



## New liquid chromatography method combining thermo-responsive material and inductive heating via alternating magnetic field

Hisashi Yagi, Kazuya Yamamoto, Takao Aoyagi\*

Department of Nanostructure and Advanced Materials, Graduate School of Science and Engineering, Kagoshima University, 1-21-40 Korimoto, Kagoshima 890-0065, Japan

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### ABSTRACT

In this study, we examined the feasibility of a novel liquid chromatography technique that combines temperature-responsive polymeric materials with inductive heating via an alternating magnetic field (AMF). We considered the following components of the technique: (i) the preparation of composite materials of magnetite and silica, (ii) their heating behavior under the AMF, (iii) the conjugation of temperature-responsive polymers for the packing materials, and (iv) the elution profiles of the model compounds in the AMF. The results showed that we could influence the elution of the model compounds by AMF induction heating generated by the surrounding coil.

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### 1. Introduction

In recent years, much attention has been focused on the use of separation technology on biomolecules such as genes, peptides, and proteins [1–3]. Of course, liquid chromatography is a very convenient and effective separation technology for such purposes, and reversed-phase chromatography has been widely used in this area [4–6]. The separation of desired compounds during chromatography is controlled by changing the polarity of the mobile phase. This has traditionally been achieved using water-soluble organic solvents, such as methanol and acetonitrile. However, many of the molecules of interest can be damaged or destroyed by pH changes and organic solvents. For biological studies and pharmaceutical manufacturing, it has therefore become increasingly important to develop an effective separation system that does not harm the effectiveness or viability of targeted molecules and compounds.

Toward this end, Okano's research group has suggested the use of temperature-responsive liquid chromatography with a unique stationary phase comprised of silica conjugated with temperature-responsive polymers [7–9]. This type of chromatography does not require an organic solvent for the mobile phase, relying instead upon water. The temperature-responsive polymer, poly(*N*-isopropylacrylamide) (PNIPAAm), which is immobilized on the surface of the stationary phase packing material, exhibits a com-

pletely reversible hydrophilic–hydrophobic change in response to temperature alteration [10,11]. This means that the elution profile can be controlled by the water temperature of the mobile phase. They also reported an ion-exchange mode using a NIPAAm-based copolymer containing the ionic groups [12–14].

It is well known that magnetite can provide an effective source of heat when acted upon by an alternating magnetic field (AMF) [15]. In a previous study, we prepared a magnetite nanoparticle that was conjugated with a NIPAAm-based, temperature-responsive polymer and that helped accurately target drug delivery to a specific site [16]. That study demonstrated both the sensitivity and the reversibility of the hydrophilic–hydrophobic change in response to AMF induced heat.

The objective of this follow up work is to demonstrate the feasibility of a novel chromatographic approach combining temperature-responsive materials with inductive heating of magnetite via AMF. Our goal was to control the polarity of the stationary phase by providing heat to the temperature-responsive polymer in the stationary phase through by AMF in a coil. That is, we hoped to show that the AMF alternation would heat the magnetic components in the packing materials and thereby control the materials' elution profiles.

To achieve these new goals, we first prepared composite materials of magnetite and silica and confirmed their heating profiles within the AMF. The temperature-responsive polymers were then conjugated for the packing materials. To modify the packing materials' surface, poly(*N*-isopropylacrylamide-co-2-carboxyisopropylacrylamide) (poly(NIPAAm-co-CIPAAm)) was

\* Corresponding author. Tel.: +81 99 285 7794; fax: +81 99 285 7794.

E-mail address: [aoyagi@eng.kagoshima-u.ac.jp](mailto:aoyagi@eng.kagoshima-u.ac.jp) (T. Aoyagi).

used. We have already reported that the copolymer is sensitive to changes in pH or temperature [17–19]. The functional groups in the copolymers were immobilized on the magnetite–silica composite surfaces. They were prepared and characterized by X-ray photoelectron spectroscopy (XPS), thermogravimetric (TG) analysis and by scanning electron microscope (SEM) measurement. Finally, we checked the elution profiles of this new chromatographic process under the influence of the AMF. The elution profiles were generated by a high-performance liquid chromatography (HPLC) system whose column was packed with materials prepared above.

