Membranes Prepared from Keratin–Polyacrylonitrile Graft Copolymers

JOHANN SCHALLER,* TAKEAKI MIYAMOTO, KAORU SHIMAMURA, and HIROSHI INAGAKI, *Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan*

Synopsis

Graft copolymerization of acrylonitrile onto a soluble wool keratin derivative was studied with the reduced and carboxymethylated low-sulfur protein fraction from wool. Copolymerization was carried out under a variety of conditions with a redox system of $(NH_4)_2S_2O_8$–Na_2SO_3 in 60% (w/w) aqueous $ZnCl_2$ solution at 10°C. Monomer conversions higher than 90% were obtained by the stepwise addition of initiator. The graft products were successfully separated into grafted copolymer and homopolyacrylonitrile (PAN) by fractional precipitation or solvent extraction with DMF. Grafted PAN were isolated by acid hydrolysis of the keratin backbone. Characterization of grafted and homo-PAN was carried out by IR spectroscopy, amino acid endgroup analysis, and viscometry. On the basis of the results, effects of polymerization conditions on grafting parameters were discussed. Membranes were prepared from 60% aqueous $ZnCl_2$ solution by using ice-cold water as the regenerating medium. All the membranes formed from the graft products were transparent. Observation by scanning electron microscopy showed that the surface consisted of rather spherical keratin domains regularly distributed in the PAN matrix. Selective hydrolysis of the keratin domains allowed a new type of porous PAN membrane to be obtained, with the inner walls of the pores being charged with amino acid residues attached to PAN chains as the endgroup.

INTRODUCTION

Although graft copolymerization of various monomers onto wool fibers has been investigated extensively,[1,2] no study has yet been reported on the grafting onto soluble keratin derivatives from wool. Recently, we have been engaged in studying the functional properties of keratin components extracted from waste wool fiber from the viewpoint of efficient utilization of wool keratin resources.[3–6] One of the studies planned in our project was to prepare the soluble graft copolymers of wool keratin and synthetic polymer and to search for possible application of the graft products. In the present work, we attempted to prepare a new type of composite membranes composed of wool keratin and polyacrylonitrile (PAN). To this end, graft copolymerization of acrylonitrile (AN) onto a soluble keratin derivative was investigated by referring to a procedure employed by Yamamoto et al.[7] for preparing soybean protein graft PAN copolymers. The low-sulfur protein fraction from reduced and S-carboxymethylated wool keratin, SCMKA, was used as the soluble keratin derivative.

This paper deals with the preparation and characterization of the keratin graft PAN copolymers and with the structure of the membranes formed from the graft copolymers.

* Present address: Institut für Biochemie, Universität Bern, CH-3012 Bern, Switzerland.

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EXPERIMENTAL

Materials

Acrylonitrile (AN) was purified by the method of Bamford and Jenkins.[8] All other chemicals were reagent grade, and deionized water was used throughout.

The S-carboxymethylated derivative of the low-sulfur protein fraction (SCMKA), which is one of the major constituents of soluble proteins from wool, was prepared from Merino 64's wool by the procedure of O'Donnell and Thompson,[9] involving extraction of the wool with 2-mercaptoethanol at pH 10.5 in the presence of 8*M* urea, alkylation of the extracted proteins with iodoacetic acid, and the fractionation by precipitation at pH 4.4 in the presence of 0.5*M* KCl. SCMKA has an average molecular weight of about 50,000.[10,11]

Graft Copolymerization

Graft copolymerization was conducted under a variety of conditions using a redox system of ammonium persulfate (APS)-sodium sulfite (SS) in 60% aqueous $ZnCl_2$ solution at 10°C. In the light of the results reported by Yamamoto et al.,[7] a constant molar ratio of 1/2.4 was adopted for APS/SS, and the initiator solution was added stepwise. The total weight of SCMKA and AN monomer was kept constant, except for sample 10N-2, while the weight ratio of SCMKA to AN monomer in the feed was changed. Table I summarizes the polymerization data. After the reaction mixture had been diluted with dimethyl sulfoxide (DMSO), the graft product was recovered by precipitation with a large excess of methanol and washed several times in 0.2% acetic acid.

