



Poly(2-*N*-carbazolyethyl acrylate)-modified silica as a new polymeric stationary phase for reversed-phase high-performance liquid chromatography

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ABSTRACT

Poly(2-*N*-carbazolyethyl acrylate) having terminal trimethoxysilyl groups was newly synthesized by radical polymerization and immobilized onto the silica surface (Sil-CEA). The chromatographic property of Sil-CEA was examined by applying polycyclic aromatic hydrocarbons as solutes. Poly(4-vinylpyridine)-modified silica (Sil-VP) and monomeric octadecylated silica (ODS) columns were used as the reference columns. Less sensitivity to molecular hydrophobicity and enhanced molecular planarity selectivity were obtained with Sil-CEA compared to ODS. On the other hand, high retention factors for the analyzed solutes and an opposite elution order for linear and disc-shaped solutes were obtained with Sil-CEA compared with Sil-VP. In this paper, the application for separation of tocopherols was also described.

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1. Introduction

Since the introduction of reversed-phase columns in 1970, bonded-phase columns have been regularly used for the separation and analysis [1]. Reversed-phase high-performance liquid chromatography (RP-HPLC) is one of the most popular and powerful separation mode because of its versatility and ability to retain and resolve a number of different types of non-volatile compounds [2–5]. New adsorbents are being introduced [6] and demands for the new types of stationary phase are still increasing and scientists are interested in the synthesis of the phases, which can provide unique separations. According to the recent review article [7], HPLC has been placed as the 3rd ranker after balance and pH meters. In spite of the high popularity, most of the HPLC separations are performed using alkyl chains-bonded silica columns [2,3,8–10]. Alkyl chains-modified silica column separation behavior is based on the hydrophobic effect, which leads the problems in the separation of compounds having similar hydrophobicity and polarity [11]. Thus, there is a need for the development of new kind of stationary phases, which can enter into further interactions besides hydrophobic effects, resulting in the separation of various kinds of isomers. Based on this basic assumption, we previously developed poly(4-vinylpyridine)-modified silica (Sil-VP) [12–16]. Sil-VP showed less sensitivity to molecular hydrophobicity and brought unique shape selectivity for polycyclic aromatic hydrocarbons (PAHs) [12–15]. However, very low retention factor for PAHs had been observed, thus the retentivity with Sil-VP should be

improved. We are interested in the development of a new stationary phase, which shows less sensitivity towards molecular hydrophobicity and brings higher retentivity for PAHs by keeping the better molecular-shape selectivity.

Poly(2-*N*-carbazolyethyl acrylate) consists of carbonyl function and carbazolyl group as a heterocyclic aromatic moiety. Carbazole-based monomers are successful charge-transferring components of the polymers [17,18], and because of the presence of nitrogen on the aromatic moiety, less sensitivity towards molecular hydrophobicity is predicted. The restricted motion of C–N bond of carbazolyl moiety has also been reported by ¹³C NMR relaxation data [19,20]. We estimate that carbazole-based phase is rigid compared to vinylpyridine due to its bulkiness and will carry out special shape selective separations. Based on these reports and predictions, we are introducing (2-*N*-carbazolyethyl acrylate) polymer for chromatographic purposes. For the purpose, we have synthesized poly(2-*N*-carbazolyethyl acrylate) with terminal trimethoxysilyl groups by radical polymerization and grafted onto the silica surface. The chromatographic property of the prepared stationary phase has been compared with poly(4-vinylpyridine)-grafted silica and monomeric octadecylated silica. To the best of our knowledge, this is the first attempt to apply poly(2-*N*-carbazolyethyl acrylate)-grafted silica (Sil-CEA) for the chromatographic application.

