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Equivalent Cross-Relaxation Rate in Magnetic Resonance Imaging. A Novel Physical Parameter for Evaluation of Conditions of Water in Synthetic Copolymer Gels

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ABSTRACT: Being similar to values of saturation transfer ratio (STR) in magnetic resonance imaging (MRI) described in our previous paper, values of equivalent cross-relaxation rate (ECR) correlated well with conditions of water in various copolymer gels composed of any two or three monomers among 2-hydroxyethyl methacrylate (HEMA), glycidyl methacrylate (GMA), *N*-vinyl-2-pyrrolidinone (N-VP), methyl methacrylate (MMA), and benzyl methacrylate (BMA). The water conditions in these copolymer gels were characterized in terms of not only total also free and constrained water contents in the matrix. The MRI measurement was performed by using an off-resonance saturation pulse under conventional field-echo imaging at frequency apart within ±75 ppm from the water resonance frequency. The ECR values were readily to determine and non-time consuming parameter for cross relaxation rate. Both the STR and ECR values at the frequency offset by 4-ppm were divided the sample gels two classes, which must correspond to hydrophilic or hydrophobic ones. In the case of 4-ppm offset, the ECR values were more sensitive parameters than the STR values for evaluation of water condition in the gels and the sensitivity was nearly equivalent to cross-relaxation rate itself. Moreover the ECR values showed excellent correlation with the HEMA as well as constrained water contents in the series of copolymers. Thus the ECR values in the MRI measurement would be adopted as a novel parameter helpful for molecular/material characterization of synthetic copolymers without invasive process to the samples.

KEY WORDS Equivalent Cross-Relaxation Rate / Saturation Transfer Ratio / Magnetic Resonance Imaging / Constrained Water / Hydrophilicity / Copolymer Gel / 2-Hydroxyethyl Methacrylate / *N*-Vinyl-2-pyrrolidinone / Methyl Methacrylate /

The chemical and physical nature of synthetic copolymer gels depends on not only the chemical structure but condition of water included in a gel network. In description about the condition of water in the hydrophilic gels, free water, constrained (but frozen) water, and non-frozen water were often referred. The balance of water in such difference conditions as well as the total amount of water involved in the copolymer gels should be significant factors for determination of the hydrophilicity of the gels.[1–7]

Nuclear magnetic resonance (NMR) technique has played an important part to elucidate various structural problems of various (co)polymers. In conventional NMR, the resonance frequency of the special nucleus containing in a sample, for example ^{1}H, ^{13}C, and so on, and its relative strength are mainly related to the structure of the sample. The magnetic resonance imaging (MRI) method is another application of NMR technique and has developed remarkably in the clinical medicine. We have already pointed out the correlation between saturation transfer ratio (STR) in MRI measurement and histological parameters of invasive ductile carcinomas in human breast.[8]

Our first interest was whether there was the correlation between STR values and traditional parameters for the hydrophilicity of synthetic copolymer gels, such as the solubility parameter. Finding any satisfactory correlation would promote to explore a new route by MRI for the structural or material characterization of artificial polymer gels containing water as well as polymeric tissue *in vivo*. We has been assessed the STR values for T_1-weighted MRI of various synthetic polymer gels, irradiating three different off resonance pulses at 75, 19, and 4 ppm far from the water resonance frequency.[9] Through those measurements, it was found that STR-75, -19, and -4 values were able to adopt as a novel parameter in evaluating hydrophilic character of the samples. The higher values of STR-19 and -4 of the samples indicated higher hydrophilicity as a whole. The absolute values of STR-19 were separable into two levels correlating with only the total water content, and meaning that the sample was hydrophilic or hydrophobic gel as a whole. STR-4 values are correlated with not only the total water content but also water conditions in more detail. Also the close relationship was found between STR-4 values and the solubility parameter of the sample polymers, meaning that these values reflected chemical structure of the polymers. Therefore both the STR value and STR image in the MRI measurement could be new useful tools for evaluation of the hydrophilicity of the synthetic copolymer gels without invasive process to the samples.

