

Formation of Molecular Membranes in Water from Ionene Oligomers and Related Amphiphiles[†]

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ABSTRACT: Ionenes were prepared by reaction of α,ω -dibromoalkanes and α,ω -bis(dimethyl-amino)alkanes at room temperature. The degree of polymerization as estimated from NMR data was 2 to 5. The oligomeric ammonium amphiphiles (ionenes) thus obtained gave molecular weights of 10^5 to 10^8 in water. Vesicles of the molecular membrane were formed by self-assembly of the C_{20} ionene. A related bis-ammonium amphiphile gave similar results. The introduction of symmetrical aromatic rings into the middle of the C_{20} chain did not change the general aggregation behavior. Differential scanning calorimetry indicated the presence of gel-to-liquid crystal phase transition in these cases. No membrane formation was observed when the methylene chain length was shorter (C_{10} and C_{12}) or the two methylene portions were uneven in length (C_{20} and C_{16}).

KEY WORDS Molecular Membrane / Ionene Oligomer / Electron Microscopy / Differential Scanning Calorimetry / Self Assembly /

Both we¹ and other research groups have found that a large variety of synthetic amphiphiles undergo spontaneous assemblage to bilayer membranes.² An interesting development in this field is the incorporation of a polymeric nature into these membranes. Ringsdorf and others³ classified the modes of the polymerization of bilayer membranes, and Fendler and Tundo⁴ summarized recent data on the preparation and utilization of polymerized bilayers. The modification of polymer surfaces by polymerized membranes is a controversial topic.⁵

We investigated the formation of molecular membranes (monolayer and bilayer) from polymeric amphiphiles such as water-soluble vinyl copolymers.^{6,7}

In this article, we describe the spontaneous formation of monolayer membranes in water from ionene oligomers and related amphiphiles. In the past, a variety of ionene poly-

mers containing aliphatic and aromatic segments were prepared, and their solution properties as polyelectrolyte studied.^{8,9} In this cases, the methylene chain or the aromatic segment connecting the ammonium groups are short (less than the equivalent of C_{10}).

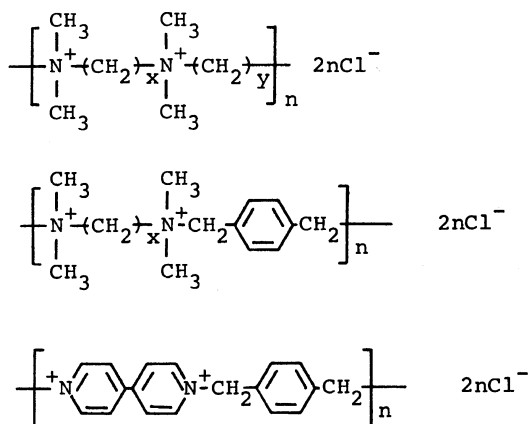


Chart 1. Typical ionene polymers.

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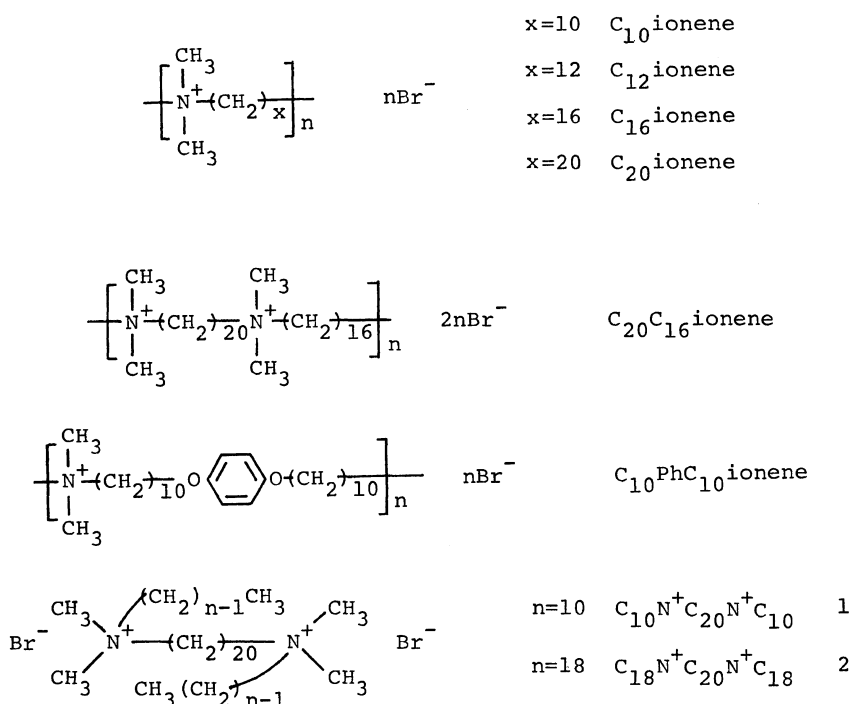