TABLE I
Preparative Conditions for SCMKA–Polyacrylonitrile Graft Copolymers[a]

Sample code	SCMKA in feed, %	Total [APS] mol/l.	Times of adding initiator[b]	Total conv. of AN to polymer, %	Isolated PAN	
					$[\eta]$ dl/g[c]	$\overline{M}_n \times 10^{-4}$ [d]
PAN-H	0	1.5	2	90	1.27	4.8
10N-1	10	1.5	2	90	4.00	22.1
10N-2[e]	10	1.5	2	90	—	—
10-IR[f]	10	2.25	3	96	—	—
20N	20	1.5	2	85	1.40	5.5
30N	30	1.5	2	90	2.17	9.8
30-IR[f]	30	2.25	3	~100	—	—
30H	30	0.38	1	50	1.55	6.3
30D	30	1.5	1	60	1.44	5.7
30D2[f]	30	1.5	1	60	1.55	6.3

[a] Reaction conditions: total weight of (SCMKA + AN) = 5.7 g/100 ml aq. $ZnCl_2$; reaction time = 1 hr; temperature = 10°C; mole ratio of APS/SS = 1/2.4.

[b] Added in equal portions at intervals of 30 min.

[c] In DMF at 25°C.

[d] Calculated with viscosity equation $[\eta] = 3.92 \times 10^{-4} \overline{M}_n^{0.75}$. From P. F. Onyon, *J. Polym. Sci.*, **22**, 13 (1956).

[e] Total weight of (SCMKA + AN) = 15 g/100 ml aq. $ZnCl_2$.

[f] Reaction time prolonged to 2 hr.

Separation and Characterization of the Graft Products

A graft product with 10% SCMKA content was fractionated at 25°C by a fractional precipitation method, using DMSO and toluene as solvent and precipitant, respectively.[12] The graft products with an average SCMKA content higher than 10% were partially insoluble in DMSO and dimethylformamide (DMF). These graft products were separated into soluble and insoluble fractions with DMF. The fractions were then subjected to selective hydrolysis of the keratin backbone.

The selective hydrolysis of the keratin backbone in the graft copolymer was performed in two steps by immersing the sample in 6*N* HCl; at first, the reaction temperature was kept constant at 25°C for 24 hr, and then it was raised to 50°C and kept constant for an additional 60 min. The two-step process improved the degree of hydrolysis of the keratin without side reaction of nitrile groups in PAN.

The dinitrophenylation (DNP-polymer) of the amino acid endgroups of the isolated polymer was carried out according to a procedure similar to that reported by Arai[13]; the isolated polymer was suspended in aqueous sodium bicarbonate and treated with a solution of 2,4-dinitrofluorobenzene in ethanol.

The purified DNP-polymers were dissolved in hexafluoroisopropanol (HFIP), and the absorption spectra were taken by using a Shimadzu UV-200S spectrophotometer with 1-cm quartz cells. The absorption spectra of the isolated polymers without dinitrophenylation were also measured for the sake of comparison. For the calculation of the number of DNP-amino acid endgroups, the molar extinction coefficient of DNP-DL-methionine in HFIP at 340 nm was used.[13]

The content of SCMKA was determined by IR spectroscopy using the CN band at 2230 cm^{-1} and amide band II at 1540 cm^{-1} as internal standards. The IR spectra were recorded on a Perkin–Elmer model 521 spectrophotometer.

Preparation of Membranes

For preparing the membranes, 60% aqueous $ZnCl_2$ solution which contained 8–10% of the graft copolymer sample was used. After bubbles in the casting solution were carefully removed, the membranes were cast with a doctor's knife on a Teflon plate and then regenerated with ice-cold water. The membranes thus obtained were washed thoroughly with 0.5% citric acid and stored in distilled water containing 20% ethanol. The resulting dry membrane thickness was about 2–4 μm. For comparison, some membranes were prepared directly from the reaction mixture for polymerization. Porous PAN membranes were prepared by selective hydrolysis of the keratin domains in the keratin–PAN graft copolymer membrane. The hydrolysis was performed in two steps by immersing the membrane in 6*N* HCl as mentioned above, but the membrane was fixed between two Teflon rings during the whole procedure, to prevent shrinking.