2. Experimental

2.1. Chemicals and reagents

All the PAH and alkylbenzene solutes were commercially available and used without further purification. Tocopherols were

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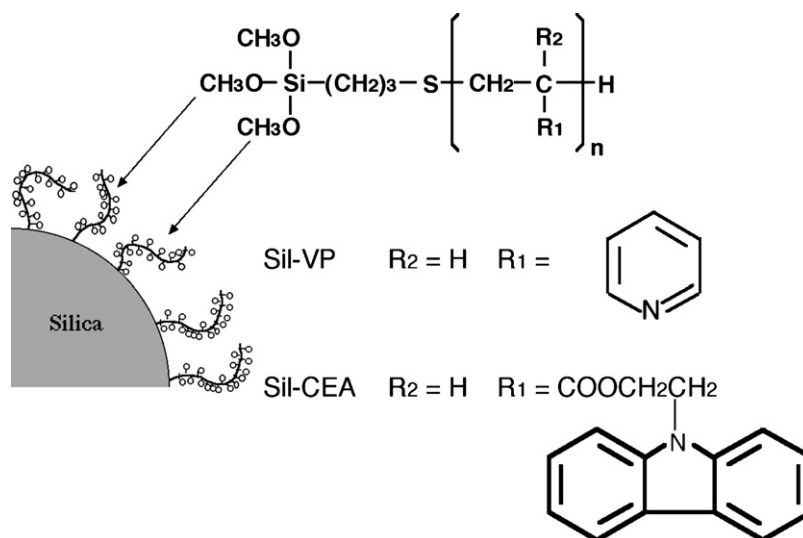


Fig. 1. Schematic illustration of the synthesized polymer-grafted silica and chemical structure of poly(2-N-carbazolyethyl acrylate) and poly(4-vinylpyridine) having reactive terminal trimethoxysilyl groups.

purchased from Calbiochem (Darmstadt, Germany). The monomer 2-N-carbazolyethyl acrylate was from Nippon Shokubai (Osaka, Japan). α,α' -Azobisisobutyronitrile (AIBN) as an initiator was from Nacalai Tesque (Kyoto, Japan) and was recrystallized from methanol prior to use. 3-Mercaptopropyltrimethoxysilane (MPS) as an anchoring group was purchased from Chisso (Tokyo, Japan). HPLC grade methanol was purchased from Wako (Osaka, Japan). Water was purified using a Milli-Q water-purification system (Millipore, Tokyo, Japan) and filtered using a mixed cellulose ester membrane filter (0.45 μm , Advantec, Tokyo, Japan) before use. Porous silica (120-S5, diameter 5 μm , pore size 120 Å, specific surface area $300 \pm 5 \text{ m}^2 \text{ g}^{-1}$) was from YMC (Kyoto, Japan).

2.2. Preparation of the stationary phases

2-N-Carbazolyethyl acrylate (15 g) was dissolved in 100 ml toluene and MPS (1.5 ml) was added to the solution. The mixture was stirred with bubbling nitrogen at room temperature for 20 min. After addition of AIBN (121 mg), the reaction was carried out under bubbling nitrogen at 65 °C for 6 h. The obtained product was concentrated in rotary evaporator and purified using $\text{CHCl}_3/\text{CH}_3\text{OH}$ as solvent and precipitant, respectively. The average polymerization degree of the prepared poly(2-N-carbazolyethyl acrylate) with terminal trimethoxysilyl groups was determined to be 19 from ^1H NMR spectrum by the integration of proton of MPS and the aromatic moiety of poly(2-N-carbazolyethyl acrylate). The obtained polymer was then grafted onto the silica surface. The schematic illustration of prepared polymer with terminal trimethoxysilyl groups and the polymer-grafted silica is shown in Fig. 1. For the immobilization of polymer on the silica surface, 5 g of the prepared polymer and 4 g of silica were mixed in 50 ml of toluene and the suspension was stirred at 115 °C for 5 days. Thus the obtained modified-silica particles were washed extensively with toluene, chloroform, methanol and lastly with water and dried in vacuum. The amount of the immobilized polymer was determined by elemental analysis to be 31.3%, w/w. Sil-CEA was then packed into stainless steel column (150 mm \times 4.6 mm I.D.). Sil-VP, having average polymerization degree of 21 and immobilization amount (17.8%, w/w) was prepared as described in our previous paper [12] and packed into stainless steel column (250 mm \times 4.6 mm I.D.). Monomeric ODS (Inertsil ODS-3, 250 mm \times 4.6 mm I.D., GL-Science, Tokyo, Japan) was used to compare the retention behaviors.

