays. The permeation of glucose was found to be dependent on the hard segment content and hydrophobicity of the polyols, whereas insulin permeation was found to vary with the hydrogen bonding and hard segment content. The permeation of albumin was almost negligible in both systems. As the synthesized nonporous polyurethanes allow the transport of the nutrients' glucose and insulin and prevent the transport of albumin with a molecular weight of 60,000 and immunoglobulins with a molecular weight of 150,000, the membranes are proposed as potential encapsulation matrices for the immunoisolation of islet cells. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 73: 1949–1954, 1999

Key words: permselectivity; polyurethane; membranes; immunoisolation; encapsulation

INTRODUCTION

Transplantation of islets or insulin-secreting cells has been attempted to restore a normal pattern of insulin secretion in diabetic individuals and thus prevent the complications of diabetes that occur in spite of long-term insulin injection therapy. The relatively low availability of human islets and the problem of immunorejection have made the pancreatic islet transplantation in humans very limited. A possible solution might be to transplant xenogenic islets surrounded by a permselective synthetic membrane, which isolates the transplant from its environment. The protection of cell transplants from being destroyed by the host's immune system is the main purpose of immunoisolation, thereby eliminating costly and dangerous immunosuppressive drug therapy.[1,2] The membrane surrounding the islets should be permeable to nutrients, glucose, and insulin and should prevent the entry of antibodies and lytic factors of the complement, as well as inward migration of white blood cells. Several permselective membranes such as alginate–polylysine alginate membrane,[3] polyvinyl chloride acrylic copolymer,[4] etc. have been used in rodents but without satisfactory results in humans so far. The dominant issue that remains is the inadequate membrane material biocompatibility. An alternative might be represented by polyurethane materials, which have shown good blood and tissue compatibility in a variety of applications such as vascular prostheses, blood filters, catheters etc.[5] In this article, nonporous polyurethane membranes have been synthesized to function as a permselective barrier and the permeation through these membranes is discussed.

Correspondence to: P. D. Nair.

Journal of Applied Polymer Science, Vol. 73, 1949–1954 (1999)
 CCC 0021-8995/99/101949-06

Table I Data on Polyurethane Synthesis

Sample No.	Composition	Molar Ratio	Hard Segment Percentage
1	TDI–PPG–BD	1.05–0.35–0.60	25
2	TDI–PPG–BD	1.05–0.25–0.70	32
3	TDI–PPG–BD	1.05–0.19–0.76	40
4	TDI–PTMG–BD	1.05–0.35–0.60	25
5	TDI–PTMG–BD	1.05–0.25–0.70	32
6	TDI–PTMG–BD	1.05–0.19–0.76	40

EXPERIMENTAL

Materials

Polyols, polytetramethylene oxide (PTMG) and polypropylene glycol (PPG), with molecular weights of 2000 were supplied from Aldrich Chemical Company, Inc., Milwaukee, USA, and 1,4-butanediol from Spectrochem Chemicals Ltd., India. Prior to use, these materials were dried in a vacuum at 60°C for 48 h. Toluene diisocyanate (TDI) (Sigma Chemicals, USA) was used as such. The catalyst used was dibutyl tin laureate (DBTL) (Fluka Chemicals, Switzerland). Dimethylformamide (DMF) (Sisco Chemicals, India, extrapure AR) was used as solvent for the polyurethane synthesis. For permeation studies, glucose (anhydrous, extrapure, S. D. Fine Chemicals Ltd., India), Insulin (insulin injection I.P., purified bovine, Boots Pharma, India), and albumin (bovine fraction V powder, Sisco Chemicals, India) were used.

Polymer Synthesis

Polymerizations were carried out using DMF as solvent using a standard two-step procedure with the molar ratios mentioned in Table I. The first step was to mix TDI with the respective polyol in a three-neck flask equipped with a condenser to which was added DBTL, 0.01% w/w, stirring rapidly at 60°C for 1 h. The prepolymer was then allowed to cool to room temperature, and 1,4-butanediol, which acts as chain extender, was added dropwise while stirring rapidly and heated at 60°C for 30 min. These reactions were carried out in a nitrogen atmosphere. After cooling and degassing, the mixture was poured on silicon oil-coated polyethylene molds and cured at 60°C for 24 h and 80°C for 17 h. The cured membranes were extracted in hexane and distilled water–ethanol (8 : 2) to remove any trapped solvents and polar/nonpolar impurities.