Callicot *et al.* have reported that cross-relaxation rate is more sensitivity parameter than STR for characteristic of human breast tissues *in vivo* NMR study.[10] How-

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ever, the imaging of cross-relaxation rate has met with complicate many calculations and procedures. Therefore Callicot *et al.* discussed human breast tissues by values of cross-relaxation rate itself. We found that equivalent cross-relaxation rate (ECR) images were able to calculate with a simple equation described after. Thus we prefer to use ECR values and ECR images for characterization and visually evaluation of water condition in the synthetic copolymer gels. The purpose of this study is to evaluation the correlation between STR, ECR, and water condition in more detail for precisely determine the hydrophilicity of synthetic copolymer gels without invasive process to the samples.

EXPERIMENTAL

Materials

Fourteen synthetic copolymers were kindly provided by Menicon Co. Ltd., which were composed of any two or three monomers among 2-hydroxyethyl methacrylate (HEMA), glycidryl methacrylate (GMA), methyl methacrylate (MMA), benzyl methacrylate (BzMA), and *N*-vinyl-2-pyrrolidinone (N-VP). These were classified into four types in Table I. Gelation was conducted by adding a very small amount ($<0.3\%$) of allyl methacrylate. Their composition was determined by an elemental analysis. The sample copolymer was shaped up 15 ϕmm columnar (length = 100 mm) for MRI measurement.

Determination of Water Contents

The total water content of the sample gels was determined as the weight difference of the gels before and after drying for 18 h at 120℃ and at pressure of 5 mmHg. Also water contents in the sample gels could be determined on the basis of data of differential scanning calorimeter (DSC), which were recorded on Seiko DSC-200 model. Samples (10 mg) were heated from ambient temperature to -60℃ and again up to 50℃ at heating rate of 5℃ min^{-1} under the nitrogen atmosphere. Since free water and constrained (but freezable) water in the gel matrices appeared as different peaks on DSC cooling curves. As for water in non-frozen state, we should determine according to the next equation.

$$\text{Non-frozen water (\%)} = \text{total water (\%)} - (\text{free water (\%)} + \text{freezable constrained water (\%)})$$

MRI Measurement

MRI picture was obtained on Signa model of GE Medical Systems equipped with 1.5 T clinical scanner and GE head coil. Axial localizer images were recorded prior to scanning of the sample gels in the coronal plane. The coronal plane images were preformed as follows. The conventional spoiled-gradient recalled acquisition in the steady state (SPGR) and then saturation-transfer-prepared SPGR (ST-SPGR) were obtained in the plane of the sample's center. We adopted the off-resonance technique for preferential saturation of the immobile protons to evaluate STR values. The single ST-pulse frequency in an interval of 18 ms was employed at the frequency apart by ±75 ppm from that of water resonance. The off-resonance saturation pulse had a bandwidth of 110 Hz and flip angle of 900 degrees. The peak pulse amplitude was employed as 3.6 μT for the ST-sequence. This technique has demonstrated a peak specific absorption rate (SAR) of 2.89 W kg^{-1}, which is satisfied within the FDA recommendation value of 8 W kg^{-1} for any tissues of 1 g. MR imaging parameters were as follows: repetition time = 50 ms; echo time = 5 ms; flip angle = 30°; matrix size = 256 × 192 dots; field of view = 16 cm; section thickness = 5 mm; intersection gap = 2 mm; and slice number = 1.

The regions of interest (ROI) for signal intensity measurement were derived at the same inside of the every sample. The ROI was ellipsoid and possessed a sampling area of 13 mm^2. The STR and ECR values were defined as the percentage of signal loss between unsaturated and saturated images according to the eqs 1 and 2;

$$\text{STR (\%)} = 100 \times (1 - M_S / M_0) \tag{1}$$

$$\text{ECR (\%)} = 100 \times (M_0 / M_S - 1) \tag{2}$$

where M_0 and M_S are the signal intensity in the usual SPGR and in the ST-SPGR images, respectively. The STR-19, -4, and ECR-19, -4 represent STR and ECR values under the irradiation of ST-plus at 19 or 4 ppm apart from water resonance frequency, respectively.