2.3. Chromatographic measurement

HPLC was run at 35 °C under isocratic condition using methanol–water as a mobile phase with a flow rate of 1 ml min^{-1} in all cases. The chromatographic system consisted of a pump (JASCO, PU-2089, Tokyo, Japan), an injector (Rheodyne, model 7725), a multiwavelength detector (JASCO, MD-910) and a column heater (Sugai U-620, type 30VP, Tokyo, Japan). 5 μl of each sample dissolved in methanol was injected and retention factor (k) was calculated using the relation described in our previous reports [14–16]. Deflection of pure methanol recorded above 400 nm was used to mark the void volume. Separation factor (α) was calculated by the ratio of the retention factors of the respective compounds.

2.4. Calculation

Positive charges on aromatic protons of β - and γ -tocopherols were calculated by Hyperchem Ver 5.1 with molecular mechanics (until the energy changes were below 0.001 kcal mol^{-1}) and following semi-empirical AM1 method.

3. Results and discussion

3.1. High retentivity for PAHs

As reported previously, Sil-VP shows very unique and high selectivity for planar/non-planar discrimination of PAHs [12], but its small retentivity should be improved. Therefore, the selectivity and retentivity for PAHs was investigated with Sil-CEA, which was newly synthesized in this work. Fig. 2 shows the results for the separation of benzene, naphthalene, anthracene and naphthacene as a series of linear and planar PAHs solutes. Sil-CEA shows sufficiently good selectivity compared with ordinal ODS. For example, α for naphthacene/anthracene is 3.09 with Sil-CEA, 2.40 with Sil-VP and 2.05 with ODS. It is also clear that the retentivity has been completely improved with Sil-CEA. For example, $\log k$ for naphthacene is 0.93 with Sil-CEA, 0.66 with ODS and 0.27 with Sil-VP. Structurally, carbazole is more bulky than pyridine, due to the presence of two phenyl rings along with five-membered heterocycle. The higher retentivity of PAHs observed with Sil-CEA can be understood from the larger number of interacting aromatic rings compared to Sil-VP.

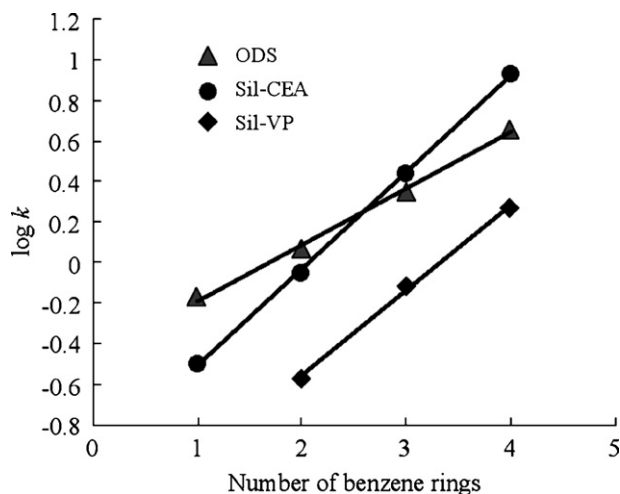


Fig. 2. Relationship between the log k and number of benzene rings with various columns. Solutes: benzene, naphthalene, anthracene and naphthacene; mobile phase: methanol–water (9:1); temperature, 35 °C; flow rate, 1 ml min⁻¹.