Amino Acid Analysis

Keratins were hydrolyzed *in vacuo* at 110°C for 24 hr with 6*N* HCl. The HCl was removed *in vacuo* and the content of amino acids in the hydrolyzate was estimated by using a Hitachi KLA-5 amino acid analyzer. The results have been expressed in terms of moles per 100 moles amino acids.

Scanning Electron Microscopy

The membrane surface was coated with a thin layer (100–150 Å) of gold by using ion coater Eiko IB-3, and was then examined in a Hitachi S-310 scanning electron microscope (SEM).

RESULTS AND DISCUSSION

Low-sulfur fraction of S-carboxymethylated wool keratin (SCMKA) is soluble in 60% aqueous $ZnCl_2$ solution, and the reaction mixture was homogeneous during the whole polymerization process. The results of graft copolymerization under various conditions are summarized in Table I, from which one finds that the total conversions of AN monomer to polymer were very high. It should be noted that total conversions in excess of 90% were attained by the stepwise addition of initiator. For the samples 10-IR and 30-IR, which were obtained by three-portionwise addition of initiator, the total conversions approached almost 100%. These samples were used as reference samples for the determination of the SCMKA content by IR spectroscopy. Table I also gives the molecular weights of PAN isolated by acid hydrolysis of the SCMKA backbone. The molecular weights of PAN formed appear to decrease as the weight ratio of SCMKA to AN in the feed increased. These results were somewhat similar to those for the soybean protein–AN system.[7] On the other hand, in our study with SCMKA, the weight ratio of SCMKA protein to AN monomer practically did not affect the total conversion of monomer to polymer within the polymerization conditions employed here.

Preliminary experiments on the solubility of the graft products indicated that the samples of series 10N were almost soluble in selective solvents for PAN, such as DMSO and DMF. The solubility behavior of the graft products gives direct evidences that true grafting to SCMKA protein had occurred and also that most of the SCMKA was grafted. Sample 10N-2 was fractionated by the fractional precipitation method, using DMSO and toluene as solvent and precipitant, respectively.[12] The fractionation result is shown in Table II, in which the molecular weights of the grafted and homo-PAN are also given. The IR spectra indicated fraction 2 to be the true grafted polymer and the other fractions to contain mainly AN homopolymers. The polymerization was accompanied by the formation of a large amount of homopolymers. The graft efficiency, i.e., weight of PAN grafted to total weight of PAN formed, was found to be 13%. On

TABLE II
Fractionation Results of Sample 10N-2 Obtained by DMSO-Toluene System

Fr. no.	Weight fraction, %	SCMKA content, %	$[\eta]$, dl/g DMF, 25°C	$\bar{M}_n \times 10^{-4}$ [a]
1	0.7	—	—	—
2	20.3	40	2.92[b]	14.5
3	49.0	~2	3.57	19.0
4	17.0	~2	1.67	6.9
5	10.0	~2	0.85	2.8
6	3.0	~5	—	—

[a] See footnote (d) in Table I.

[b] For grafted PAN obtained by acid hydrolysis.

the other hand, the graft products, for which the weight ratio of SCMKA in the feed was more than 10%, were partially insoluble in DMSO and DMF, although they were completely soluble in 60% aqueous $ZnCl_2$ solution.

In Table III the DMF extraction results from the samples with higher SCMKA contents are given. In order to characterize the fractions, the PAN chains were isolated by acid hydrolysis of the SCMKA backbone. The IR spectra of the fractions soluble in DMF showed them to be mostly homo-PAN, while according to amino acid endgroup analysis in isolated PAN as discussed later, it was proved that the insoluble residues consisted mainly of grafted polymer. The yield of the grafted polymer, insoluble in DMF, increased with increasing SCMKA content but decreased with increasing total conversion of AN monomer to polymer. The graft efficiencies are those calculated from the ratio of soluble and insoluble fractions.