## 2. Experimental

### 2.1. Materials

Poly(NIPAAm-co-CIPAAm) with a 10 mol% of CIPAAm content was synthesized according to the methods described in a previous study [17]. The silica particles (Wakogel® C-200, particle size: 75–150  $\mu\text{m}$ ), iron(II) chloride ( $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ ), iron(III) chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ), toluene, and 28% ammonia solution were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The model drugs hydrocortisone, prednisolone, dexamethasone, hydrocortisone acetate, testosterone, ibuprofen, ketoprofen, and naproxen were purchased from the same company. A silane coupling agent, 3-aminopropyltriethoxysilane (APTES) was obtained from the Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) and used as received. The coupling agent, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), was obtained from Dojindo Co. (Kumamoto, Japan). All other chemicals and solvents were commercially available and of analytical grade.

### 2.2. Preparation of magnetite–silica composite

The ratio of Fe ions to silica particles was changed to obtain samples with different compositions. For example, 9.0 g of  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ , 24.0 g of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , and 5.0 g of silica particles were dispersed into 50 mL of distilled water. The mol ratio of Fe(II) to Fe(III) was about 1:2. During stirring, a 28% ammonia aqueous solution (100 mL) was added and the resulting solution was then stirred at 75 °C for 30 min. The precipitated magnetite–silica composite was then thoroughly washed with distilled water and ethanol and finally dried *in vacuo* for 24 h to provide 15.0 g of the composites as a black powder.

### 2.3. Preparation of APTES-modified magnetite–silica composite

The magnetite–silica composite prepared above (7.5 g) was dispersed in 75 mL of toluene. A 2.5 mL aliquot of APTES was then added to the solution and it was stirred at room temperature for 24 h. The resulting composites were exhaustively washed with toluene and ethanol. After drying *in vacuo* for 24 h, 8.3 g of the APTES-modified magnetite–silica composites were obtained as a black powder.

### 2.4. Preparation of polymer-modified magnetite–silica composite

The production method of the temperature-responsive polymer was as follows. To the aqueous solution containing 0.25 g of poly(NIPAAm-co-CIPAAm), 50 mg of EDC was added, and the mixture was stirred at 4 °C. A 1.5 g sample of the APTES-modified magnetite–silica composite was added to the solution, and it was stirred at 4 °C for 12 h and then at ambient temperature for 24 h. After the reaction was completed, the polymer-modified composite was repeatedly washed with a large amount of water. Finally, the resulting composite was dried at room temperature under reduced

pressure for 24 h. A 1.52 g sample of the packing material was obtained as a brown powder.

### 2.5. Measurements

The CIPAAm content in the copolymer was determined from  $^1\text{H}$  NMR (JMN-GSX400, JEOL, Tokyo, Japan). The lower critical solution temperature (LCST) of the poly(NIPAAm-co-CIPAAm) in an aqueous solution (1.0%, w/v) was estimated by transmittance measurement using a UV–vis spectrometer (V-550, Jasco, Tokyo, Japan) equipped with a temperature controller. The heating rate was 1.0 °C/min, and the wavelength was fixed at 500 nm. The LCST of the polymer solution was defined as the temperature when the transmittance was 50%.

The successful preparations in each step were confirmed by scanning electron microscopy (H-7010A, Hitachi, Tokyo, Japan), X-ray diffraction (XRD) (RINT2200 HK/PC, Rigaku, Tokyo, Japan), and X-ray photoelectron spectroscopy measurements (ESCA-1000, Shimadzu, Kyoto, Japan).