IR Studies

The ATR–FTIR spectra of polyurethane films were recorded with a Nicolet Impact 410 FTIR spectrometer.

Scanning Electron Microscopy (SEM)

Scanning electron micrographs of gold-sputtered polyurethane films were used to study the surface characteristics of the membrane using a Hitachi S2400 SEM.

Mechanical Properties

The mechanical properties of the membranes were studied using an Instron Universal Testing Machine Model 1011 (UK) using the procedure ASTM D882 for thin films at a speed of 100 mm/s.

Contact Angle Studies

Underwater air and octane contact angles were measured by the Hamilton technique[6] with a Rame–Hart model contact angle goniometer. The surface free energy was calculated from the contact angle using Young's equation.

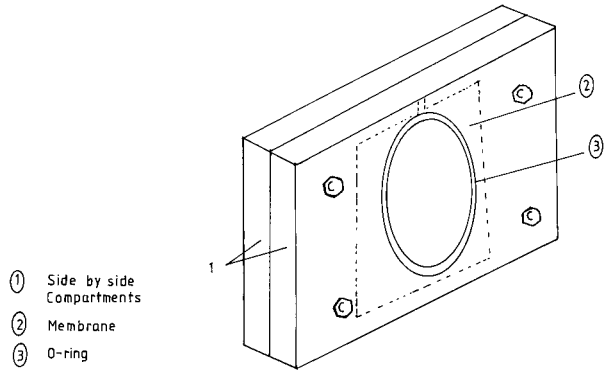

Figure 1 The diffusion cell consisting of donor and receptor half cells.

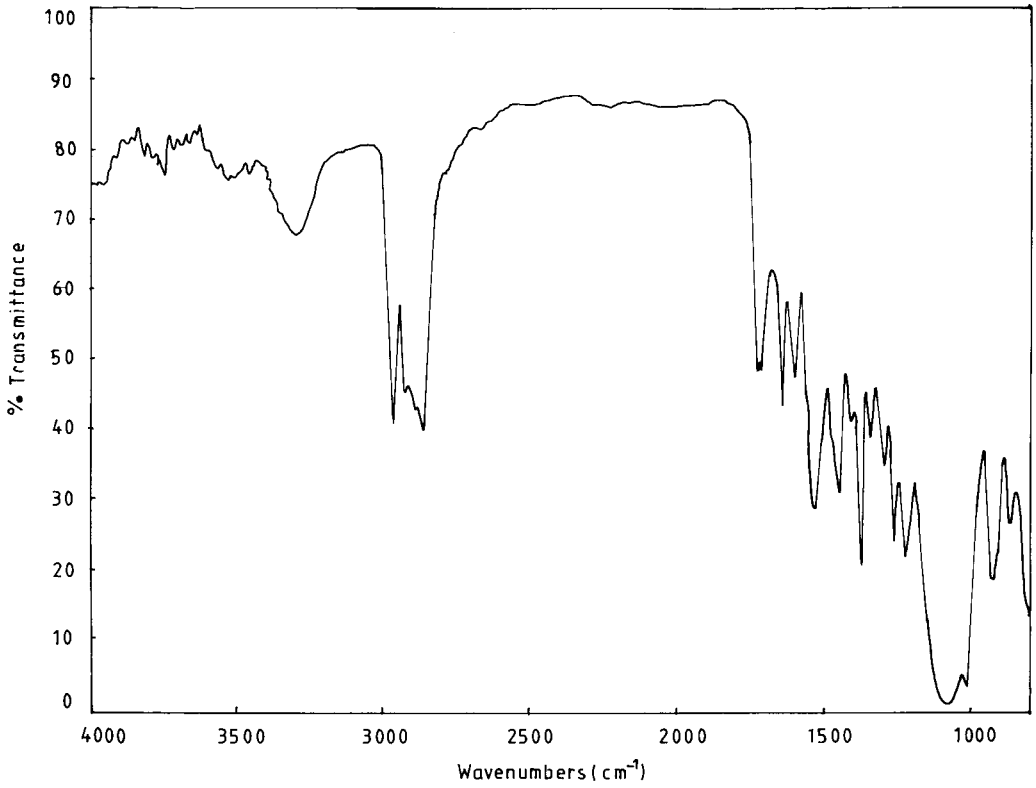

Figure 2 Representative infrared spectra of polyurethane (polytetramethylene glycol system).

$$\gamma_{sv} - \gamma_{sw} = \gamma_{wv} \cos \theta$$

where γ is the interfacial free energy for hydrated gel–water vapor (γ_{sv}), hydrated gel–water (γ_{sw}), γ_{wv} is the interfacial free energy for water–water vapor, γ_{wv} = 72 erg/cm^2 at 25°C, and θ is the contact angle for which Young's equation holds.