Table III also gives the molecular weights of isolated PAN. In the homogeneous graft copolymerization system, it is known that the growing radicals are subjected to the same terminating mechanism, and little difference can be observed between the molecular weights of homopolymer and the corresponding grafted polymer.[14] In the present system, however, the average molecular weights of homo-PAN are rather less than those of the grafted PAN. The results obtained here might be explained by considering the similar effect as observed in the heterogeneous graft copolymerization system.[15] The viscosity of an aqueous 60% $ZnCl_2$ solution is very high by nature, and those of the polymerization solutions were extremely high at high conversions of monomer. Such a high viscosity of the polymerization solution and the difference in solubility characteristics of SCMKA and PAN in the reaction mixture might be correlated with the observed effect, resulting in the difference in the molecular weights of the grafted and homo-PAN.

Additional support for true grafting can be obtained through the detection

TABLE III
Characterization Results on DMF-Soluble and DMF-Insoluble Fractions

Sample code	DMF extract, %	SCMKA content, %	Isolated PAN[a]		Graft efficiency, %
			$[\eta]$, dl/g	$\bar{M}_n \times 10^{-4}$	
20N		22	1.40	5.5	21
Insoluble (20N-I)	36	58[b]	1.49	5.9	
Soluble (20N-S)	64	~3	1.30	5.0	
30N		32	2.17	9.8	48
Insoluble (30N-I)	66	47[b]	2.72	13.2	
Soluble (30N-S)	34	~2	1.52	6.1	
30D		41	1.44	5.7	53
Insoluble (30D-I)	70	57[b]	1.51	6.0	
Soluble (30D-S)	30	~3	1.31	5.0	

[a] See footnote (d) in Table I.
[b] Calculated from the SCMKA content in the soluble fraction.

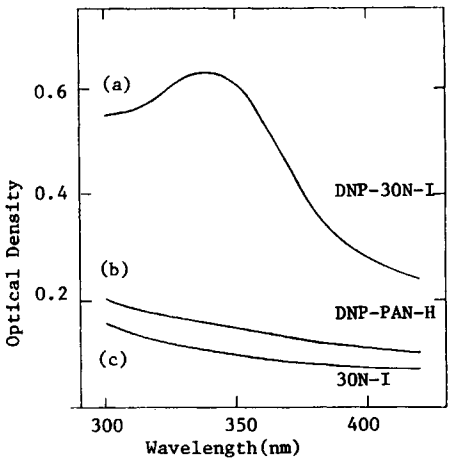

Fig. 1. UV absorption spectra of (a) DNP-polymer (DNP-30N-I), (b) dinitrophenylated homo-PAN (DNP-PAN-H), and (c) isolated PAN (30N-I).

of amino acid endgroups in isolated PAN chains. This method is based on the dinitrophenylation of amino acid endgroups incorporated in the isolated polymer.[13] A typical UV absorption spectrum for DNP-polymer (DNP-30N-I) is shown in Figure 1 together with those of isolated PAN (30N-I) without dinitrophenylation and of a sample of PAN-H dinitrophenylated under identical conditions. The absorption maximum around 350 nm is characteristic of DNP-amino acids.[13]

From the amino acid endgroup analysis, one can also estimate the number-average molecular weight $\overline{M}_n$ of grafted polymers per amino acid endgroup; the value of $\epsilon_m/E_{1\text{ cm}}^{0.1\%}$ gives $\overline{M}_n$ of grafted PAN per DNP-amino acid endgroup. Here, ϵ_m is the molar extinction coefficient of DNP-DL-methionine at 340 nm in HFIP[13] and $E_{1\text{ cm}}^{0.1\%}$ is the optical density of DNP-endgroups in 0.1% w/v polymer solution at 340 nm. The value of $\overline{M}_n$ per DNP-amino acid endgroup corresponds to that of grafted PAN, in case that the number of DNP-amino acid endgroups per polymer chain is unity. The results on samples 20N-I, 30N-I, and 30D-I are shown in Table IV. It can be seen that the values of $\overline{M}_n$ per DNP-amino acid endgroup are not far from those of grafted PAN from viscosity measurement. Taking it into consideration that the molecular weight of SCMKA is ca. 5×10^4, the value of SCMKA contents of the samples indicates that the number of grafting sites is, at most, 1.0 per SCMKA molecule.

TABLE IV
Number-Average Molecular Weights of Grafted PAN Calculated from Viscosity Measurement and Amino Acid Endgroup Analysis

Sample code	SCMKA[a] content, %	Number-average molecular weight of grafted PAN, $\overline{M}_n \times 10^{-4}$	
		Viscosity[a]	Amino acid endgroup analysis
20N-I	58	5.9	3.5
30N-I	47	13.2	11.0
30D-I	57	6.0	5.0

[a] See Table III.