Chart 2. Bis-ammonium and ionene.

Stable bilayer membranes are formed from dialkylammonium amphiphiles with alkyl tails of at least C₁₀. The total alkyl chain length corresponding to the bilayer thickness should thus be at least C₂₀. We previously reported that stable monolayer membranes are produced from two-headed single-chain amphiphiles.¹¹ It should be of interest to see whether the covalent connection of two double-chain molecules at one of the alkyl chain end could give membrane-forming compounds. For this purpose, we prepared bisammonium amphiphiles (C₁₀N⁺C₂₀N⁺C₁₀, **1** and C₁₈N⁺C₂₀N⁺C₁₈, **2**) and a series of ionene polymers and examined their aggregation behavior.

EXPERIMENTAL

Materials

α,ω -Dihydroxyalkanes, as purchased or prepared from the corresponding dicarboxylic acids *via* esterification and LiAlH₄ reduction,

were converted to α,ω -dibromoalkanes by HBr gas¹² or by concentrated hydrobromic acid in ligroin¹³; 1,12-dibromododecane, bp 125–127°C/0.04 mmHg; 1,16-dibromohexadecane, mp 50–52°C; 1,20-dibromoeicosane, mp 66–68.5°C. lit¹⁴ mp 67.4–68.0°C. Subsequently, 1,20-dibromoeicosane was dissolved in benzene and allowed to react with a large excess of dimethylamine in a sealed ampoule at 90°C for 118 h. The reaction mixture was neutralized with dilute alkali and the product was recovered from the organic layer and recrystallized from acetone: yield 80–90%, mp 40–41.5°C.

Anal. Calcd for C₂₄H₅₂N₂: C, 78.19%; H, 14.22%; N, 7.59%. Found: C, 78.15%; H, 14.20%; N, 7.30%.

1,16-Dibromohexadecane was similarly converted to the dimethylamino derivative by reaction at 60°C for 43 h: colorless oil, yield 36%. The NMR spectrum confirmed the conversion. 1,12-Dibromododecane was allowed

to react with dimethylamine in ethanol at room temperature for one week. After solvent removal, the solid residue was neutralized with ethanolic KOH and extracted with benzene, and the oily residue was distilled: colorless oil, yield 50%, bp 143–145°C/0.2 mmHg.

Anal. Calcd for $C_{16}H_{36}N_2$: C, 74.92%; H, 14.14%; N, 10.92%. Found: C, 74.56%; H, 14.15%; N, 10.56%.

A similar procedure was used to convert 1,10-dibromodecane to the dimethylamino derivative: colorless oil, yield 30%, bp 131–134°C/1 mmHg.

Anal. Calcd for $C_{14}H_{32}N_2$: C, 76.11%; H, 14.12%; N, 12.26%. Found: C, 73.11%; H, 14.10%; N, 12.01%.

Hydroquinone (10 g, 0.09 mol, Kishida Chemicals, reagent grade) in 200 ml of ethanol, 10 g (0.18 mol) of KOH in ethanol, and 150 g (0.5 mol) of distilled 1,10-dibromodecane were refluxed under N_2 for 5 h. The reaction mixture was poured into water and the precipitates were recrystallized repeatedly from ethyl acetate: colorless powders, yield 16.6 g (40%), mp 75–78°C.

Anal. Calcd for $C_{26}H_{44}O_2Br_2$: C, 56.94%; H, 8.09%. Found: C, 58.99%; H, 8.31%.