Permeation Studies

Permeation of glucose, insulin, albumin, and limited studies of immunoglobulins were studied using a diffusion cell. The diffusion cells used were side-by-side cells consisting of donor and receptor half-cells as shown in Figure 1. Amounts of known concentrations (5 mL) of the solution containing the solute of interest (glucose, 16.5 mmol/L; insulin, 8 U/mL; albumin, 8 g/dL; immunoglobulin, 8 g/dL) were placed in the donor com-

Table II IR Peak Assignments for Polyurethanes

Peak (cm^{-1})	Assignments
3307	NH str.
2960	CH_2, CH_3 str.
1722	Amide I, C═O str.
1536	Amide II, NH def.
1376	C—CH_3, sym., def.
1598	Phenyl
1221	—C—O Polyether

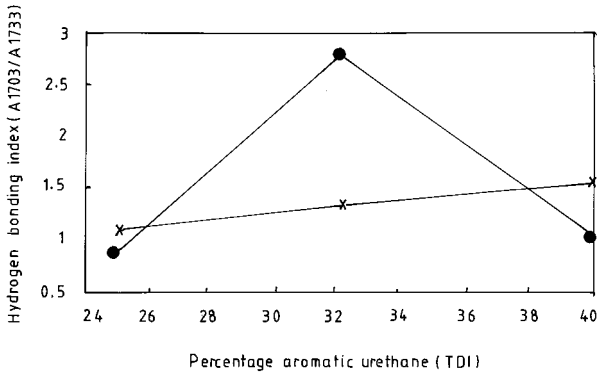

Figure 3 (a) The relationship of hydrogen bonds for different hard segments (x) (polytetramethylene system). (b) The relationship of hydrogen bonds for different hard segments (●) (polypropylene system).

partment. The pure solvent was kept in the receptor compartment. The whole setup was placed in an orbital shaking incubator at an rpm of 100 and temperature of 37°C. Periodically the contents of the receptor cell were removed and replaced by fresh solvent. The aliquot removed was analyzed using UV–VIS spectrophotometer using an o-toluidine method[7] for glucose at 630 nm, Lowry's method[8] at 640 nm for albumin, insulin, and immunoglobulin.

The solute permeability coefficient P was determined using the following equation:

$$\ln (2\mathrm{Co}/\mathrm{Ct} - 1) = 2APt/Vl$$

In this expression, Co is the initial concentration in the donor cell, Ct is the solute concentration in the receptor cell at time t, V is the volume of each half cell, l is the swollen membrane thickness, and A is the effective area of permeation. A plot of $Vl/2A$ ln (2Co/Ct − 1) versus t yielded a slope from which the value of permeability coefficient was calculated[9] in each case.

RESULTS AND DISCUSSION

IR Studies

The characteristic peaks of polyurethanes such as 3307, 2960, 1722, 1536, 1376, 1598, 1221 cm^{-1} were obtained for the synthesized polyurethane, confirming that the synthesized polymers were polyurethanes. A representative IR spectrum is shown in Figure 2. Table II gives the IR peak assignments for polyurethane. The extent of hydrogen bonding in the synthesized polyurethane[5] was estimated from the ratio of the absorbances of the peaks at 1703 and 1733 cm^{-1}. Figure 3(a) shows the relationship of hydrogen bonds for different hard segment content. We observed that the hydrogen bonding increased with hard segment content for the PTMG system. However, in the PPG system, instead of increasing trends for the hydrogen bond with hard segment content, we also observed that the hydrogen bonding for the 40% hard segment decreased [Fig. 3(b)].

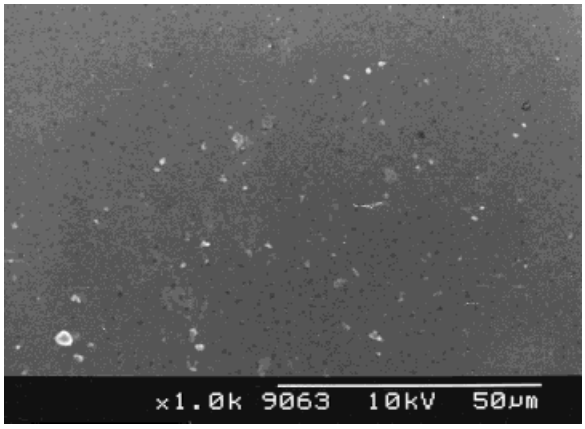

Figure 4 Representative scanning electron microscopy of a polyurethane membrane.

