# Poly(4-vinylpyridine) based novel stationary phase investigated under supercritical fluid chromatography conditions

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ABSTRACT

A novel poly(4-vinylpyridine) based stationary phase was investigated for its performance under supercritical fluid chromatography (SFC) mode. Due to its unique structure, this stationary phase has high molecular planarity recognition ability for aromatic samples possessing the same number of aromatic rings and $\pi$-electrons. Taking advantage of the planarity recognition ability observed, separations of structurally similar polycyclic aromatic hydrocarbons and steroids were achieved. This novel stationary phase afforded good peak symmetry for both acidic and basic active pharmaceutical ingredients even when excluding the use of additives such as acids, bases, and salts. These findings may be attributed to the polymeric pyridyl groups covalently-attached on silica gel, which will effectively shield the undesirable interaction between residual silanol groups on the surface and the analytes. Moreover, the properties of pyridyl group on the selector can be reversibly tuned to cationic pyridinium form by eluting trifluoroacetic acid containing modifier. Column robustness toward cycle durability testing was also confirmed.

## 1. Introduction

Supercritical fluid chromatography (SFC) is increasing in use in the analytical and preparative separation field [1]. SFC uses supercritical or subcritical mobile phases consisting of pressurized carbon dioxide ($CO_2$), usually mixed with a miscible organic solvent (e.g. an alcohol). This technology has major advantages over more conventional liquid chromatography (HPLC) or gas chromatography (GC), because it has a low viscosity allowing high diffusivities and limited pressure drop. Therefore, high flow rates can be applied without losing efficiency [2–8]. In addition, the "green" aspect is a significant motivation for SFC because $CO_2$ is a nontoxic recycled material and generates no waste disposal issues. The high-throughput potential together with ecological advantages contribute to making SFC attractive technology for a wide range of applications, not only for chiral [9–15], but also in the achiral field [16–30].

The retention and separation mechanisms in SFC are likely to depend on a combination of both mobile phase and stationary phase (SP)[5]. A variety of SPs are currently available for use in SFC mode. Most of these phases have been developed in and transferred from the commercially available portfolios of HPLC SPs (e.g.

reverse phase, normal phase, and/or HILIC). In parallel, there are some activities to develop novel SPs specifically designed for SFC use [31]. One of the most recognized SP dedicated to achiral SFC separation is 2-ethylpyridine (2-EP) bonded silica phase. This 2-EP SP affords good peak shapes especially for basic compounds, without any additive in the mobile phase [32]. Other novel SPs for SFC have been developed by academic and industry groups [33–38].

Most of the SPs used for achiral SFC separations are composed of low-molecular-weight selectors covalently bonded onto a solid support, usually silica gel. Polymer type selectors would be expected to interact with analytes by utilizing multiple and cooperative mechanisms and in addition possess high durability. However, only a very limited number of examples have been introduced that utilize polymer-based ligands for achiral SFC separation [36].

Based on the experience of our research team in the polymeric field, we recently developed a novel poly(butylene terephthalate) based column, which exhibited unique molecular recognition ability together with high robustness in cycle durability tests [38]. We considered that these features may be attributed to the associated macromolecular effect and as a result we decided to develop various polymer-based SPs and to evaluate their performance.

For the design of the novel polymer stationary phase series, we attempted to prepare several polymers based on the ethylpyridine moiety, mainly as the commercial phases containing such synthon are considered as benchmarks for many researchers. In

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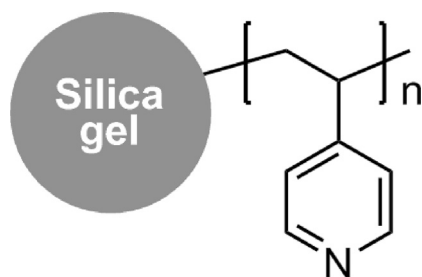  *E-mail address:* kn_nagai@jp.daicel.com (K. Nagai).

**Fig. 1.** Structure of poly(4-vinylpyridine) or P4VP-based selector of the column DCpak P4VP.

this perspective, moving from a monomeric to a polymeric selector, we were expecting that the molecular recognition ability may be improved by multiple concerted interactions between the more abundant polymeric pyridyl ligand interactions with the analyte sample. Similar to the recently reported poly(butylene terephthalate) selector, these polymer type selectors were anticipated to display a high durability as the polymer layer on silica gel should effectively shield any undesirable chemical interaction.

The HPLC separation behavior of pyridine containing polymer SP has been studied by Ihara [39–41]. This SP showed good selectivity particularly for planar and disk-like aromatic molecules in reverse and normal phase HPLC modes. The investigations of the polymeric phase in HPLC mode was the objective of this work.

In an earlier study, we focused on novel vinylpyridine polymers and related vinyl heteroaromatic polymers, and evaluated their performance in SFC mode. We tested various poly(vinylpyridine isomers), including poly(2-vinylpyridine), poly(3-vinylpyridine), and poly(4-vinylpyridine), together with poly(vinylimidazole) [42,43]. These SPs afforded distinctive molecular recognition abilities, particularly for structurally-similar isomeric samples. Among them, poly(4-vinylpyridine) (P4VP) SP was found to provide better molecular shape recognition performance (Fig. 1). The present article focuses on this P4VP column and describes its separation behavior by using various samples. Based on these results, its characteristics and suitable chromatographic conditions are discussed.

