

ORIGINAL ARTICLE

Complete NMR assignment of a sulfonated aromatic block copolymer via heteronuclear single-quantum correlation, heteronuclear multiple-bond correlation and heteronuclear single-quantum correlation total correlation spectroscopy

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A sulfonated aromatic block copolymer (SABC), consisting of hydrophobic and hydrophilic blocks, was analyzed by heteronuclear single-quantum correlation (HSQC), heteronuclear multiple-bond correlation (HMBC) and HSQC total correlation spectroscopy (HSQC–TOCSY). Because of its complicated chemical structure with five different phenylene rings, 12 types of ¹H signals and 24 types of ¹³C signals were observed in a narrow chemical shift range (7.0–8.0 p.p.m. for ¹H and 118–162 p.p.m. for ¹³C). To improve the ¹H signal separation, the temperature conditions for the ¹H nuclear magnetic resonance (NMR) experiments were optimized. Moreover, ¹H and ¹³C NMR signal assignments for the hydrophobic blocks were performed using HSQC and HMBC, with reference to the assignments of a model oligomer. For the hydrophilic blocks, furthermore, HSQC–TOCSY techniques were applied. As a result of these studies, complete ¹H and ¹³C NMR signal assignments were made for the SABC. The ion-exchange capacity (IEC) and the copolymerization composition were calculated using the ¹H NMR assignments for the SABC, and the IEC value obtained in this way was consistent with that obtained via titration.

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INTRODUCTION

Proton exchange membrane (PEM) fuel cells (PEMFCs) are expected to be used as alternative power sources, especially for applications in electric vehicles and residential units. PEMs are an essential component of PEMFCs. Although perfluorinated sulfonic acid polymers, such as Nafion, are most often used as PEMs, nonfluorinated aromatic ionomers have been extensively investigated. Sulfonated aromatic block copolymers (SABCs) are one of nonfluorinated PEM materials, and their excellent physical properties have been reported.^{1–5} To further improve the properties of SABCs, it is crucial to understand how their physical properties depend upon their chemical structure. In addition, for practical applications, the degradation mechanisms during PEMFC operation, resulting in changes of the chemical structure, should be investigated in detail. A necessary first step is to determine the precise chemical structures of pristine membranes. As nuclear magnetic resonance (NMR) is a powerful tool for determining the chemical structures of polymers, it

is possible to elucidate the chemical structures of pristine and post-test membranes. The complex structure of SABCs makes the NMR analysis difficult: for example, the present case contains five different phenylene rings. Here, we report a successful NMR assignment of pristine SABC based on heteronuclear single-quantum correlation (HSQC),^{6–9} heteronuclear multiple-bond correlation (HMBC)^{10–12} and HSQC total correlation spectroscopy (HSQC–TOCSY)^{13,14} techniques.

EXPERIMENTAL PROCEDURES

Materials

The chemical structure, number average molecular weight (*M_n*), molecular weight distribution (*M_w/M_n*), copolymer composition (*w/z*) and calculated IEC of the SABC are shown in Figure 1. The SABC consists of hydrophobic and hydrophilic blocks. The hydrophilic blocks contain a substructure of sulfonated fluorenylidene biphenylene. The SABC was synthesized with F-terminated telechelic oligomers¹ (DP (degree of polymerization) = 21) and

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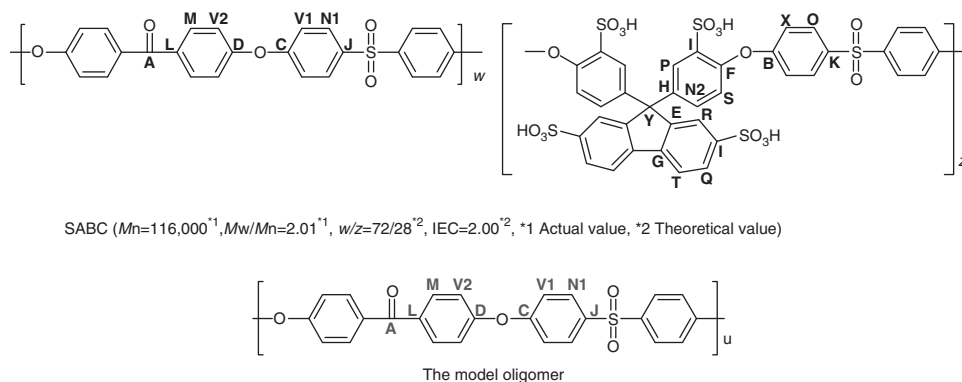


Figure 1 The chemical structures of the SABC and the model oligomer.