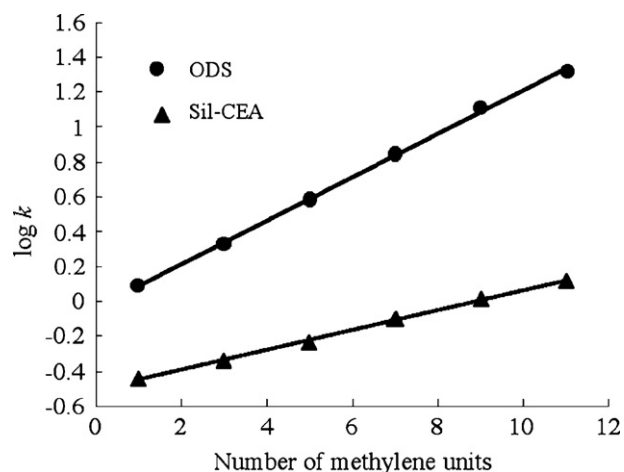


Fig. 3. Relationship between the log k and number of methylene units with Sil-CEA and ODS. Solutes: ethyl-, butyl-, hexyl-, octyl-, decyl-, dodecylbenzenes; mobile phase: methanol–water (9:1); temperature, 35 °C; flow rate, 1 ml min⁻¹.

3.2. Less interaction for alkylbenzenes

One of the main objectives of our investigation is to prepare the stationary phase having lower hydrophobic effects, which arises specially due to the dispersive or van der Waals interactions between the solute and stationary phases. In order to investigate on the extent of hydrophobic effect of Sil-CEA, a series of alkylbenzenes were used as solutes. As shown in Fig. 3, extremely lower retentivity was observed with Sil-CEA ($k = 0.36$ – 1.32). ODS phase, which work mainly based on the hydrophobic effect, showed much higher retention factors for the examined solutes compared with

Table 1

Retention (k) and separation factor (α) for planar and non-planar PAHs with various columns at 35 °C.

PAHs	Sil-VP		Sil-CEA		ODS	
	k	α	k	α	k	α
<i>o</i> -Terphenyl (C ₁₈ H ₁₄)	0.22		1.37		2.52	
Triphenylene (C ₁₈ H ₁₂)	2.63	12.0	6.94	5.07	3.79	1.50
<i>o</i> -Terphenyl (C ₁₈ H ₁₄)	0.22		1.37		2.52	
<i>p</i> -Terphenyl (C ₁₈ H ₁₄)	0.65	2.95	3.63	2.65	3.60	1.43
<i>cis</i> -Stilbene (C ₁₄ H ₁₂)	0.18		0.89		1.92	
<i>trans</i> -Stilbene (C ₁₄ H ₁₂)	0.41	2.28	1.59	1.79	2.05	1.07
<i>cis</i> -Stilbene (C ₁₄ H ₁₂)	0.18		0.89		1.92	
Phenanthrene (C ₁₄ H ₁₀)	0.82	4.56	2.46	2.76	2.10	1.09

Mobile phase: methanol–water (9:1).

the other reference columns. On the other hand, Sil-VP showed almost no retention for all solutes ($k < 0.1$). When graph was plotted between the number of methylene units and log k , small slope was obtained with Sil-CEA (0.06), whereas ODS showed higher slope (0.12). Less interactions of Sil-CEA for alkylbenzenes promise us a distinct advantage because the polymer main chain does not work as hydrophobic interaction site for solutes and thus the selectivity due to the CEA phase is not disturbed by a hydrophobic effect.

3.3. Molecular-shape selectivity for PAHs

The molecular planarity selectivity for PAHs solutes on Sil-CEA was investigated by using four-pairs of planar/non-planar solutes. As shown in Table 1, Sil-CEA provided high selectivity ($\alpha = 1.79$ – 5.07) with high retentivity compared with ordinal ODS ($\alpha = 1.07$ – 1.50) although Sil-VP showed furthermore higher selectivity but with small retentivity. Triphenylene and *o*-terphenyl have same number of carbon atoms and π -electrons, but triphenylene is planar and *o*-terphenyl is non-planar. For this pair higher selectivity was observed with Sil-CEA and Sil-VP compared to ODS. Between the *o*- and *p*-terphenyl, *o*-terphenyl is highly non-planar due to sterical hindrance of two phenyl rings at the *ortho* position compared to *p*-terphenyl. Higher selectivity for the pair was also observed with Sil-CEA and Sil-VP compared to ODS phase. Since the selectivity order are similar with each other but the selectivity for *p*-*o*-terphenyl and triphenylene/*o*-terphenyl are not so different with ODS phase as shown in Table 1. Whereas, lower selectivities were observed in Sil-VP and Sil-CEA for *p*-*o*-terphenyl compared to triphenylene/*o*-terphenyl. From this result, it can be estimated that high-density polymer network on silica can recognize the extent of molecular bulkiness.