Table I. Chemical composition and longitudinal relaxation time of samples

Sample No.	Composition	Unit / %					Water Content / %*		Longitudinal relaxation time at frequency offset of 4 ppm		
		HEMA	GMA	N-VP	MMA	BzMA	Total	Free	T_1/sec	T_1^*/sec	T_{is}/sec
1	HEMA-*stat*-GMA	65	35				58.0	8.0	0.346	0.154	0.283
2	HEMA-*stat*-GMA	83	17				48.0	7.1	0.223	0.091	0.159
3	N-VP-*stat*-GMA/MMA		32	41	27		59.0	2.9	0.456	0.249	5.61
4	HEMA-*stat*-N-VP	20		80			83.0	66.2	2.48	2.08	18.5
5	HEMA-*stat*-N-VP	40		60			74.0	52.0	1.68	1.24	5.08
6	HEMA-*stat*-N-VP	60		40			61.0	7.5	0.565	0.278	0.559
7	HEMA-*stat*-N-VP	80		20			47.0	6.9	0.254	0.105	0.184
8	N-VP-*stat*-MMA			88	4	8	79.2	58.6	2.32	1.97	22.2
9	N-VP-*stat*-MMA			79	13	8	73.2	51.3	1.59	1.30	10.8
10	N-VP-*stat*-MMA			70	22	8	64.7	42.6	1.51	0.995	5.95
11	N-VP-*stat*-MMA			61	34	5	55.0	22.0	0.925	0.619	2.01
12	N-VP-*stat*-MMA			52	40	8	40.0	7.6	0.507	0.325	0.961
13	N-VP-*stat*-MMA			43	49	8	28.5	0.0	0.337	0.201	0.529
14	N-VP-*stat*-MMA			34	58	8	18.4	0.0	0.217	0.126	0.335

*See text.

The cross-relaxation rate ($1/T_{is}$) was calculated according to the eq 3;

$$1/T_{is} = 1/T_1{}^* - 1/T_1 \qquad (3)$$

where $1/T_1$ and $1/T_1{}^*$ are the longitudinal relaxation rate unsaturated and saturated images. At the steady state, M_S / M_0 is given by Akasaka[11] as follows;

$$M_S / M_0 = T_1{}^* / T_1 \qquad (4)$$

The signal intensity of SPGR was defined as the eq 5;

$$S = [(1-E_1)/(1-E_1\cos\alpha)] \times (M_0 E_2{}' \sin\alpha) \qquad (5)$$

where $E_1 = \exp(-TR/T_1)$, $E_2{}' = \exp(-TE/T_2)$, α = flip angle.

Therefore T_1 and $T_1{}^*$ could be calculated by the two factors, signal intensity and flip angle.[12] In this study, T_1 and $T_1{}^*$ were calculated by an exponential curve fitting at the flip angle 1, 5, 10, 20, 30, and 40 degree.