The heating profiles of the magnetite–silica composite suspension (3 g of the composite/10 mL of water) were measured in a coil made from copper tubing (the inside and outside diameters were 11 and 12 cm, respectively, and there were 12 turns in the coil). An alternating current with a frequency of 300 kHz and 1 kW of power was applied to induce the AMF in the coil. The current was generated by a high-frequency power unit (T162-6023A, Thamway, Shizuoka, Japan).

### 2.6. Elution profile studies in the AMF

The polymer-modified composite materials were suspended in 45 mM phosphoric acid (pH 3.0) that is the same as the mobile phase. The materials were then packed into a glass column (100 mm length  $\times$  4.6 mm diameter), through which the liquid was pumped during the liquid chromatography (Tosoh CCPD, Tokyo, Japan). The elution behaviors of the model drugs were recorded at a flow rate of 0.5 mL/min, both with and without the AMF.

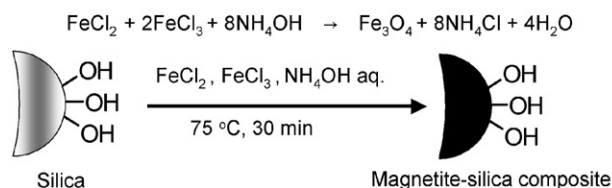
## 3. Results and discussion

### 3.1. Preparation and characterization of magnetite–silica composites

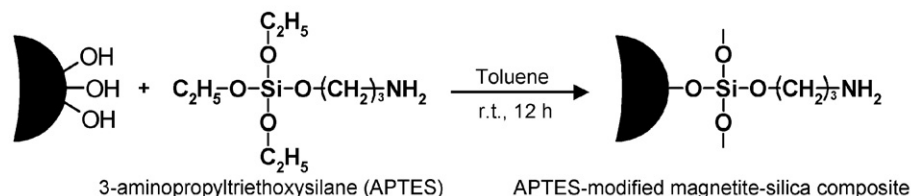
Fig. 1 shows the preparative scheme of the packing materials for this new chromatographic method. In each reaction, XPS and TG analyses were used to confirm the desired reactions. It is well known that the co-precipitation method is very effective for the preparation of magnetite particles [20–22], so, to obtain the magnetite–silica composites, we applied the same method in the presence of silica particles. The preparative data and yields are summarized in Table 1. Fig. 2 shows the X-ray diffraction pattern of each magnetite and silica composition ratio. As seen in the figure, the diffraction patterns of the composites were similar to those of the magnetite alone. This suggests that the co-precipitation reaction for the magnetites and silica occurred satisfactorily, and that the co-existing silica particle did not interfere with the precipitation process. The same figure shows that the magnetite–silica ratio in the resulting composites can be tuned by changing the feed ratios of the iron ions and co-existing silica particles.

Prior to the next reaction, we studied the heating profiles of the different compositions of the magnetite–silica composite. For this analysis, the electrical power was fixed at 1000 W to generate the AMF. Fig. 3 shows the experimental conditions and the AMF heating profiles. The results clearly indicated that the heating rate increased

## i. Preparation of magnetite-silica composite



## ii. Introduction of aminopropyl groups



## iii. Polymer modification

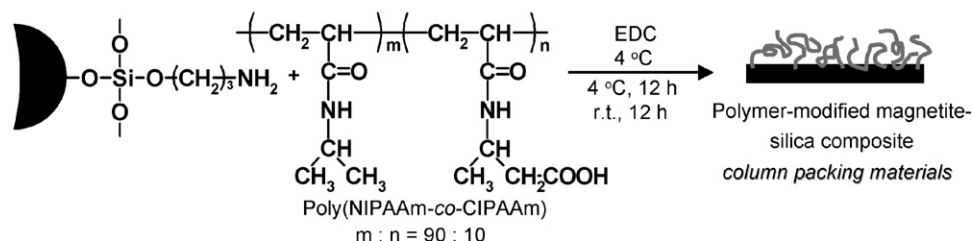


Fig. 1. Preparation scheme of the column packing materials for the new chromatographic method.