In an attempt to elucidate the grafting sites on the SCMKA molecule, we have examined the amino acid composition of grafted SCMKA. Table V shows the average amino acid composition of grafted SCMKA from five different graft products together with that of original SCMKA. Slight decreases in the contents of SCM-Cys, Met, and Tyr in the grafted SCMKA suggest merely that these amino acid residues might be grafting sites. This result is necessarily associated with the fact that the number of grafting sites was, at most, 1.0 per SCMKA molecule. No exact information was obtained concerning the actual grafting sites on the SCMKA molecule from amino acid analyses of the grafted SCMKA. Recently, Kojima et al.[16] have indicated that the basic amino acids, such as histidine and lysine, may play an important role in the grafting of methyl methacrylate by tri-*n*-butylborane onto blood proteins. In the present case, the contents of these basic amino acids in the grafted SCMKA remained unchanged.

The keratin–PAN graft copolymer membranes were prepared from 60% aqueous $ZnCl_2$ solution by using ice-cold water as the regenerating medium. All the membranes were transparent. On the other hand, it was impossible to prepare the transparent membranes from simple mixtures of SCMKA and PAN. Figures 2(a) and 3(a) show scanning electron micrographs of 10N-1 and 30N membranes. For the sake of comparison, Figure 4 shows that of homo-PAN membrane prepared under identical conditions. As can be seen in Figures 2(a) and 3(a), the 10N-1 and 30N membranes have relatively flat surfaces where the dark domains are dispersed in the lighter matrix. The darker and lighter phases correspond to the keratin and PAN phases, respectively. The formation of keratin domains is the result of incompatibility of keratin and PAN, and the domain size of keratin increases with SCMKA content when the SCMKA content does not exceed 30%. In the case of the 30N membrane, the surface structure consists of rather spherical keratin domains regularly distributed in the PAN

TABLE V
Amino Acid Composition of SCMKA and Grafted SCMKA[a]

Amino acid	SCMKA	Grafted[b] SCMKA
Lys	2.9	3.2 ± 0.1
His	0.6	0.5
Arg	6.9	7.4 ± 0.5
SCM-Cys	6.8	5.4 ± 1.0
Asp	8.0	8.3 ± 0.3
Thr	4.7	5.1 ± 0.2
Ser	9.3	9.4 ± 0.3
Glu	16.4	14.2 ± 0.5
Pro	3.8	5.1 ± 0.3
Gly	8.5	10.4 ± 0.5
Ala	6.0	6.6 ± 0.1
Val	5.0	5.9 ± 0.2
Met	0.7	tr.
Ile	9.3	9.0 ± 0.2
Tyr	5.0	3.1 ± 0.5
Phe	2.6	2.7 ± 0.1

[a] Values given as moles/100 moles amino acids.
[b] Average values for five different graft products.