OH-terminated telechelic oligomers¹ (DP = 8). The IEC of the SABC was measured by titration after the ion exchange, resulting in a value of 1.78 meq g⁻¹.

We used F-terminated telechelic oligomer as the model oligomer for the NMR assignment of the SABC. The chemical structure of the model oligomer is also shown in Figure 1.

NMR experiments

All NMR spectra were acquired with a UNITY INOVA 500 (Varian, Inc., Palo Alto, CA, USA) in deuterated dimethyl sulfoxide (using tetramethylsilane as the internal reference) at frequencies of 499.8 and 125.7 MHz for ¹H and ¹³C NMR, respectively. The concentration of the sample solutions was 50 mg in 0.6 ml. ¹H NMR experiments were conducted at 25, 50, 70, 90 and 110 °C. All NMR experiments were executed at 60 °C. In the HSQC, HMB and HSQC–TOCSY experiments, which were all gradient-selected experiments, a total of 256, 400 and 256 spectra, each containing 2048 data points, were accumulated, respectively.

RESULTS AND DISCUSSION

¹H NMR spectrum of the SABC and the temperature effect

Figure 2a shows the ¹H NMR spectrum for the SABC at 25 °C. The lower-case letter labels in Figure 2 represent ¹H signals (the detail is described later). Because of the complex structure, with five different phenylene rings, 12 types of ¹H signals were observed in the narrow chemical shift range between 7.0 and 8.0 p.p.m. As these ¹H signals are overlapping or close, a precise NMR assignment was not possible with such insufficient ¹H signal separation. Therefore, the following two strategies were executed to separate the signals:

- The solvent and the temperature effect for ¹H NMR experiments were examined. By optimizing the solvent and temperature conditions, the signals were sharpened and separated.
- The ¹H and ¹³C signals of the hydrophobic blocks were assigned in advance using the model oligomer, and then those of the hydrophilic blocks were assigned. Thus, the signals of the hydrophobic and hydrophilic blocks were separated.

First, the solubility of the SABC in other solvents (pyridine, 1,1,1,3,3,3-hexafluoroisopropanol and methanol) was examined. However, the SABC was insoluble in any of these solvents. Next, the effect of temperature on the ¹H NMR experiments was examined. Figure 2b–e show the ¹H NMR spectra of SABC at 50, 70, 90 and 110 °C, respectively. In general, signals become sharper at higher temperatures. The separation of the signals within the temperature range is as follows: The signal ‘q’ was separated from ‘m’ and ‘o’ at 25, 50 and 70 °C, and the signal separation was better at lower