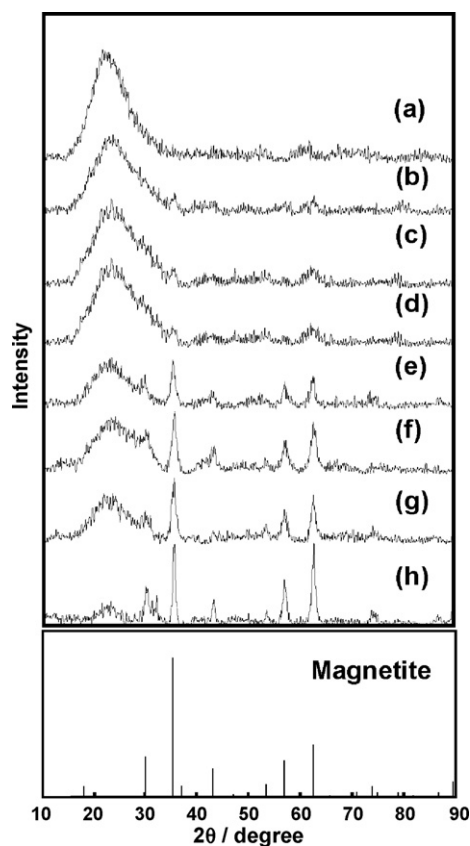


Fig. 2. XRD patterns of the magnetite-silica composites. (a) Silica; (b–g) magnetite-silica composites; (b) magnetite:silica = 1:3, (c) 1:2, (d) 1:1, (e) 1:0.75, (f) 1:0.5, (g) 1:0.3; (h) magnetite.

proportionally with the percentage of magnetite in the composition. As we describe later, temperature-responsive polymers can change drastically in their surface hydrophilicity and hydrophobicity around 30 °C. Hence, according to the heating profile, the composites are quite useful as a heating source for temperature-responsive polymers. In separate experiments, we also controlled the heating profiles by adjusting the electrical power (data not shown).

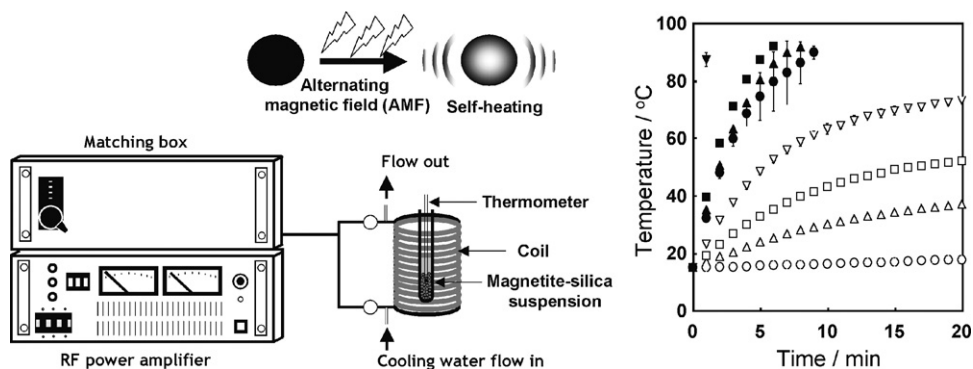
## 3.2. Polymer modification on the composite surfaces

In the next step, the amino groups were introduced into the magnetite-silica composite surface by the reaction with 3-aminopropyltriethoxysilane (APTES). Fig. 4 shows that the peak intensity was higher after the modification than before, based on the  $N_{1s}$  of the nitrogen atom in the XPS. The weight loss traced in the TG analysis also showed clear evidence of a successful reaction. The weight loss around 100 °C is due to the water evaporation adsorbed on the material surfaces. The weight loss around 300 °C