SEM Studies

SEM studies show no phase separation and a smooth nonporous membrane at all compositions. A representative SEM picture is shown in Figure 4.

Mechanical Properties

On comparison of the data on mechanical properties shown in Table III, the PTMG system is found to be tougher than the PPG system. We find that the value of stress at break for PU (32%) and PU (25%) (in the PPG system) is less than that for

Table III Mechanical Properties of Synthesized Polyurethanes

Membrane Type	Stress at Peak (MPa)	Stress at Break (MPa)	% Strain at Break (%)	% Strain at a Maximum Load
PPG system				
PU (25%)	5.786	5.772	443.1	421
PU (32%)	1.822	1.822	193.2	192.6
PU (40%)	2.047	1.047	58.76	56.25
PTMG system				
PU (25%)	2.521	2.517	893.3	895.5
PU (32%)	15.94	15.96	785.3	784.6
PU (40%)	4.819	4.792	204.2	201.4

Table IV Surface Parameters for Synthesized Polyurethanes

Membrane Type	Air–Water Contact Angle, θ_{air} (°)	Octane–Water Contact Angle, θ_{octane} (°)	γ_{sw} (interfacial free energy between solid and water)
PPG System			
PU (25%)	42.75	142	1.3934
PU (32%)	47	133	1.8843
PU (40%)	52.25	124.72	2.77
PTMG System			
PU (25%)	29	136	1.260
PU (32%)	49.85	131	2.3419
PU (40%)	46.25	99.2	13.577

PU (25%), whereas in the PTMG system, the stress of PU (25%) and PU (40%) is less than PU (25%). This was contrary to expectation and is believed to be due to local crystallinity effects with the increase in the hard segment content.

Contact Angle Studies

A decrease of the surface air–water contact angle (θ_{air}) and increase of the octane water contact angle (θ_{octane}) is suggestive of hydrophilicity[6] in a system. The polyurethane with a hard segment of 25% with θ_{air} of 42.75° is more hydrophilic in the PPG series. The polyurethane with 25% hard segments is also found to be most hydrophilic in the PTMG series. Between the PPG and PTMG, the polyurethane prepared by using PTMG as glycol is more hydrophilic. The data for contact angle studies are shown in Table IV.

Permeation Studies

Robert et al.[10] has reported on nonporous polyurethane membranes (consisting of aromatic diisocyanate alkyl diamine hard and soft segments of polyalkylene oxide) that are readily permeable to both glucose and insulin but impermeable to immunoglobulins. Robert et al.[10] have also reported that the permeation constants, P, for glucose as 1.1×10^7 cm^2/s, insulin as 0.226×10^7 cm^2/s, and albumin as 0.00116×10^7 cm^2/s through the nonporous polyurethane membranes. Serum-dependent cell lines (RAJI and MOPC-31C) could be grown and maintained successfully in these nonporous dense polyurethane membranes for at least 2 months. However, the permeation constants of glucose and insulin reported by them are low. In the present study, novel polyurethanes consisting of aromatic diisocyanate alkyl diol hard and soft segments of hydrophilic polyalkylene oxide were synthesized. We have observed higher permeation for glucose and insulin (Table V) through these polyurethanes. In general, the permeation of hydrophilic solutes like glucose should be higher in more hydrophilic sys-

Table V Permeation Coefficient for Glucose and Insulin

Membrane Type	Glucose[a]	Insulin[a]
PPG System		
PU (25%)	4.2	1.8
PU (32%)	1.3	4
PU (40%)	6.6	3.8
PTMG System		
PU (25%)	25	0.28
PU (32%)	18	2.3
PU (40%)	4	1.6

[a] Values are permeability $\times 10^7$ (cm^2/sec).