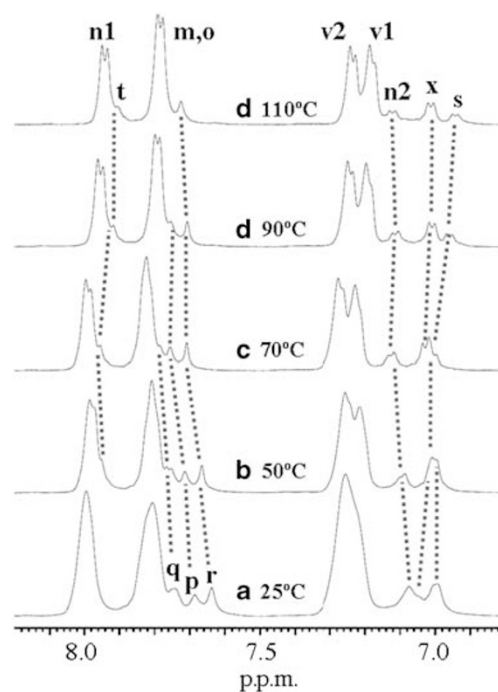


Figure 2 ¹H NMR spectra of the SABC at (a) 25 °C, (b) 50 °C, (c) 70 °C, (d) 90 °C and (e) 110 °C. The lower-case letter labels attached to the ¹H signals (from ‘m’ to ‘x’) correspond to the upper-case letter labels (from ‘M’ to ‘X’) shown in the chemical structure of the SABC (Figure 1).

temperatures. The signals ‘s’, ‘x’, ‘n2’, ‘v1’ and ‘v2’ were separated from other signals above 50 °C, and the separations were better at higher temperatures. Accordingly, 50 and 70 °C were the most suitable for the ¹H NMR experiment. It was determined that all the NMR experiments, which took more than 5 days, would be conducted at 60 °C so that the chemical structure of the SABC would not change during the experiments.

NMR assignment for the SABC

Figure 3 shows the ¹H NMR (top), ¹³C NMR (middle) and HSQC (bottom)^{6–9} spectra of the SABC at 60 °C.

In the ¹H NMR spectrum at 60 °C, the lower-case letter labels from ‘m’ to ‘x’ (except for ‘u’ and ‘w’) are attached to all ¹H signals, and they represent the ¹H signals (described in detail later).

In the ¹³C NMR spectrum, 24 different types of signals arising from five different phenylene rings are observed from 117.8 to 161.6 p.p.m..