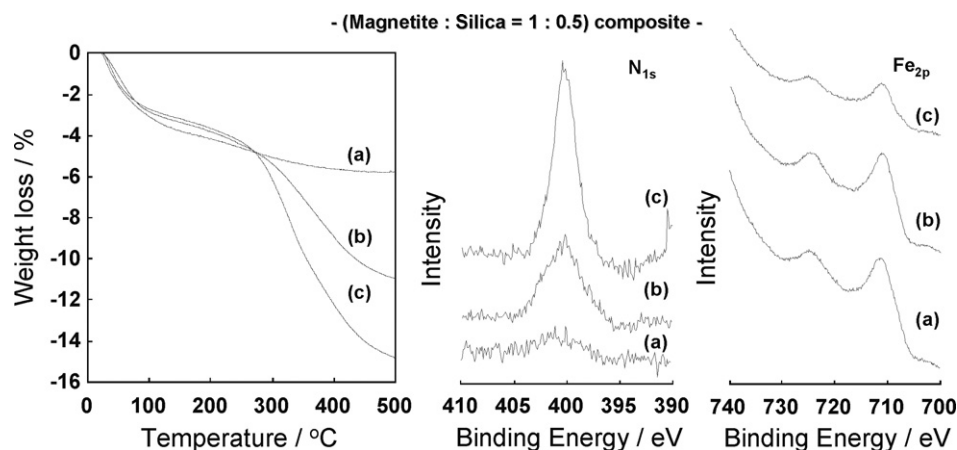
Table 1  
Preparation of magnetite-silica composite.

Code	In feed (g)			Yield (g)	Magnetite content (wt%) <sup>a</sup>
	FeCl <sub>2</sub> ·4H <sub>2</sub> O	FeCl <sub>3</sub> ·6H <sub>2</sub> O	Silica		
Silica 30	9	24	30	40.1	25.2
Silica 20	9	24	20	30.0	33.3
Silica 10	9	24	10	20.3	50.7
Silica 7.5	9	24	7.5	17.5	57.1
Silica 5	9	24	5	15.2	67.1
Silica 3	9	24	3	13.0	76.9
Magnetite	9	24	0	10.3	100

<sup>a</sup> Estimated by ((magnetite-silica (yield) (g) – silica (in feed) (g))/magnetite-silica (yield) (g)) × 100.



**Fig. 3.** Temperature profiles of the magnetite–silica composite in the AMF. (Open circles) silica, (open triangles)–(closed squares) magnetite–silica composite, (triangles) magnetite:silica = 1:3, (open squares) 1:2, (open reverse triangles) 1:1, (closed circles) 1:0.75, (closed triangles) 1:0.5, (closed squares) 1:0.3, (closed reverse triangles) magnetite. Conditions: 3 g magnetite–silica composite/10 mL water, external AMF: 300 kHz, 1000 W.



**Fig. 4.** TG curves and XPS spectra of (a) magnetite–silica composite, (b) APTES-modified magnetite–silica composite, and (c) polymer-modified magnetite–silica composite.

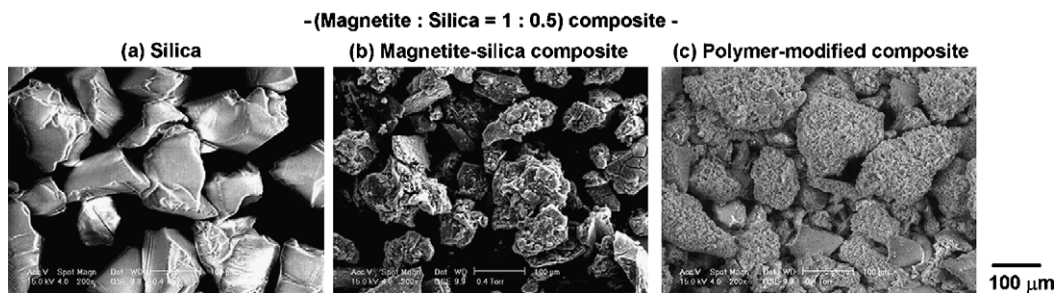
corresponds to the burning out of the organic components, that is, the aminopropyl groups. The TG charts strongly suggested the successful introduction of a useful functional group for the polymer immobilization.