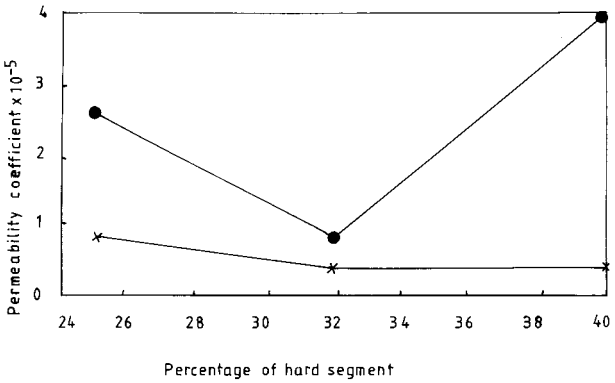

Figure 5 Variation of permeability with percentage of hard segments in polyurethanes (x) (polytetramethylene and (●) polypropylene systems).

Table VI Amount Permeated in 1 h

Membrane Type	Glucose (mol/L)	Insulin (mol/L)	Albumin (mol/L)	Immunoglobulin (mol/L)
PPG System				
PU (25%)	0.11	0.0021	0.00010	0
PU (32%)	0.10	0.00082	0.0035	—
PU (40%)	0.15	0.00082	0.0000821	—
PTMG System				
PU (25%)	0.14	0.00054	0.0000328	0
PU (32%)	0.10	0.0015	0.000073	—
PU (40%)	0.09	0.0030	0.00020	—

tems [in this case, the 25% of PPG and PTMG series (Table IV)]. However, we observed that the permeation was also dependent on the hard segment content of the polyurethanes for both PPG and PTMG series. The hydrogen bonding tendency increased with the hard segment content in the case of the PTMG, but the permeation of glucose in this system decreases with the increase of hydrogen bonds (Fig. 5). As stated earlier, the hydrogen bonding in the case of PPG was not uniformly increased except for the 32% hard segment content. Permeation constants for glucose proportionately decreased with increased hydrogen bonding. Although hydrogen bonds are weak, they may form virtual crosslinks that in turn reduce the permeation.

In the case of insulin, the permeation constant is found to be independent of the hydrogen bonding. The permeation constant for insulin is also higher than that determined by Robert et al.[10] It seems, therefore, that the permeation of glucose with a molecular weight of 180 (being a small molecule) is affected by hydrogen bonding of polyurethanes, but the permeation of molecules such as insulin with a molecular weight of 6000 and a coiled structure will not be affected by hydrogen bonding in the matrix.

The permeation constants for albumin and immunoglobulin were not determined because the concentration of albumin permeated was negligible. Table VI gives the actual amount permeated through these membranes in a 1-h interval in moles per liter. It is evident that a higher amount of glucose can permeate through these membranes. The membranes are also permeable to insulin but impermeable to albumin and immunoglobulins. We are now carrying out further studies for establishing the cell contact nature of these membranes.

CONCLUSIONS

Permeation of glucose and insulin through the synthesized linear polyurethane membrane was found to vary over different ranges with hydrogen bond and hard segment content. As the permeation of albumin is negligible, the membranes are also expected to be impermeable to the immunoglobulins with the molecular weight of 150,000. Hence they are possible candidate materials for use as immunoisolation barriers for islet cell encapsulation.

The authors are grateful to Dr. K. Mohandas, Director, SCTIMST, and Dr. R. Sivakumar, Head, BMT Wing, for providing the facilities to carry out the work. S. G. acknowledges the award of the SCTIMST fellowship.

REFERENCES

1. Lim, F.; Sun, A. M. Science 1980, 210, 908.
2. Sefton, M. V.; Stevenson, T. K. Adv Polym Sci 1993, 107, 143.
3. O'Shea, G. M.; Goosen, M. F. A.; Sun, A. M. Biochem Biophys Acta 1984, 804, 133–136.
4. Altman, J. J.; Houlbert, D.; Callard, P.; McMillan, P.; Solomon, B. A.; Rosen, J.; Galletti, P. M. Diabetes 1986, 35, 625–633.
5. Lelah, M. D.; Cooper, S. C. Polyurethanes in Medicine; CRC Press: Boca Raton, FL, 1986.
6. Hamilton, W. C. J Colloid Int Sci 1974, 47, 672.
7. Caraway, W. T. in Fundamentals of Clinical Chemistry; Tietz, N. W., Ed.; Saunders: Philadelphia, 1976; p 234.
8. Lowry, O. H.; Rosenbrough, N. J.; Farr, A. I.; Randall, R. J. J Bio Chem 1951, 193, 265.
9. Stringer, J. L.; Peppas, N. A. J Controlled Release 1996, 42, 195.
10. Robert et al. ASAIO J 1993.