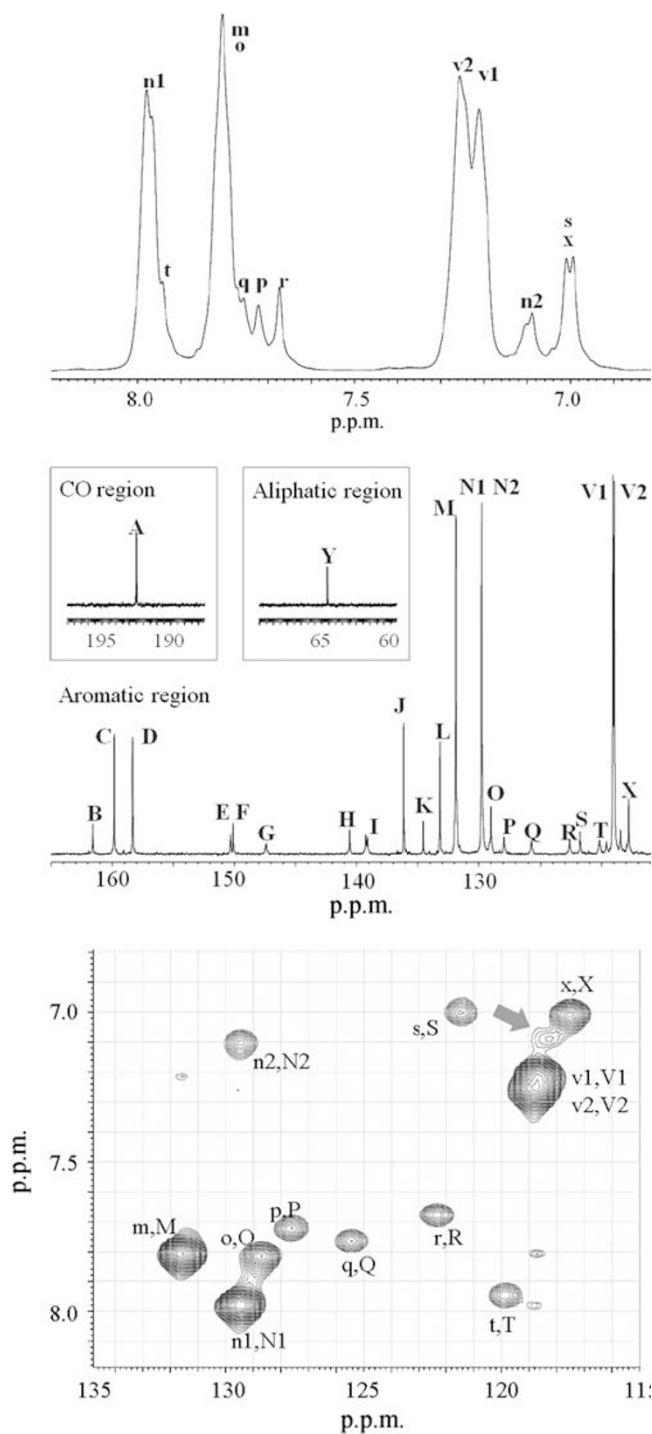


Figure 3 ^1H NMR (top), ^{13}C NMR (middle) and HSQC (bottom) spectra of the SABC at 60 °C. In HSQC, the vertical axis represents the ^1H chemical shift, and the horizontal axis represents the ^{13}C chemical shift. $^1J_{\text{CH}} = 140\text{ Hz}$ was used.

The upper-case letter labels from 'A' to 'Y' (except for 'U' and 'W') are attached to the ^{13}C signals from 192.5 to 64.4 p.p.m., in order. The upper-case letter labels represent the ^{13}C signals. The chemical shifts of many ^{13}C signals are close. 'N1' and 'N2' completely overlap, and 'V1' and 'V2' almost overlap in the ^{13}C NMR spectrum. The two pairs of signals are separated in HSQC and HMBC

Table 1 ^1H and ^{13}C chemical shift assignments for the SABC and the model oligomer

	SABC		The model oligomer			
	^{13}C chemical shift (p.p.m.)	^1H chemical shift (p.p.m.) ^a	^{13}C chemical shift (p.p.m.)	^1H chemical shift (p.p.m.)		
A	192.5	—	A	193.8	—	—
B	161.6	—	—	—	—	—
C	159.8	—	C	160.5	—	—
D	158.3	—	D	159.0	—	—
E	150.3	—	—	—	—	—
F	150.1	—	—	—	—	—
G	147.4	—	—	—	—	—
H	140.6	—	—	—	—	—
I	139.2, 139.1	—	—	—	—	—
J	136.1	—	J	136.6	—	—
K	134.5	—	—	—	—	—
L	133.2	—	L	133.7	—	—
M	131.9	m	M	132.4	m	7.85
N1	129.8	n1	N1	130.0	n1	7.95
N2	129.8	n2	—	—	—	—
O	129.0	o	—	—	—	—
P	127.9	p	—	—	—	—
Q	125.7	q	—	—	—	—
R	122.6	r	—	—	—	—
S	121.8	s	—	—	—	—
T	120.2	t	—	—	—	—
V1	119.1	v1	V1	119.1	v1	7.15
V2	119.0	v2	V2	119.1	v2	7.12
X	117.8	x	—	—	—	—
Y	64.4	—	—	—	—	—

^a ^1H chemical shifts of the SABC were obtained from HSQC spectrum.

(shown later). Therefore, they are indicated as 'N1', 'N2', 'V1' and 'V2'. 'I', which will later be assigned to two ^{13}C signals, is not separated into the two ^{13}C signals in HMBC and does not have an HSQC correlation (shown later). Therefore, 'I' is not indicated by 'I1' and 'I2'.

The HSQC spectrum gives correlations between ^1H and ^{13}C nuclei that are one bond apart. All 12 HSQC correlations are observed in Figure 3. The lower-case letter labels are attached to the ^1H signals, corresponding to the upper-case letter labels of the ^{13}C signals with which the ^1H signals correlates in the HSQC spectrum, respectively. The HSQC spectrum indicates that the signals of 'x' and 's' still overlap, and other ^1H signals are observed to be very close together. The correlations of 'x-X' and 's-S' were identified by the intensity of the correlations with 'X' and 'S'.

The following analysis for the hydrophobic blocks was performed using the model oligomer, based on the HMBC technique.