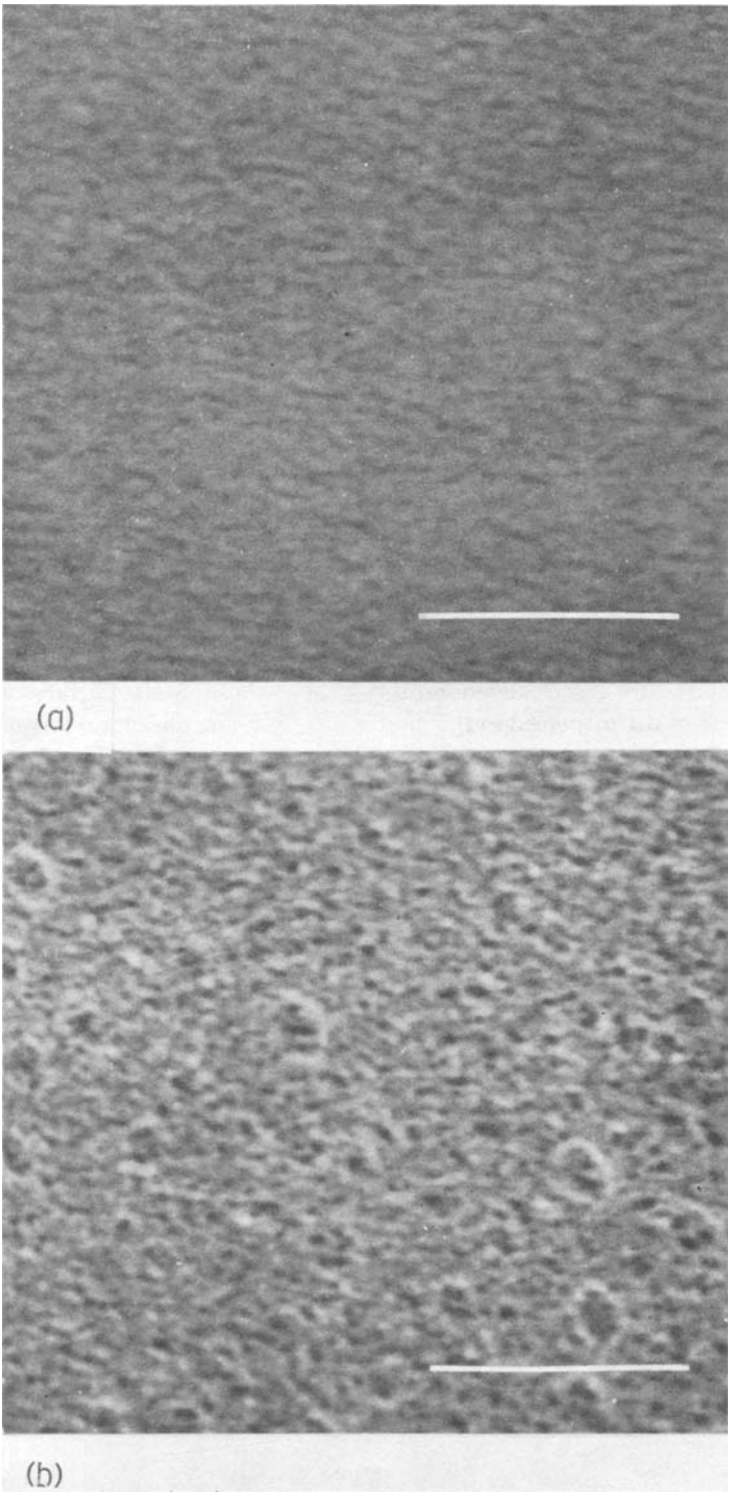

Fig. 2. Scanning electron micrographs of (a) 10N-1 membrane and (b) porous membrane obtained by selective hydrolysis of the keratin domains in 10N-1 membrane. The scale bar indicates 1 μm length.