In the last preparation step, the thermo-responsive poly(NIPAAm-co-CIPAAm) was reacted with the amino groups on the magnetite–silica composites. The polymer conjugation was traced using the XPS and the weight loss during the TG analysis, and the results are shown in Fig. 4. It is clear that the reaction with the aminopropyl groups significantly increased the  $N_{1s}$  peak intensity and also decreased the  $Fe_{2p}$  peak. This strongly suggests that the polymer effectively reacted and bonded to the composite surfaces, and extent of the weight loss around 300 °C also supports this conclusion. Separately, we measured the IR spectra before and after the reaction to evaluate the immobilization of the polymers

on the surface. The clear adsorption peaks at 1655 and 1549  $cm^{-1}$  based on the amide groups also suggested a satisfactory modification (data not shown). The polymer used here shows a clear phase transition at 28 °C, detected by the transmittance measurement, in the same medium as the mobile phase.

### 3.3. Surface morphology of the packing materials

We observed the morphology of the obtained column packing materials by SEM. The images are shown in Fig. 5. As seen in the images, the co-precipitated magnetite particles were accumulated on the silica surface (Fig. 5(b)). This suggests that the composite formation by the magnetite co-precipitated reaction occurs using the silica as cores or scaffolds. The surface was smoother after the polymer reaction via the amino group introduction (Fig. 5(c)). As



**Fig. 5.** SEM images of (a) silica, (b) magnetite–silica composite, and (c) polymer-modified magnetite–silica composite.



already mentioned, the temperature-responsive polymers that can change the surface properties cover the composites.

The analytical data and heating profiles of the sequence of prepared composites strongly indicate that the different magnetite–silica composite ratios can be used for a feasibility study of the new chromatography.

#### 3.4. Feasibility of the new chromatographic concept

Prior to the AMF phase of the study, we investigated the temperature-response of the packing materials. The temperature of the column was controlled by circulating constant temperature water in a water jacket surrounding the column, after which the chromatographic studies were performed. The retention times of the model compound elution are plotted as a function of the temperature in Fig. 6. In this experiment, five kinds of steroids having different polarities were used as model compounds. The figure shows that longer elution times are associated with increasing temperature. The most hydrophobic compound produced the longest elution time, suggesting that the partition of the compound to the stationary phase from the mobile phase would improve. In the composite only case, without the temperature-responsive polymer, no change was observed in the retention time. When testosterone was used, the retention time decreased, which would be due to the lower viscosity from heating.

In this experiment, we used a phosphoric acid solution with pH 3.0 as a mobile phase. To modify the surface properties, the temperature-responsive poly(NIPAAm-co-CIPAAm) was used. After the reaction, some unreacted carboxyl groups would remain because of the bulkiness of the polymer chains. In a solution of a neutral pH, these remaining groups could dissociate and turn into the more hydrophilic carboxylate ions, which we feared might obstruct the hydrophilic–hydrophobic change brought on the AMF