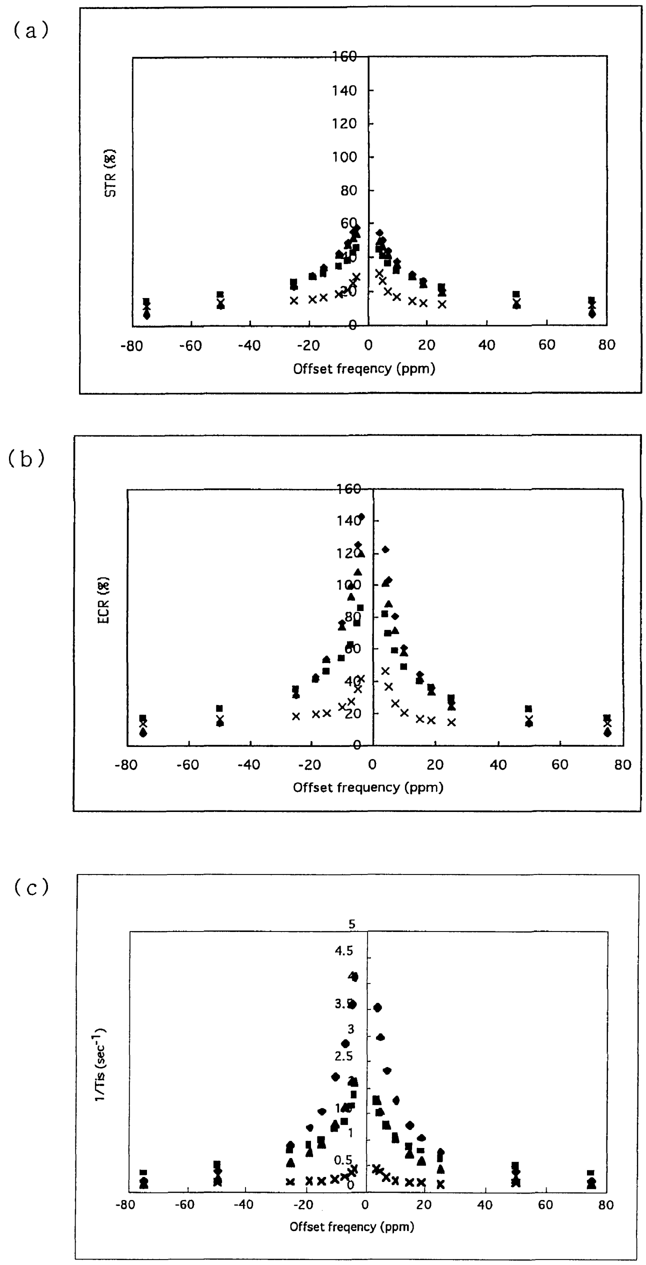

Figure 1. Correlation between offset frequency and STR, ECR, or $1/T_{is}$ in the synthetic copolymer gels. (a) Correlation between STR and offset frequency. (b) Correlation between ECR and offset frequency. (c) Correlation between $1/T_{is}$ and offset frequency. ◆: poly(HEMA-*stat*-GMA)s, ■: poly(N-VP-*stat*-GMA/MMA)s, ▲: poly(HEMA-*stat*-N-VP)s, ×: poly(N-VP-*stat*-MMA/BzMA)s.

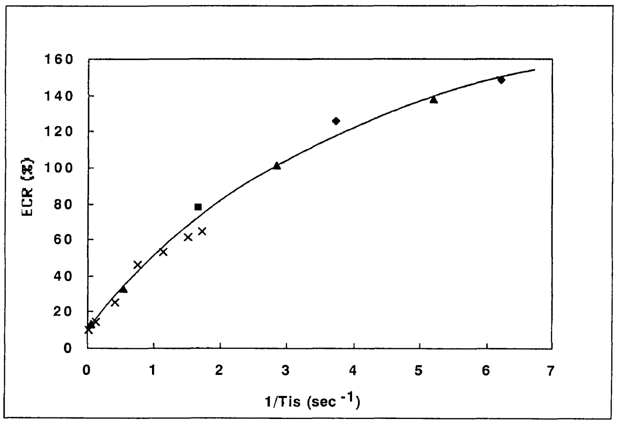

Figure 2. Correlation between ECR and $1/T_{is}$ in the synthetic copolymer gels at frequency offset of 4 ppm. ◆: poly(HEMA-*stat*-GMA)s, ■: poly(N-VP-*stat*-GMA/MMA)s, ▲: poly(HEMA-*stat*-N-VP)s, ×: poly(N-VP-*stat*-MMA/BzMA)s.

RESULTS AND DISCUSSION

Table I shows the composition and longitudinal relaxation of the copolymer samples. The sample 1—7 were hydrophilic gels and 8—14 are hydrophobic gels in the common sense.

Figure 1 illustrates three kinds of correlation between offset frequency and STR, ECR, and cross-relaxation rate of sample No.1 [poly(HEMA-*stat*-GMA)], No.3 [poly(N-VP-*stat*-GMA/MMA)], No.6 [poly(HEMA-*stat*-N-VP)], and No.11 [poly(N-VP-*stat*-MMA/BzMA)] symbolized as ◆, ■, ▲, and ×, respectively. These samples were involved the nearly equal contents (55—60 wt%) of water irrespective of different chemical structure. At the frequency offset below 10 ppm from the water resonance one, ECR values and $1/T_{is}$ show higher sensitivity than STR values in the hydrophilic gels. Furthermore shapes of ECR *vs.* offset frequency (Figure 1b) were very similar pattern to that of intermolecular cross-relaxation rate *vs.* offset frequency (Figure 1c), suggesting that the cross-relaxation rate may be replaced by the ECR values as a short-cut parameter. In practice, both numerical values gave an almost straight line, as is demonstrated in Figure 2.