The hydrophobic blocks

Table 1 gives the chemical shift assignments of the model oligomer. These data were obtained based on the results of ^1H NMR, ^{13}C NMR, HSQC, correlation spectroscopy (COSY) and HMBC of the model oligomer, which are shown in Supplementary Figures SII–SI5 of the supplementary information. The correspondence of the signals of the model oligomer to SABC was presumed and is shown in Table 1. The chemical shift assignment of the hydrophobic blocks was confirmed, mainly by HMBC, as follows. The HMBC spectra of SABC are shown

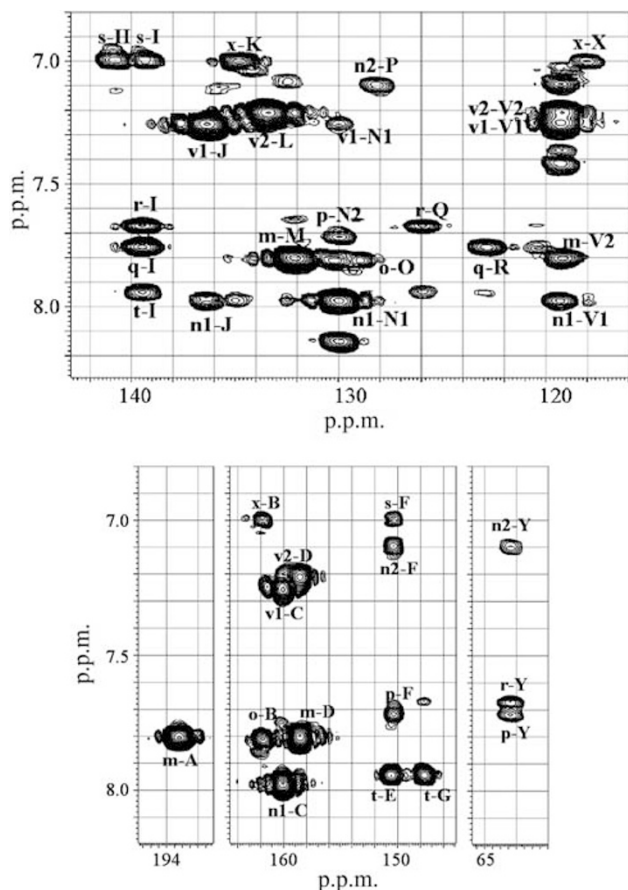


Figure 4 HMBC spectra of the SABC at 60 °C. The vertical axis represents the ^1H chemical shift, and the horizontal axis represents the ^{13}C chemical shift. $^1J_{\text{CH}} = 140\text{ Hz}$ and $^nJ_{\text{CH}} = 8\text{ Hz}$ were used.

in Figure 4. The HMBC spectrum yields correlations between ^1H and ^{13}C nuclei that are two or three bonds apart. All the ^1H and ^{13}C signals from the hydrophobic blocks were confirmed by correlations with the ^1H signals of 'v1', 'v2', 'm' and 'n1' acquired from the HMBC spectra. Thus, the ^1H and ^{13}C signals of the hydrophobic blocks were assigned.

The hydrophilic blocks

We then tried to assign the hydrophilic blocks of the SABC in the same way as for the hydrophobic blocks. However, we were not successful for the following two reasons:

- Some HMBC correlations of n2-S, q-T, r-E, r-G, q-G, n2-H, p-H and o-X are not acquired. The reason was assumed to be that $^nJ_{\text{CH}}$ (8 Hz) was not appropriate for all of the ^1H and ^{13}C signals of the SABC.
- As some of HMBC correlations were not identified for the overlapping between 'x' and 's', 'x' and 's' could not be assigned.

Then, we applied HSQC–TOCSY, in which the correlations are observed without any relation to $^nJ_{\text{CH}}$. It was expected that all HSQC–TOCSY correlations would be acquired and that HSQC–TOCSY would lead to the assignments of 'x' and 's'.