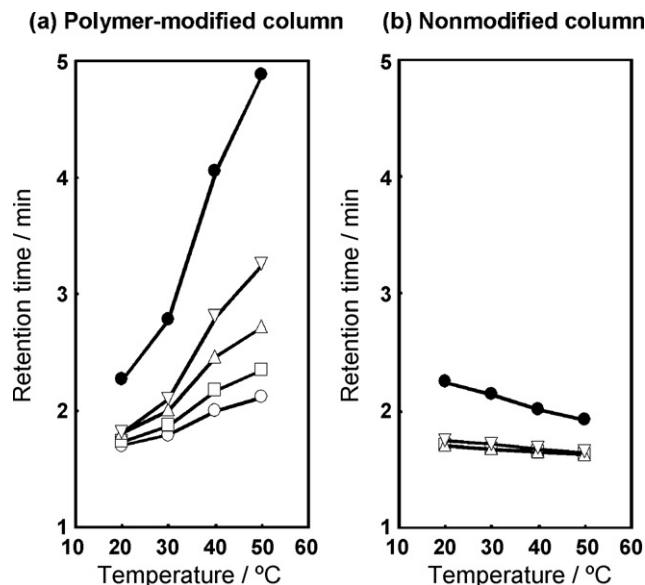


Fig. 6. Temperature-dependent retention time changes of the steroids on (a) polymer-modified column and (b) nonmodified column. (Open circles) hydrocortisone, (open squares) prednisolone, (open triangles) dexamethasone, (open reverse triangles) hydrocortisone acetate, (closed circles) testosterone.

application. Therefore, we used a low pH condition to maintain the protonation of the carboxyl groups.

To analyze the effectiveness of the process, we checked the change in retention times brought on by alternation of the AMF. Fig. 7 shows that the AMF prolonged the retention times of all the model compounds. Compared to the previously mentioned temperature-controlled chromatography, we were able estimate

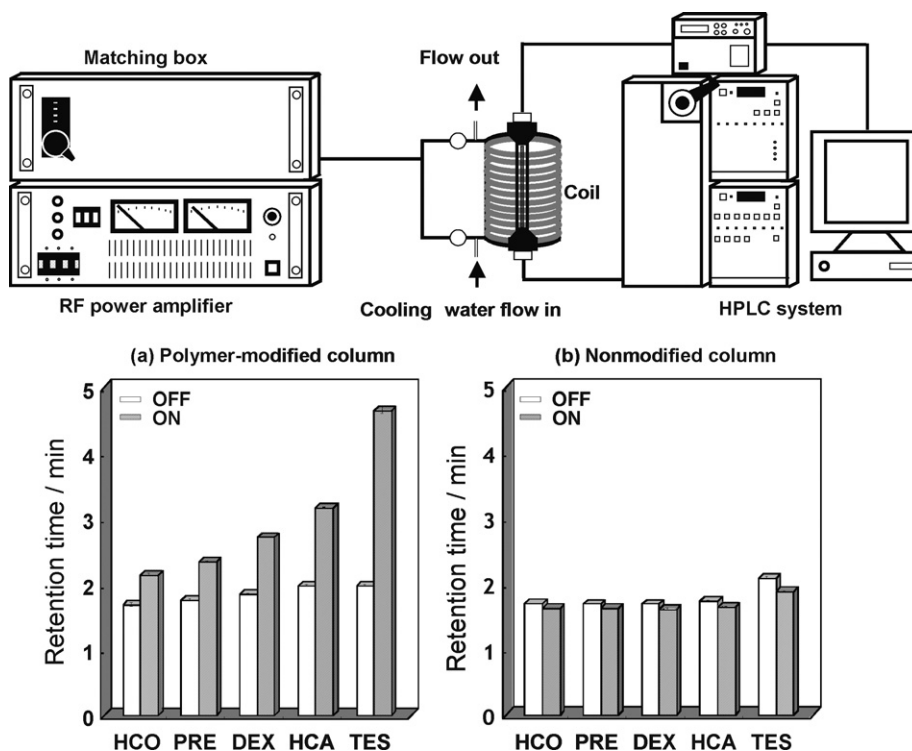
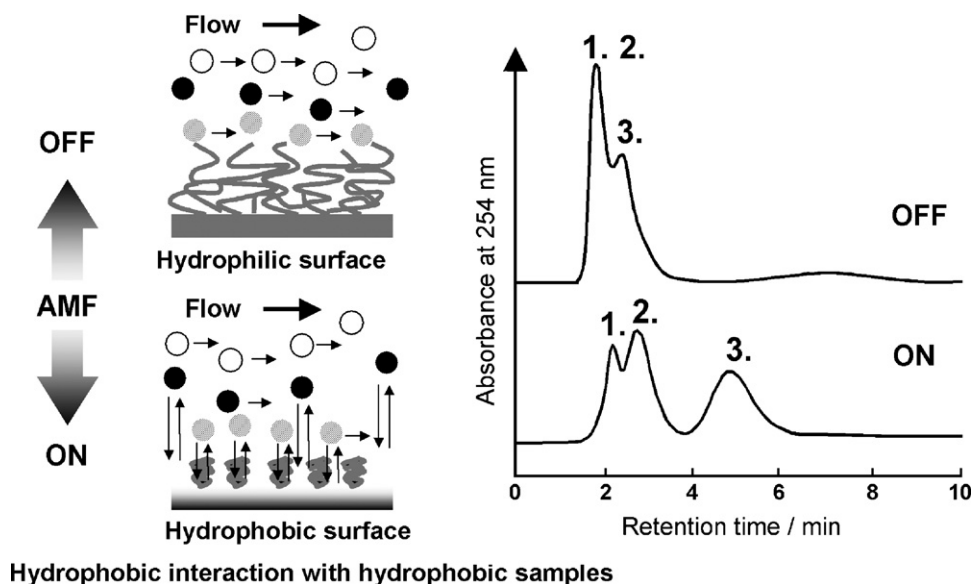