Figure 3 shows correlation between (a)STR-4, (b)ECR-4 values, as well as (c)$1/T_{is}$ at the frequency offset by 4 ppm and total water contents in two series of copolymer gels, poly(HEMA-*stat*-N-VP) [▲] and poly(N-VP-*stat*-MMA/BzMA)[×]. In the correlation between total water content and STR, its linearity was not so high. However, the correlation curves between total water content and ECR (Figure 3b) or $1/T_{is}$ (Figure 3c) was on more straight lines and resulted again in the similar information. It seems that the sensitivity of ECR was obviously

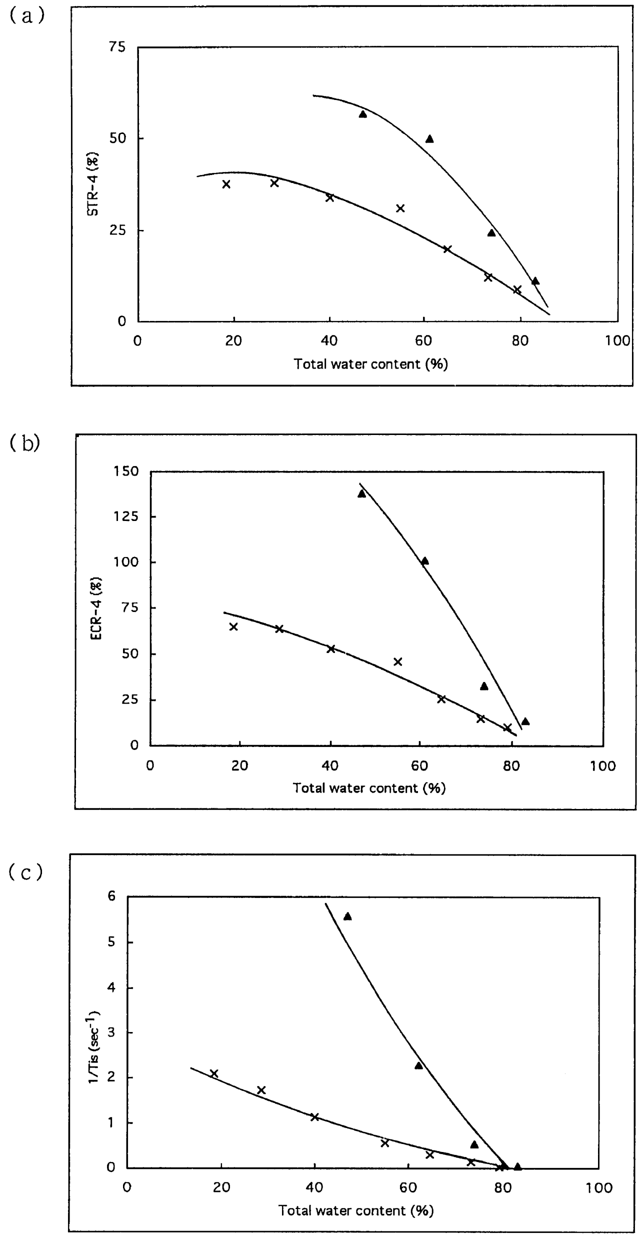

Figure 3. Correlation between total water content and STR, ECR, or $1/T_{is}$ in the synthetic copolymer gels at frequency offset of 4 ppm. (a) Correlation between STR and total water content. (b) Correlation between ECR and total water content. (c) Correlation between $1/T_{is}$ and total water content. ▲: poly(HEMA-*stat*-N-VP)s, ×: poly(N-VP-*stat*-MMA/BzMA)s.

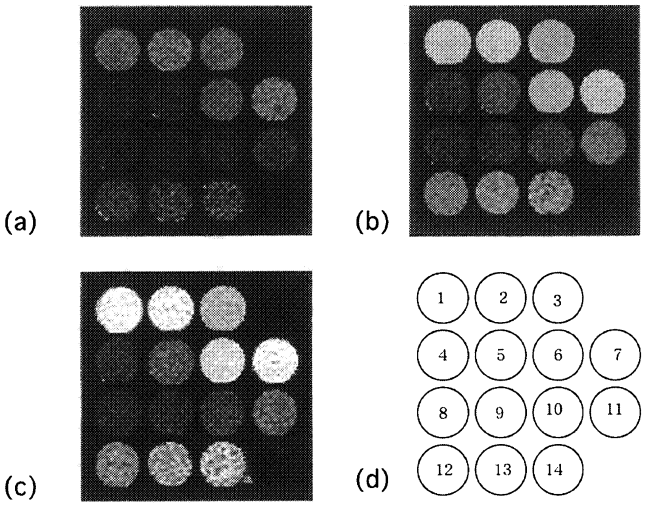

Figure 4. MR images of the synthetic copolymer gels. (a) Calculated STR image at frequency offset of 19-ppm. (b) Calculated STR image at frequency offset of 4-ppm. (c) Calculated ECR image at frequency offset of 4-ppm. (d) Samples No. in the image.