On the other hand, the other unique chromatographic property in Sil-VP is to show an opposite retention order for molecular linearity (L/B) [12]. In this work, the same behavior was observed in the separation of the sample sets of phenanthrene–anthracene and triphenylene–naphthacene as shown in Table 2. On the contrary, Sil-CEA followed that in ODS. The mechanism for molecular-shape selectivity is discussed in the next section.

Table 2

Retention (k) and separation factor (α) for linear and disc-like PAHs with various columns at 35 °C.

PAHs	L/B	Sil-VP		Sil-CEA		ODS	
		k	α	k	α	k	α
Phenanthrene (C ₁₄ H ₁₀)	1.46	0.82	0.94	2.46		2.10	
Anthracene (C ₁₄ H ₁₀)	1.57	0.77	(1.06) ^a	2.74	1.11	2.22	1.06
Triphenylene (C ₁₈ H ₁₂)	1.12	2.63	0.70	6.94		3.79	
Naphthacene (C ₁₈ H ₁₂)	1.89	1.85	(1.42) ^a	8.48	1.22	4.54	1.20

Mobile phase: methanol–water (9:1).

^a Inverse value of the selectivity.

3.4. Retention mechanism

Uniqueness of Sil-VP as well as Sil-CEA is to show less interaction for alkylbenzenes in RP-HPLC mode. This is a quite interesting point because various polymer-grafted silica such as poly(styrene)-[21], poly(methyl acrylate)- [22], poly(octadecyl acrylate)-grafted silicas [23] showed relatively higher retentivity. The hydrophobicity of polymers is partially derived from their polymer main chains. Therefore, the less interaction for alkylbenzenes in CEA and VP gives us important information on their physical states on silica. As a supporting result, it should be noted that VP shows a good masking effect for the silanol residue on silica. Residual silanol causes peak-broadening and excessive retention for basic substances. We have proposed that this masking effect can be induced by hydrogen bonding between a silanol proton and a pyridine moiety [12]. Therefore the main chain is not flexible but rather rigid on silica because of multiple-bindings with the silanol groups. As connecting such a physical state with less interaction for hydrophobic substances, it is considered that the polymer main chains form high-density network on silica and more hydrophilic pyridine groups faces toward a polar mobile phase than hydrophobic polymer chains. Similar to this estimation, CEA as a weak basic moiety can perform hydrogen-bonding interaction with silanol protons, but its more hydrophobic property causes to show a little higher retentivity for hydrophobic substances than VP. Supporting our assumption, no dispersive interaction has also been reported with ethylpyridine phases [24].

The estimated physical state of CEA on silica is probably related to molecular-shape selectivity for PAHs. Here, the specificity in selectivity is summarized: Sil-CEA shows (1) high retentivity for aromatic hydrocarbons, (2) high selectivity in planar/non-planar discrimination, and (3) opposite selectivity for molecular linearity (*L/B*) against Sil-VP. The main driving force for interaction with PAHs is possibly based on benzene H-to-benzene π interaction as shown in Fig. 4. We have clarified by calculation that a benzene H-to-benzene π interaction ($1.78 \text{ kcal mol}^{-1}$) is stronger than a benzene π -to-benzene π interaction as a plane-to-plane inter-