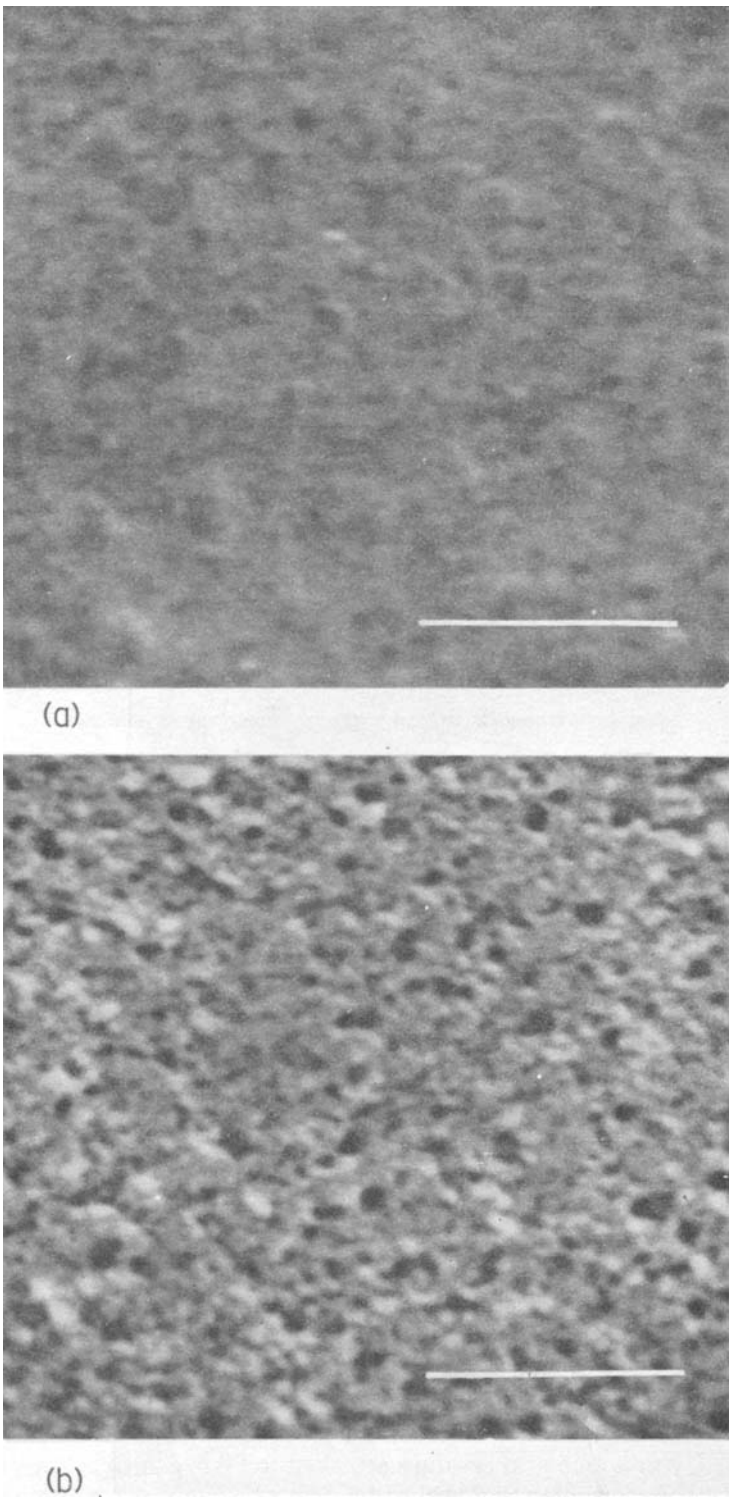

Fig. 3. Scanning electron micrographs of (a) 30N membrane and (b) porous membrane obtained by selective hydrolysis of the keratin domains in 30N membrane. The scale bar indicates 1 μm length.

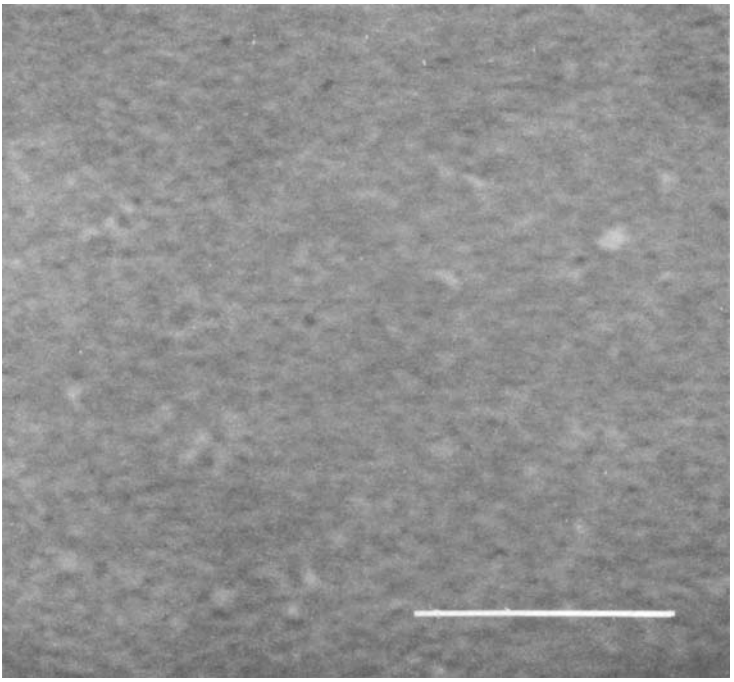

Fig. 4. Scanning electron micrograph of homopolyacrylonitrile.

matrix. Almost no difference was observed between front and back sides of the membranes. It should be mentioned here that in the case of monomer conversions higher than 90%, membranes could be successfully prepared directly from the polymerization solution, having a surface structure similar to that shown in Figures 2(a) and 3(a).