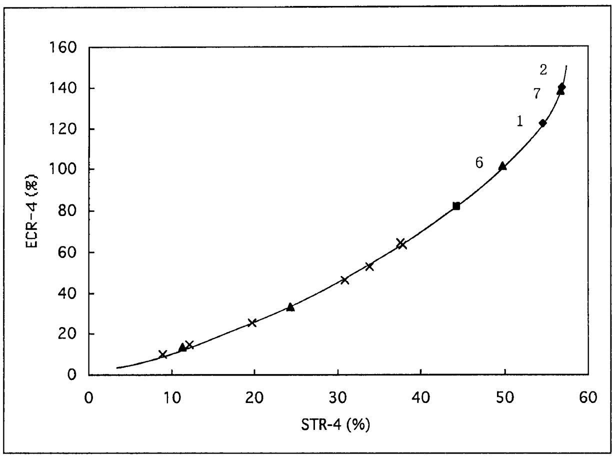

Figure 5. Correlation between STR and ECR in the synthetic copolymer gels at frequency offset of 4 ppm. ◆: poly(HEMA-*stat*-GMA)s, ■: poly(N-VP-*stat*-GMA/MMA)s, ▲: poly(HEMA-*stat*-N-VP)s, ×: poly(N-VP-*stat*-MMA/BzMA)s.

higher than STR. It is theoretically reasonable because the ECR and STR can be connected by the next eq 6;

$$\mathrm{ECR} = (T_1 / T_1^*)\,\mathrm{STR} \tag{6}$$

where T_1 and T_1^* are the longitudinal relaxation time for the unsaturated and saturated images, respectively, and in general T_1 become longer than T_1^* owing to the reduction of T_1^* through the ST effect.[11, 13]

In addition to the calculated STR-19 (a) and -4 (b) imaging pictures, ECR-4 picture for the samples are shown in Figure 4c. Both the STR and ECR pictures were drown on the basis of the percentage of signal loss between unsaturated and saturated images, calculated according to the eqs 1 and 2, respectively. The ECR imaging picture has clearer contrast than STR imaging pictures. The contrast of the imaging pictures must be important to visually evaluate the hydrophilic characteristics in the sample gels.

Figure 5 shows correlation between ECR-4 and STR-4 for the synthetic copolymer gels measured under irradiating at the frequency offset by 4 ppm. Symbol ◆, ■, ▲, and × corresponds to poly(HEMA-*stat*-GMA), poly(N-VP-*stat*-GMA/MMA), poly(HEMA-*stat*-N-VP), and poly (N-VP-*stat*-MMA/BzMA), respectively. The samples of No.1, 2, 6, and 7, which are all composed of large amounts of HEMA unit, rapidly deviates from linear correlation curve to larger ECR side.

Relations of STR or ECR *vs.* monomer contents in the various copolymer gels is shown in Figure 6, in which the symbol ■ and ◆ are ECR-4 and ECR-19, then □ and ◇ are STR-4 and STR-19 value, respectively. The ECR-4 values were relatively largest parameter in every