1,4-Bis(10-bromodecyloxy)benzene (5 g, 9 mmol) in benzene was allowed to react with 32 g (0.18 mol) of ethanolic dimethylamine in an ampoule at 80°C for 21 h. After solvent removal, the colorless residue was treated with KOH and recrystallized from ethanol: colorless powders, yield 60%, mp 59–62°C. An NMR spectrum of the product (in $CDCl_3$) was consistent with the bis-dimethylamino structure and thin layer chromatography indicated the absence of the starting material.

Preparation of Amphiphiles

In an ampoule were placed 3.5 g (0.009 mol) of 1,20-bis(*N,N*-dimethylamino)icosane in 100 ml of ethanol and 7.0 g (0.03 mol) of distilled 1-bromodecane (bp 113–116°C/12 mmHg) in ethanol. The sealed ampoule was immersed in an oil bath (80°C) for 72 h. A

colorless solid was obtained upon solvent removal and recrystallized from acetone to give 1,20-bis(*N,N*-dimethyldecylammonio) eicosane: yield, 87%, mp 40→155–165°C (the arrow indicates the liquid crystalline range).

Anal. Calcd for $C_{44}H_{94}N_2Br_2 \cdot H_2O$: C, 63.74%; H, 11.67%; N, 3.38%. Found: C, 64.14%; H, 11.57%; N, 3.49%.

The reaction of 1,20-bis(*N,N*-dimethylamino)icosane and 1-bromooctadecane was similarly conducted in an ampoule at 80°C for 90 h. Colorless powders of 1,20-bis(*N,N*-dimethyloctadecylammonio)icosane were obtained in 71% yield after recrystallization from acetone and acetone/ethanol: mp 50→145→170°C.

Anal. Calcd for $C_{60}H_{126}N_2Br_2 \cdot H_2O$: C, 68.41%; H, 12.25%; N, 2.66%. Found: C, 68.17%; H, 11.99%; N, 2.80%.

NMR and IR spectra were consistent with the respective structures.

Polymerization

The following procedure was typical. Equimolar amounts (2×10^{-3} mol) of 1,20-dibromoeicosane and 1,20-bis(*N,N*-dimethylamino)icosane were dissolved in 20 ml of purified tetrahydrofuran (THF) and stirred magnetically at room temperature (18–23°C) for 264 h. The precipitates were centrifuged, washed with THF, and dried. The solid dissolved in warm dimethyl sulfoxide was added dropwise to THF with magnetic stirring. Stirring was continued for additional 5 h. The polymer (0.5 g) was recovered by decantation and centrifugation. The other ionenes were prepared in a similar manner. The polymerization results are summarized in Table I.

Measurement

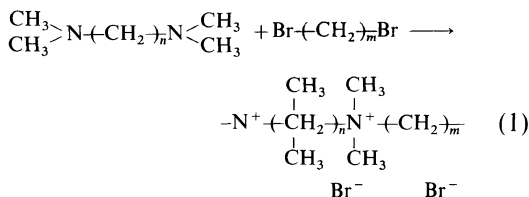
Electron microscopy was performed using a Hitachi H-500 instrument, as described elsewhere.¹⁵ Differential scanning calorimetry was carried out with a Daini-Seikosha SSC-560 instrument. Sample solutions were sealed in silver pans and the measurement was repeated

from 0 to 80°C at a rate of 2°C/min. The details are described elsewhere.¹⁶ Molecular weights were determined by a small-angle light scattering apparatus with a He-Ne laser source (Toyo Soda, LS-8).¹⁷ The sample concentration was 5 mg/50 ml (2.5×10^{-4} – 3.8×10^{-4} unit M).

RESULTS AND DISCUSSION

Polymerization

The polymerizations were carried out at room temperature to assure predominance of the quaternization reaction.



The products were powders or semi-solids. High polymers were not obtainable under the present conditions, and some of the low molecular-weight fractions may have been lost during reprecipitation. The complete removal of the starting materials (dibromides and diamines) from the polymers was ascertained by thin layer chromatography.