## 2. Materials and methods

### 2.1. Chemicals

The modifier used in this study was Japanese Industrial Standard special grade methanol (MeOH) obtained from Nacalai Tesque Inc. (Kyoto, Japan). Carbon dioxide of industrial grade (over 99.5%) was purchased from Tatsumi Industry Co., Ltd. (Hyogo, Japan). Ammonium formate was obtained from Wako Pure Chemical Industries (Osaka, Japan).

*o*-Terphenyl, triphenylene, anthracene, phenanthrene, pyrene, chrysene, perylene, theobromine, *trans*-cinnamic acid, 3-phenylphenol, adenine, and diethylamine were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). 1,3,5-Tri-*tert*-butylbenzene, 2-acetylanthracene, 9-acetylanthracene, 3-acetylphenanthrene, 9-acetylphenanthrene, paraxanthine, fenoprophen, ketoprofen, naproxen, alprenolol, propranolol, atenolol, pindolol, and cyanocobalamin were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Trifluoroacetic acid (TFA), naphthacene and dexamethasone were purchased from Nacalai Tesque Inc. (Kyoto, Japan). 2-Propanol (IPA), prednisone, estrone, prednisolone, estradiol, estriol, caffeine, theophylline, nicotinamide, and pyridoxine were purchased from Wako Pure Chemical Industries (Osaka, Japan). *N*-hexane (nHex) was purchased from Kanto Chemical Co. (Tokyo, Japan)

### 2.2. Instrumentation and chromatographic conditions

DCpak P4VP column (initially launched as DCpak SFC-B), sized 150 mm × 4.6 mm (i.d.), was supplied by DAICEL Corporation (Tokyo, Japan). This selector is composed of immobilized P4VP on 5 μm silica particle (N.B. also available on 3 μm). A Silica 2-ethylpyridine (2-EP) column of 5 μm particle, sized 150 mm × 4.6 mm (i.d.), was purchased from Waters Corporation (Milford, MA, USA). The SFC instrument used in this study is Nexera-UC supplied by Shimadzu Corporation (Kyoto, Japan) equipped with a $CO_2$ pump, a modifier pump, a vacuum degasser, a column oven, a multiple wavelength UV detector, and automated back pressure regulator (ABPR). Lab Solutions software (V 5.89) was used for system control and data acquisition. Chromatographic conditions, such as modifier, column temperature, ABPR pressure, total flow rate, detection wavelength, sample concentration, and injection volume were described in each figure, respectively.

### 2.3. Data analysis

Relative retention factor ($k$) and separation factor ($\alpha$) were calculated with the equations below.

$$k = (V/V_0) - 1, \tag{i}$$

$$\alpha = k_2/k_1 \tag{ii}$$

where $V$ is the elution volume of an analyte and $V_0$ is the column void volume. $V_0$ was estimated by injecting 1,3,5-tri-*tert*-butylbenzene as a non-retained marker. $k_1$ and $k_2$ in Eq. (ii) are the retention factors of the first and second eluted peaks, respectively.

## 3. Results and discussion

### 3.1. Planarity recognition of aromatics

Considering the structure features of the poly(4-vinylpyridine) SP, it was expected to interact with planar aromatic samples as a result of the multiple aromatic pyridyl units covalently attached on silica gel. Non-planar *o*-terphenyl (**1**) and planar triphenylene (**2**) with the same number of aromatic rings and π-electrons will provide detailed perception of the planarity recognition ability, because they have been considered as indicator for molecular planarity recognition in HPLC [44,45] and SFC [46]. Fig. 2A shows the SFC chromatogram of **1** and **2** by using P4VP, when its performance was compared with commercially available 2-EP SP under isocratic conditions. The retention time of non-planar **1** was almost identical for the new selector and 2-EP, while that of planar sample **2** significantly increased for P4VP. The separation factor for P4VP selector between **1** and **2** ($\alpha$: $k_2/k_1$) reached 30.6, whereas that obtained by 2-EP is 4.4. This result indicates that π-electron rich planar **2** could strongly interact with vinylpyridine polymer selector via π–π interaction.

Taking advantage of this high planarity recognition ability, commercially available polycyclic aromatic hydrocarbons (PAHs) were analyzed. Fig. 3 shows the SFC chromatogram of eight PAHs (**2–9**) under gradient condition. Eight peaks were well separated on the P4VP column. Of particular note is that anthracene (**4**) and phenanthrene (**5**) have the same molecular weight and similar molecular size and polarity. Therefore, these two compounds cannot be distinguished by MS detector, which mean that the only method to separate **4** and **5** must be by column separation. This new selector achieved a baseline resolution for **4** and **5**. On the other hand, when these compounds were analyzed by 2-EP SP under the same condition, **4** and **5** co-eluted. The slight adjustment of gradient conditions was necessary to separate **4** and **5** in isocratic mode, immediately after eluting **4** and **5**, a linear gradient program started.