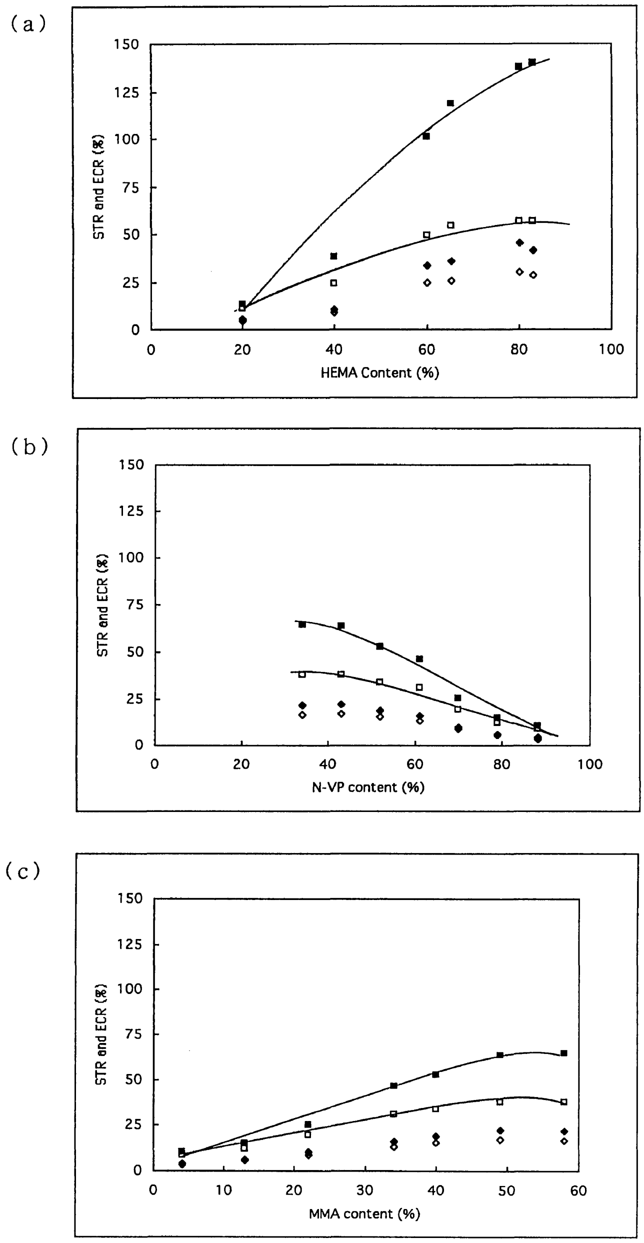

Figure 6. Correlation between STR, ECR values and monomer content in the synthetic copolymer gels. ECR-4 (■), ECR-19 (◆), STR-4 (□), or STR-19 (◇) values *vs.* (a) HEMA, (b) N-VP, (c) MMA content.

plot and STR-4 values were next higher one. In the Figures 6b and 6c, the samples are a series of copolymer consisted of the same monomer units, N-VP, MMA, and BzMA, but their different molar ratio. Both ECR and STR values monotonously increased or decreased, depending upon which monomer unit was a dominant component. Although similar situation can be seen more clearly in Figure 6a, it is worthy to note that the samples are various type copolymers besides containing HEMA unit. It seems that HEMA contents, namely $-CH_2CH_2-OH$ group contents, mainly determine ECR or STR values. Era *et al.* have already suggested that the number of hydroxyl group may in the samples correlated closely with the ST effect at frequency offset below 10 ppm from the water resonance frequency (=4.70 ppm from TSP).[14,15] In the paper, we would like to insist that the

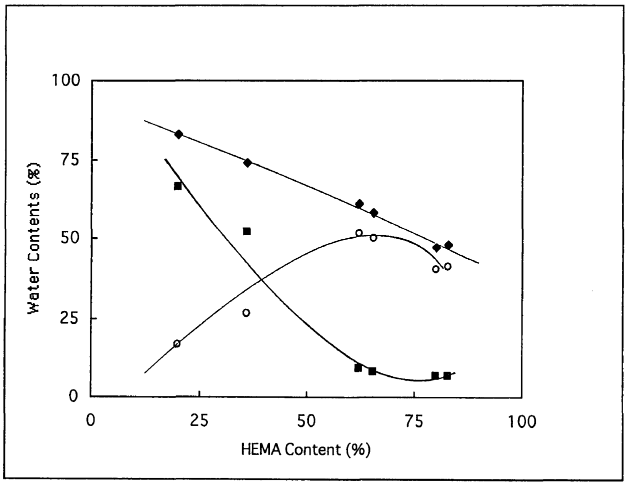

Figure 7. Correlation between water contents and HEMA content in the synthetic copolymer gels. (◆) total water, (■) free water, and (○): bound water content.

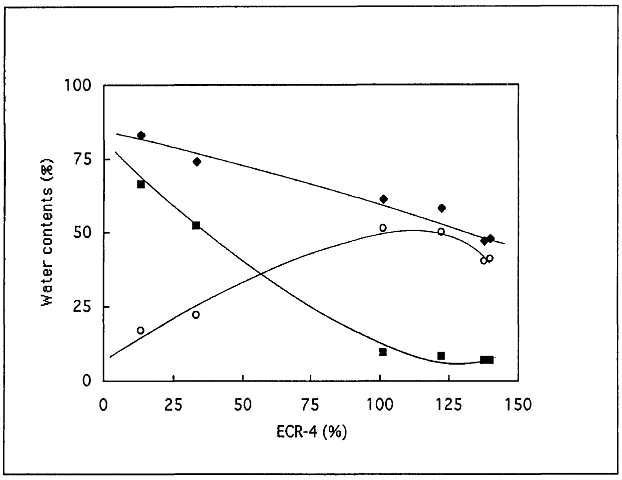

Figure 8. Correlation between water contents and ECR-4 in the synthetic copolymer gels. (◆) total water, (■) free water, and (○): bound water content.