NMR spectra were consistent with the ionene structure. Figure 1 shows an NMR

spectrum of C₂₀ ionene. The major peak assignments are given in the insert. There are peaks apparently attributable to unreacted terminal units (peaks c and d), in addition to the peaks of monomer unit in polymer (peaks a and b). The degree of polymerization (DP) (length of the ionene unit) can be estimated from the NMR peak ratio. Assuming the polymer chain end to consist of unreacted BrCH₂ and/or (CH₃)₂NCH₂ groups, the relative amount of terminal and main-chain units in the C₂₀ ionene are given by 1/2 (area of peak c) + 1/8 (area of peak d) and 1/36 (area of peak b), respectively. The degree of polymerization calculated from these data is given in

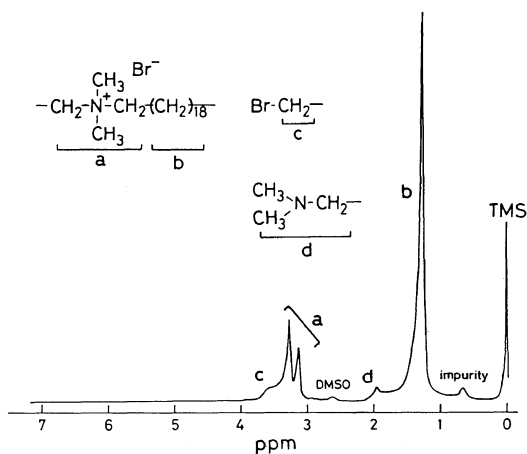


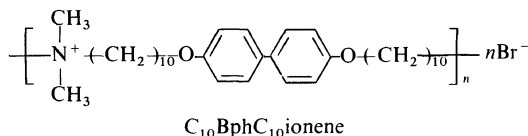
Figure 1. ¹H NMR spectrum of C₂₀ ionene in CDCl₃.

Table I. Polymerization^a

Entry	Polymer	Total monomer concn	Solvent	Temp	Time	Recovery	\overline{DP}
		M		C	h	%	
1	C ₂₀ ionene	0.2	THF	18–23	246	30	3–4
2	C ₂₀ ionene	0.6	THF	ca. 18	100	58	—
3	C ₁₆ ionene	0.3	CHCl ₃	26–32	161	63	2.5–3.5
4	C ₁₂ ionene	0.2	Benzene	ca. 25	286	17	2.2–3.2
5	C ₁₀ ionene	0.96	Dry ethanol	ca. 22	216	—	—
6	C ₂₀ C ₁₆ ionene	0.2	THF	26–32	173	72	—
7	C ₁₀ PhC ₁₀ ionene	0.2	THF	ca. 27	144	45	4–5

^a Anal. Calcd for C₂₂H₄₆NBr 0.5H₂O: C, 63.90%; H, 11.46%; N, 3.39%. Found (entry 1): C, 63.78%; H, 11.18%; N, 3.21%. Found (entry 2): C, 63.36%; H, 11.29%; N, 3.17%. Calcd for C₁₈H₃₈NBr H₂O: C, 59.00%; H, 11.00%; N, 3.82%. Found (entry 3): C, 59.43%; H, 10.76%; N, 3.67%.

Table I. A similar estimation was made for other ionenes. DP was 3–4 for C₂₀ ionene and smaller for C₁₆ and C₁₂ ionenes. Analogous values were found for ionenes containing the aromatic unit (C₁₀PhC₁₀ ionene and C₁₀BphC₁₀ ionene.¹⁸)



Aggregation Behavior

Table II summarizes the aggregation behavior of ionene oligomers and their analogs. Their molecular weights in water are very large. Since the molecular weights of the ionene oligomers are in the range of 600 to 2400, the light scattering data indicate that approximately 10³–10⁴ ionene molecules are aggregated in water. In the case of simple ionenes (C_n ionenes), the molecular weight increases with methylene chain length from 10⁵ to 10⁶. C₂₀ ionene has a molecular weight close to those of typical bilayer membranes of dialkylammonium salts. It was noted previously that the molecular weight of bilayer aggregates of double-chain amphiphiles is one million or more.¹⁹

Vesicle formation was observed by electron microscopy for the aqueous aggregates of C₁₈N⁺C₂₀N⁺C₁₈ and C₂₀ ionene, as typically shown in Figure 2 (a and b). The thickness of the vesicle wall is *ca.* 100 Å in both cases. This