Figure 5 shows the HSQC–TOCSY spectrum of the SABC. In the HSQC–TOCSY spectrum, correlation signals among all ^1H signals

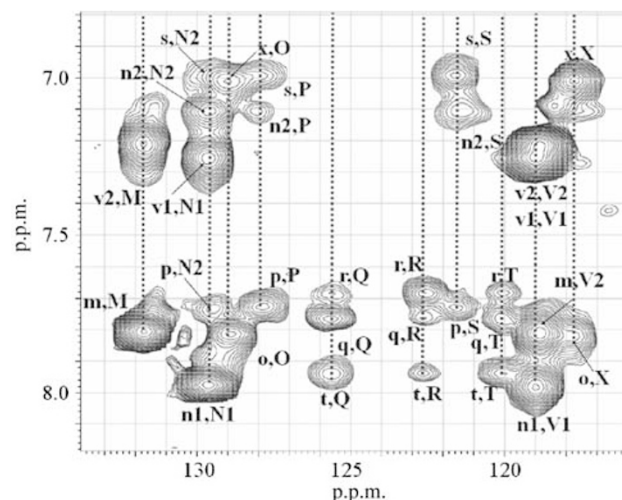


Figure 5 HSQC–TOCSY spectrum of the SABC at 60 °C. The vertical axis represents the ^1H chemical shift, and the horizontal axis represents the ^{13}C chemical shift. A mixing time of 100 ms was used.

having spin–spin coupling (TOCSY correlations) and HSQC correlations are acquired. All HSQC–TOCSY correlations of the hydrophilic blocks were acquired with good separation and were identified as follows: r-Q, q-Q, t-Q, r-R, q-R, t-R, r-T, q-T, t-T, n2-P, p-P, (s-P), n2-S, p-S, s-S, n2-N2, p-N2, (s-N2), o-X, x-X, o-O and (x-O). The correlations shown in parentheses, (s-P), (s-N2) and (x-O), were not identified by the correlations alone because of the overlapping between 's' and 'x'. However, they can be identified by other correlations of p-S, n2-S and o-X. On the basis of the HSQC–TOCSY correlations, the following three groups of proton signals of [r, q, t], [n2, p, s] and [o, x] were determined to exist on the same phenylene rings, respectively. Moreover, 'p', 's' and 'n2' were assigned based on the HMBC correlations for n2-P, n2-Y and p-Y, and 'r', 'q' and 't' were assigned based on the HMBC correlations for q-R and r-Y. Consequently, the HMBC correlations from 's' and 'x' of s-F, s-H, s-I, x-B, x-K and x-X were identified, and they led to the assignment of 's' and 'x'. Thus, all ^1H and ^{13}C signals were assigned by other HMBC correlations without inconsistency. The results are summarized in Table 1.

As described above, the complete NMR assignment of the SABC was performed, and it was supported by the following two facts:

- In Figure 2d and e, 'n2', 'x' and 's' indicate doublet splitting and the ^1H signal strengths.
- In Figure 2b, 'q' indicates doublet splitting.

Generally, in NMR spectra of block copolymers, NMR signals based on the junction points for each block are observed separately. However, in the NMR spectra of the SABC under the experimental conditions in this study, the signals of the junction points for each block were not separated from each other for the following reason. In both the hydrophobic blocks and the hydrophilic blocks of the SABC, the 1,4-phenylene ether-sulfone (ES) unit is present. That is, two types of ES units neighboring the 1,4-phenylene ether-ketone unit and neighboring sulfonated fluorenylidene biphenylene exist, and they have already been assigned. Thus, the ^1H and ^{13}C NMR signals of the ES unit separated based on diad sequence and did not separate based on triad-or-higher sequences. The NMR signals of junction points for

Table 2 The integrated intensities of the ^1H signals and the formula for calculating the copolymer composition and the IEC