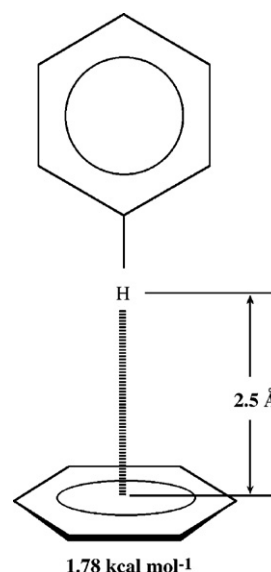


Fig. 4. Benzene H-to-benzene π interaction estimated by ab initio MO/MP2 calculation.

action ($0.49 \text{ kcal mol}^{-1}$) [25]. This calculation result can be applied for a solute H-to-carbazole π interaction. Therefore, the CEA polymer network can provide a specific microenvironment suitable for multiple benzene H-to-benzene π interactions, especially for compact and planar PAHs such as triphenylene compared to non-planar PAHs, such as *o*-terphenyl.

To understand the molecular linearity selectivity with Sil-CEA, we focus on the structure of carbazole moiety. Fig. 5 shows possibility of multiple interactions to explain the specificity in CEA. We focus on the fact that a carbazole moiety is rigid and consists of two benzene rings along with five-membered heterocycle. π -Electrons

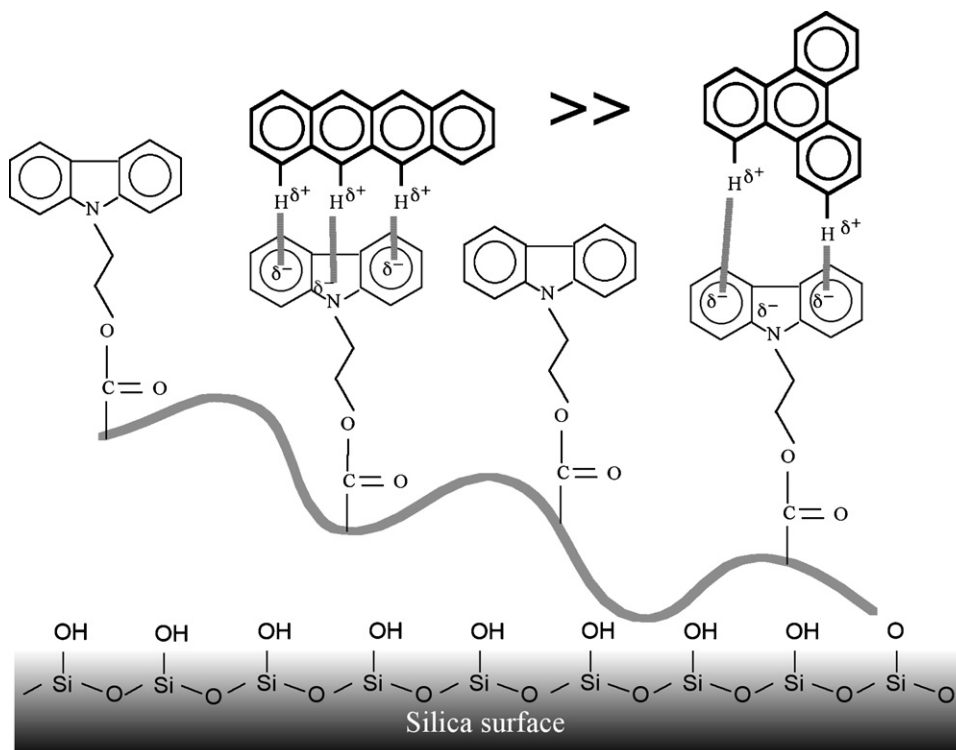


Fig. 5. Proposed illustration for higher preference for linear PAHs solute compared to disc-like one in Sil-CEA.