In addition to the formation of keratin domains, all the membranes obtained would presumably have micropores of less than 100 Å diameter, because these membranes were prepared from concentrated aqueous salt solution by using water as the regenerating medium. Unfortunately, micropores of less than 100 Å diameter cannot be seen directly by scanning electron microscopy (SEM).

Finally, we attempted to prepare a new type of porous PAN membrane by selective hydrolysis of the keratin domains. Figure 5 shows the IR spectra of a 10N-1 membrane and its hydrolyzed membrane. The spectrum of the former shows characteristic absorption bands at 2230 and 1540 cm^{-1}, which may be assignable to the nitrile group in PAN and to amide band II in SCMKA, respectively, while amide band II is not found in that of the latter. Figures 2(b) and 3(b) show scanning electron micrographs of the 10N-1 and 30N membranes, respectively, after selective hydrolysis of keratin domains. The darker areas are due to the pores originating from keratin domains. Micropores of 200 to 500 Å diameter for the 10N-1 membrane and of 500 to 1000 Å for 30N, respectively, can be directly observed. The inner wall of their micropores is considered to be charged with amino acid residues attached to PAN chains as the endgroup. In fact, the presence of amino acid endgroups was confirmed by dinitrophenylation of isolated PAN chains as mentioned above.

Preliminary experiments showed that these porous PAN membranes exhibited

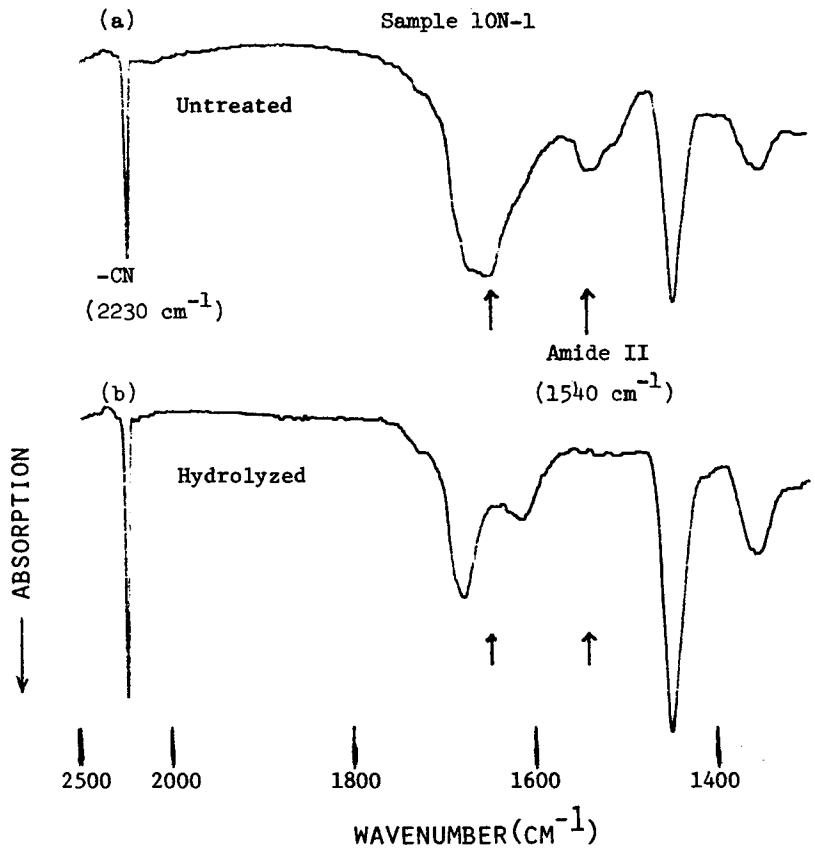

Fig. 5. IR spectra of (a) 10N-1 membrane and (b) hydrolyzed 10N-1 membrane.

a much greater permeability for sulfobromophthalein sodium tetrahydrate with a molecular weight of 833 in comparison with unhydrolyzed membranes. A detailed study on the permeability of solute through these membranes is in progress.

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