Fig. 7. Retention time changes in the steroids on (a) polymer-modified column and (b) nonmodified column. (HCO) hydrocortisone, (PRE) prednisolone, (DEX) dexamethasone, (HCA) hydrocortisone acetate, (TES) testosterone. Conditions: external AMF: 300 kHz, 1000 W.



**Fig. 8.** Chromatograms of a mixture of three steroids with 45 mM phosphoric acid (pH 3.0) as the mobile phase and AMF. Peaks: 1, hydrocortisone; 2, dexamethasone; 3, testosterone. HPLC conditions: flow rate, 0.5 mL/min; monitoring, UV at 254 nm. External AMF: 300 kHz, 1000 W.

that the chromatography was performed around 48 °C. This means that the temperature-responsive polymer could utilize the heat generated by the AMF and would thus dehydrate and become more hydrophobic. The hydrophobic surface improved the interaction with the hydrophobic model compounds.

We wanted to measure the surface temperature of the packing materials, but it was found that accurate measurement was very difficult. Therefore, we decided to compare temperature control via not only heating of the mobile phase but also via AMF application. This led us to understand that temperature modification of the mobile phase is required for elution control even with the inductive heating by AMF.

Finally, the model compounds were separated using hydrocortisone, dexamethasone, and testosterone. Fig. 8 shows the elution profiles with and without the application of the AMF. Without the AMF, the three compound peaks could not be separated. With the application, peaks were clearly isolated. However, the observed peaks seem to be broad. One of the reasons may be the lack of uniformity in the size and shape of the composite particles, as we used crushed silica gels for the feasibility study. We are currently studying methods of the preparing sphere-type packing materials of a uniform size.

To confirm the viability of this system, we used the anti-inflammatory drugs ibuprofen, ketoprofen, and naproxen. As expected, the retention times of the model drugs were definitely prolonged by the application of the AMF. Thus, AMF improved the hydrophobicity of the surface of the packing materials. It is understood that, for practical use, the separation efficiency or specificity of the process must be improved. With that goal in mind, we are now preparing the spherical packing materials with magnetite and temperature-responsive polymers.

#### 4. Conclusions

In this study, we examined a new chromatographic procedure that couples a thermo-responsive polymer with inductive heating via an AMF. The AMF generated this heat by acting upon column packing materials made up of a magnetite–silica composite whose surface had been modified by a thermo-

responsive *N*-isopropylacrylamide-based functional copolymer. When the AMF was applied, the elution time increased in the chromatogram. Mixed model compounds were also separated using this system.

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