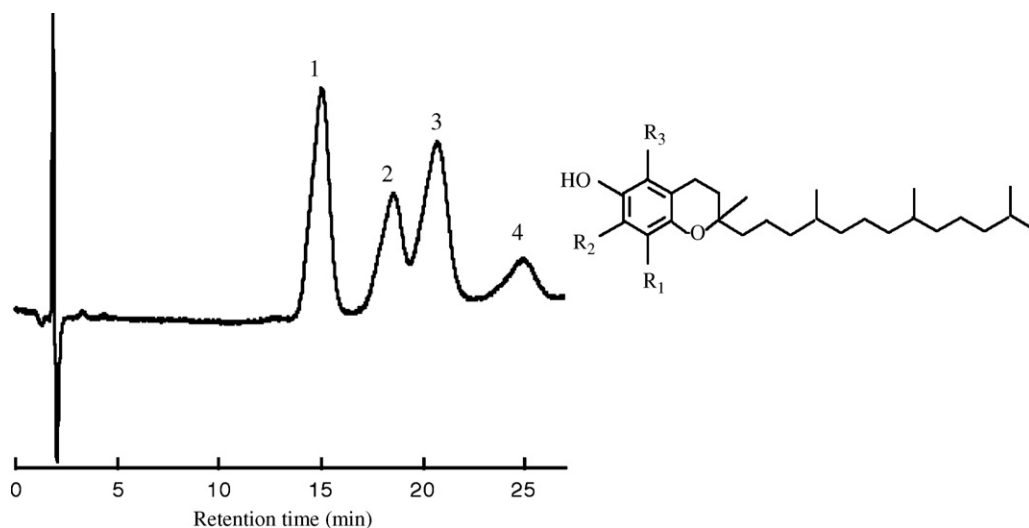


Fig. 6. Typical chromatogram for a mixture of tocopherols with Sil-CEA. Mobile phase: methanol–water (8:2); temperature, 35 °C; flow rate, 1 ml min⁻¹; solute: δ -tocopherol (1, R₃ = H, R₂ = H, R₁ = CH₃), γ -tocopherol (2, R₃ = H, R₂ = CH₃, R₁ = CH₃), β -tocopherol (3, R₃ = CH₃, R₂ = H, R₁ = CH₃) and α -tocopherol (4, R₃ = CH₃, R₂ = CH₃, R₁ = CH₃).

are not localized but rather conjugated on three rings. Therefore, Fig. 5 indicates that a carbazole moiety can interact with multiple interaction sites toward linear solutes such as naphthalene.

3.5. Application of Sil-CEA

The shape selective separation behaviors based on solute H-to-carbazole π interaction of the prepared stationary phase can be examined by using tocopherols as the solutes. Tocopherols constitute a major part in vitamin E groups and consist of four homologues, namely α -, β -, γ - and δ -tocopherol, showing different bioactivity. Between the different isomers of tocopherol because of similar hydrophobicity, ODS phase cannot separate β - and γ -tocopherol. However, aromatic protons of β - and γ -tocopherol show different chemical shifts in ¹H NMR spectra [10]. Calculation also showed the higher positive charge in the aromatic proton of β -tocopherol (0.147) compared to the aromatic proton of γ -tocopherol (0.126). The chromatogram of tocopherol using Sil-CEA as stationary phase is shown in Fig. 6. By using Sil-CEA, the separation of α -, β -, γ - and δ -tocopherols were performed in good selectivity. Especially, the separation factor of β - and γ -tocopherols ($\alpha_{\beta\text{-tocopherol}/\gamma\text{-tocopherol}} = 1.13$) is remarkable. Thus, the separation of β - and γ -tocopherol along with higher retentivity of β -tocopherol support our estimated mechanism of benzene H-to-carbazole π interaction. From this result, it is estimated that Sil-CEA has a higher potential to separate the aromatic compounds, which have similar hydrophobicity, but different aromaticity and shape.

4. Conclusion

Poly(2-*N*-carbazolyethyl acrylate)-modified silica as a RP stationary phase described in this study, showed higher preference towards molecular aromaticity to molecular hydrophobicity and brought the shape selective separation. It has been estimated that solute H-to-benzene π interaction between the solute and the prepared stationary phase has enhancing effect on retention and molecular-shape selectivity.

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