ECR-4 values were more sensitive parameters than the STR values in order to evaluate amounts of hydroxyl group in the synthetic copolymer gels.

Figure 7 shows the correlation between HEMA content and water condition in the synthetic copolymer gels. Symbols ◆, ■, and ○ mean total water, free water, and bound water (=freezable constrained water+non-frozen water) content, respectively. Only the total water content gives linear plots with HEMA contents. However, contrary our expectation, the total water contents decreased as the HEMA content increased. The amount of free (mobile) water is predominant in the range of rather small HEMA content and the bound water become predominant in the range over 60% HEMA contents, suggesting that the tight (or chemical) interaction of water with hydroxyl group in the gel matrix occur exclusively. Next Figures 8 shows correlation between ECR-4 and water condition in the synthetic copolymer gels (symbols ◆, ■, and ○ are the same as that of Figure 6). The most striking fact is the similar characteristic of Figures 8 and 7 as a whole. Again the ECR-4 values instead of

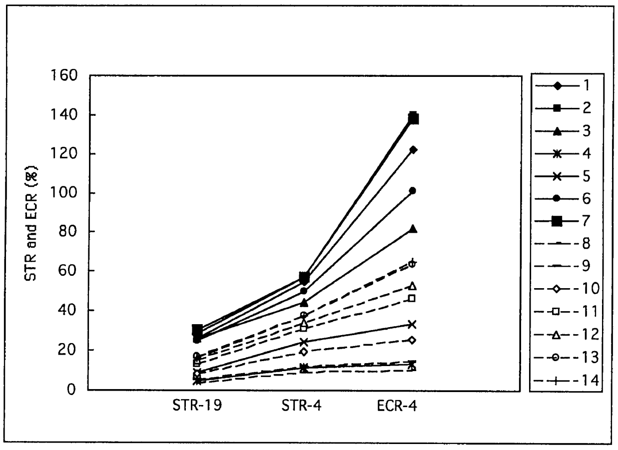

Figure 9. Correlation between STR-19, -4, and ECR-4 in the synthetic copolymer gels. Solid and broken lines mean hydrophilic and hydrophobic gels, respectively.

HEMA content in Figure 7 precipitously increased as the total water contents decreased, and it may be proper to consider that the higher ECR-4 values essentially reflect the more abundant bound water content in the gels. In the samples in this work, the contents of bound water can be controlled closely by the hydroxyl group in the HEMA unit. At last, STR-19, -4, and ECR-4 values for all sample gels are shown Figure 9. We can read one simple principle that hydrophilic gel systems (solid line) result in higher values and hydrophobic gel systems (broken line) keep lower values. Especially the ECR-4 values for the hydrophilic gels with HEMA unit give us the reliable information, amplifying slight differences in each sample.

Anyhow the above results suggest that ECR-4 values are good parameter for existence of the bound water in the synthetic copolymer gels, in other words in the HEMA content. Thus we insist that the ECR-4 value and ECR-4 image must be a reliable numerical index and visible information about the water condition in the synthetic gels, respectively. Accumulation of ECR or STR data for various types of the sample in future would bring to us the valiable information concerning water conditions in the synthetic polymer gels, and further the relationship between their chemical structure and hydrophilicity.

CONCLUSIONS

Through MRI measurement for various synthetic copolymer gels, it was found that ECR-4 as well as STR-19 and -4 values are able to adopt as a new parameter in evaluating hydrophilic character of the samples. The ECR-4 values were always higher and more sensitive than corresponding STR values. ECR-4 values are correlated with not only the total water content but also its conditions in more detail. The higher values of ECR-4 were associated with more abundant bound water constrained by the chemical interaction to the gel matrix and also higher HEMA content in the viewpoint of chemical structure. Therefore these modified values and images in MRI measurement could be new useful analytical tools for evaluation of the hydrophilicity and chemical structure of the synthetic copolymer gels without invasive process to the samples.

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