The integrated intensities of the ^1H signals	
$I(v1, v2)^a$	310.57
$I(s, x, n2)^b$	100.00
The calculating formula	
w (mol%):	
$I(v1, v2) \times 100 / (I(s, x, n2) + I(v1, v2))$	
z (mol%):	
$I(s, x, v2) \times 100 / (I(s, x, n2) + I(v1, v2))$	
IEC (eq g^{-1}):	
$\frac{z \text{ mol\%} \times 4}{(w \text{ mol\%} \times \text{m.w.HPO}^c + z \text{ mol\%} \times \text{m.w.HPI}^d)}$	

^aIntegrated intensities of 'v1' and 'v2' signals.^bIntegrated intensities of 's', 'x' and 'n2' signals.^cm.w. of the hydrophobic blocks ($\text{C}_{25}\text{H}_{16}\text{O}_5\text{S}$): 428.46.^dm.w. of the hydrophilic blocks ($\text{C}_{37}\text{H}_{24}\text{O}_{16}\text{S}_5$): 884.90.

each block arise when the NMR signals of the ES unit are separated on the basis of triad-or-higher sequences.

Copolymer composition and IEC

The IEC and copolymer composition (w/z mol%) were calculated based on the integrated intensities of some ^1H signal areas. Table 2 gives the integrated intensities of some ^1H signal areas and the calculating formula. The copolymer composition of $w/z = 76/24$ (mol%) was calculated. The IEC obtained by NMR was 1.81 meq g^{-1} , which was in good agreement with that obtained by titration (1.78 meq g^{-1}). Consequently, the validity of the NMR assignment was supported.

As mentioned above, the theoretical composition ratio is $w/z = 72/28$ (mol%). In contrast, $w/z = 76/24$ (mol%) was obtained from the NMR results. The composition ratio of z (mol%) obtained from NMR was smaller than the theoretical one. It was assumed that the difference in the two values was based on the following reasoning: The SABC was synthesized by sulfonation after copolymerization of the two telechelic oligomers. The theoretical composition ratio (w/z) is 72/28 when all the fluorenylidene biphenylene substructures are substituted by four sulfo groups. Sulfonated fluorenylidene biphenylene substructures with three or fewer sulfo groups existed, and the NMR signals were broadened or were not observed all. That is because the sulfonated substructures with three or fewer sulfo groups do not dissolve easily in DMSO. The HSQC signal indicated by an arrow in Figure 3 was presumed to be assigned to one of the less sulfonated substructures.

CONCLUSIONS

Complete NMR assignment of the SABC was performed using HSQC, HMBC and HSQC–TOCSY. Owing to the complex structure of the SABC with sequenced hydrophilic and hydrophobic blocks, the ^1H NMR spectrum of the SABC at 25°C demonstrates insufficient separation of ^1H signals. The signal separation was improved by optimizing the temperature conditions for the ^1H NMR experiment, and all NMR experiments were conducted at 60°C . The ^1H and ^{13}C NMR chemical shift assignments of the hydrophobic blocks were executed based on HSQC and HMBC with reference to those of a

model oligomer. For the ^1H and ^{13}C NMR chemical shift assignments of the hydrophilic blocks, HMBC correlations that were necessary and sufficient for the complete assignment were not acquired, and some HMBC correlations were not identified. Then, HSQC–TOCSY was applied. All HSQC–TOCSY correlations were obtained, and HSQC–TOCSY identified the unidentified HMBC correlations. Thus, all of the ^1H and ^{13}C signals of the hydrophilic blocks were successfully assigned. The copolymer composition ($w/z = 76/24$ mol%) and the IEC (1.81 meq g^{-1}) for the SABC were obtained based on the chemical shift assignment and the integrated intensities of some ^1H NMR signals. The IEC value obtained from the ^1H NMR spectra was consistent with that obtained via titration (1.78 meq g^{-1}).

The authors performed a complete NMR assignment of an SABC. This approach is applicable to other emerging aromatic polymer with complex structures and the post-test membrane. The application to post-test membranes elucidates the changes in the chemical structure and the degradation mechanism of the polymer membranes, which will further promote the development of high-performance PEM materials.

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Supplementary Information accompanies the paper on Polymer Journal website (<http://www.nature.com/pj>)