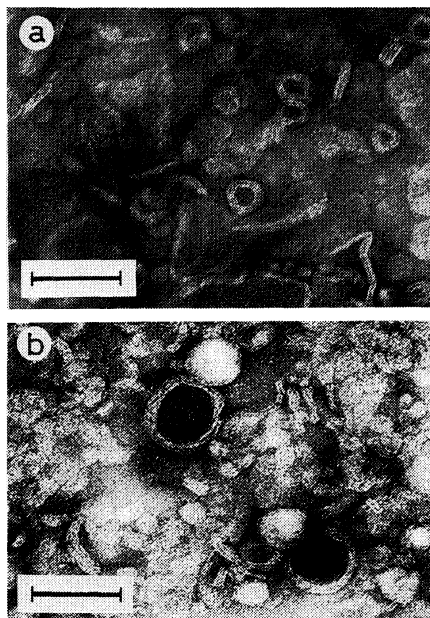


Figure 2. Electron micrographs, a; C₁₈N⁺C₂₀N⁺C₁₈ b; C₂₀ ionene. The sample solution (5 mg ml⁻¹) was stained by uranyl acetate (pH 4), Magnification × 24,000. The bar represents 1000 Å.

Table II. Aggregation behavior

Amphiphile	Molecular weight in water	Electron micrograph	DSC	
			Peak top/°C	ΔH/kcal mol ⁻¹
C ₁₈ N ⁺ C ₂₀ N ⁺ C ₁₈	(4–7) × 10 ⁶	Vesicle	70	6.6 (1.5 mM) ^b
C ₁₀ N ⁺ C ₂₀ N ⁺ C ₁₀	—	Not clear	None	
C ₂₀ ionene	2 × 10 ⁶	Vesicle	53	7.3 (80 unit mM) ^b
C ₁₆ ionene	1.2 × 10 ⁵	(Lamella) ^a	26	4.7 (60 unit mM) ^b
C ₁₂ ionene	(1–2) × 10 ⁵	Not clear	Broad (40–50)	
C ₁₀ ionene	(1–1.5) × 10 ⁵	Not clear	None	
C ₂₀ C ₁₆ ionene	—	No structure	None	
C ₁₀ PhC ₁₀ ionene	1.5 × 10 ⁷	(Disk-like) ^a	63	1.8 (60 unit mM) ^b
C ₁₀ BphC ₁₀ ionene ^c	1 × 10 ⁸	Disk-like	62	0.8

^a Parenthesis indicates the morphology to be ill-defined.

^b Figures in parenthesis are the sample concentrations for the DSC study.

^c DP = 3–4.

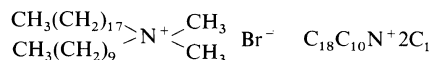
value is much longer than the distance of the C_{20} chain between the two ammonium groups (45 Å). The observed wall may consist of double molecular layers. The structure (molecular membrane) formation is less apparent for C_{16} ionene, C_{12} ionene, C_{10} ionene, and $C_{10}\text{PhC}_{10}$ ionene. There are no indications of membrane formation in the case of $C_{10}\text{N}^+C_{20}\text{N}^+C_{10}$ and $C_{20}C_{16}$ ionene. Disk-like structures were found in an electron micrograph of the aggregate of $C_{10}\text{BphC}_{10}$ ionene.

The crystal-to-liquid crystal phase transition is a fundamental property of the bilayer membrane, and has been observed for representative synthetic bilayers.¹⁶ Figure 3 displays a DSC chart for C_{20} ionene. The endothermic peak (peak top, 53°C) is apparently attributed to the phase transition. The enthalpy change (ΔH) estimated from the peak area is 7.3 kcal mol⁻¹, in reasonable agreement with ΔH values for bilayers of typical double-chain ammonium amphiphiles. This value becomes smaller, however, on lowering the sample concentration. A sharp DSC peak was similarly found for C_{16} ionene, but C_{12} ionene gave a weak, broad peak at 40–50°C and C_{10} ionene did not show any peak. Aromatic ionenes ($C_{10}\text{PhC}_{10}$ and $C_{10}\text{BphC}_{10}$) gave small peaks. $C_{18}\text{N}^+C_{20}\text{N}^+C_{18}$ produced a distinct DSC

peak at 70°C, but no peak was detected in the case of $C_{10}\text{N}^+C_{20}\text{N}^+C_{10}$.

Membrane Formation

The aggregation behavior summarized in Table II suggests that molecular membranes are formed from several ionenes (C_{20} ionene, $C_{10}\text{PhC}_{10}$ ionene and $C_{10}\text{BphC}_{10}$ ionene) and from $C_{18}\text{N}^+C_{20}\text{N}^+C_{18}$. The latter compound may be regarded as that derived by connecting two double-chain ammonium amphiphiles at one of the alkyl tails. We showed previously that stable bilayers are produced from double-chain ammonium salts when the two alkyl tails are not shorter than C_{10} .¹⁰ $C_{18}\text{N}^+C_{20}\text{N}^+C_{18}$ corresponds to two molecules of $C_{18}C_{10}\text{N}^+2C_1$ combined, and naturally belongs to a class of membrane-forming amphiphiles. $C_{10}\text{N}^+C_{20}\text{N}^+C_{10}$ does not form stable membranes probably because the alkyl tail is too short.



The same holds true for ionenes. C_{20} ionene gives stable molecular membranes, but not its lower homologs (C_{16} , C_{12} , and C_{10} ionenes), though C_{16} ionene may produce some ordered aggregates. When two hydrocarbon chains of different lengths are included ($C_{20}C_{16}$ ionene), the ordered aggregate is not produced, as anticipated. The introduction of benzene or biphenyl unit into ionenes does not interfere with membrane formation. In fact, the incorporation of aromatic rigid segments into single-chain amphiphiles was found to promote bilayer formation.¹⁷

Schematic illustrations of membrane formation from ionenes and related amphiphiles are shown in Figure 4, and compared with those of ordinary ammonium bilayers. The representative molecular membranes contain; a, bilayers of double-chain amphiphiles; B, bilayers of single-chain amphiphiles; and C, monolayers of single-chain amphiphiles. It is readily apparent that the molecular membrane of a bis-ammonium amphiphile is produced

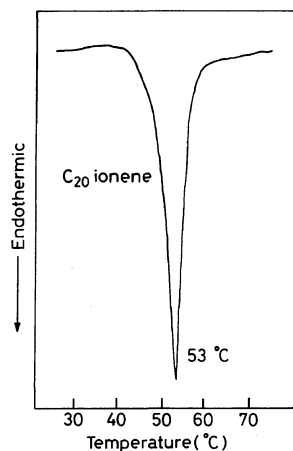


Figure 3. DSC thermogram of C_{20} ionene, heating rate 2.0°C/min⁻¹. The sample solution (20 mM) was prepared by sonication.

Molecular Membranes of Ionene Oligomers

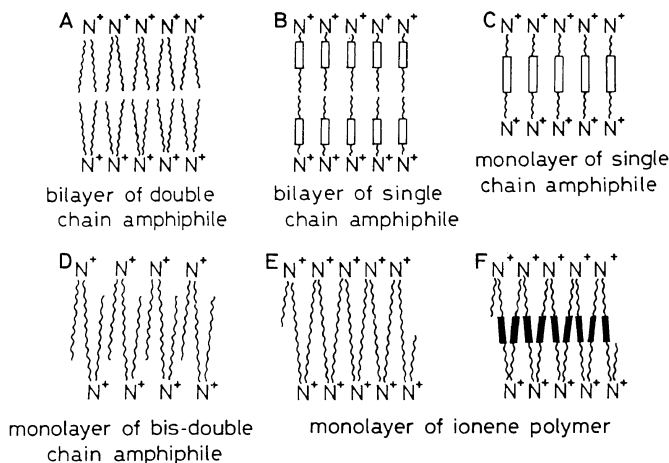


Figure 4. Schematic illustrations of the membrane formation from ionenes and related amphiphiles.

from bilayer A by connecting two double-chain component molecules at one end. When both of the alkyl chain ends are connected among different components of bilayer A, ionene-type membranes result. The monolayer F is obtainable from bilayer B in a similar manner, and should possess the general structural characteristics in between those of membranes B and C.

CONCLUSIONS

Our data show that stable molecular membranes are formed spontaneously from ionene-type amphiphiles. As described before,^{6,7} some vinyl copolymers undergo spontaneous assemblage to bilayer membranes. The present results are the second example of membrane-forming polymeric (oligomeric) amphiphiles. The polymerization of pre-formed bilayer vesicles is being actively studied by many research groups; however, examples of bilayer formation by pre-formed polymers are relatively rare. It is expected in the future that molecular membranes will be obtainable from other types of polymers such as polyesters and polyamides.

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