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Recognition mechanism of aromatic derivatives resolved by argentation chromatography: The driving role played by substituent groups

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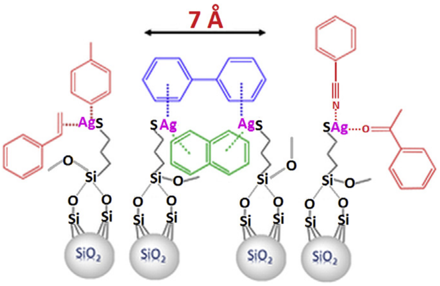
HIGHLIGHTS

- The recognition mechanism of silverthiolate phases towards aromatics is investigated.
- The found retention and selectivity was fully rationalized through DFT calculations.
- Effect of electron withdrawing (EW) and electron donating (ED) groups was considered.
- Groups with strong EW effect directly interact with the silver-selector.
- ED groups induce silver to interact with the aromatic ring.

GRAPHICAL ABSTRACT



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ABSTRACT

Argentation chromatography is widely used nowadays as a powerful tool to separate complex mixtures of analytes containing unsaturated and/or aromatic fragments. Here we present the results of chromatographic and computational studies on a silver-thiolate stationary phase, in which the silver metal is covalently bonded to mercaptopropyl silica particles. The exceptionally high selectivity displayed by this organometallic moiety prompted us to deeply investigate its molecular recognition properties. The interactions of the silver atom with a series of benzene derivatives was investigated to gain information on the mechanism by which the different ring substituents modulate retention factors and selectivity. The experimental trend was fully rationalized by means of quantum-mechanical Density Functional Theory (DFT) calculations, which allowed us to elucidate the chromatographic results in the light of unusual and unexpected substituent effects.

1. Introduction

The ability of transition-metal cations, such as Ag$^+$, Au$^+$ and Cu$^+$, to interact with unsaturated bonds by forming weak complexes stabilized by charge-transfer interactions has promoted their application in liquid chromatography as suitable Stationary Phases (SPs) useful to effectively resolve complex mixtures of organic molecules of analytical or pharmaceutical interest. They are

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able to perform this kind of discrimination on the basis of number, position and stereochemistry of unsaturated bonds [1–3].

Such an approach is commonly and successfully employed in food chemistry, in order to discriminate and define structures of lipids or of other natural products [4–7].

The silver thiolate stationary phase, in which the silver atom is covalently linked to the sulphur atom of mercaptopropyl silica gel [8–10] (MPSG-Ag), has been recently introduced for the separation of unsaturated and/or aromatic compounds as alternative to the common SPs used in *argentation chromatography* (also known as *silver ion chromatography*). The increasing interest for this kind of SP in argentation chromatography is undoubtedly related to the substantially covalent anchorage involving the silver cation (Ag^+) onto the thiolate moiety of the MPSG-Ag. In fact, when compared to the SPs derived by the direct process of impregnating of the silica surface with silver cations, as well as to those based on the use of strong cation exchange resins, the new silver-thiolate SPs display a consistent improvement of chemical stability. The reasons of such different characteristic are associated to the ionic nature of the interaction between Ag^+ and the matrix surface in the older types of SPs. Indeed, this makes such SPs prone to undergo both silver ion leaching (which leads to scarce reproducibility of retention times) and easy reduction of the metal cation, from Ag^+ to Ag^0, responsible for oxidation of target compounds [8,9].

The exceptionally high selectivity displayed by this SP is mostly due to the ability of the silver to form weak charge-transfer complexes with unsaturated/aromatic analytes. This is usually imputable to three different contributions: electrostatic interactions (ion-dipole), electron donation from occupied π-orbitals of the multiple bond to empty 5s- and 5p-orbitals of the transition metal and a back-donation from filled metal 4d-orbitals to unoccupied π^*-orbitals of the unsaturated bond [4,11–16].

With the aim to better define the characteristics of the molecular recognition exhibited by silver thiolate stationary phases towards aromatic compounds, a series of twelve benzene derivatives (**Ar-G$_i$**) were used as molecular probes in runs of argentation High Performance Liquid Chromatography (Ag-HPLC). The different **Ar-G$_i$** derivatives feature electron withdrawing (EW) or electron donating (ED) groups, whose substructure contains or not unsaturated bonds. Starting from the peculiar retentions and selectivities observed on a MPSG-Ag phase for our **Ar-G$_i$** probes, we designed Density Functional Theory (DFT) calculations to rationalize the experimental results. A very satisfactory interpretation of the unexpected and non-trivial substituent effects governing the chromatographic retention mechanism was achieved and comprehensively discussed.

2. Material and methods

2.1. Synthesis of MPSG stationary phase

1 g of imidazole was dissolved in 60 mL of dry toluene at 60 °C and then 5.5 g of silica (Kromasil 5 μm, 100 Å, 340 m^2/g previously dried under vacuum at 120 °C for 2 h) were added to the solution. To this silica dispersion it was added dropwise a solution of 2.7 mL of 3-mercaptopropyl-trimethoxysilane ($C_6H_{16}O_3SSi$, Mw: 196.34 u.m.a.; d: 1.039 g/cm^3; 2.835 g corresponding to 13.55 mmol) in 20 mL of dry toluene. The resulting mixture was maintained at 60 °C for 20 h under N_2 atmosphere and mechanical stirring. After cooling, modified MPSG silica was filtered, washed with toluene, methanol and dichloromethane and dried under vacuum at 60 °C.

Elemental analysis: 4.49%C, 1.03%H, 2.90%S, corresponding to 1.05 mmol/g silica (0.9 mmol/g matrix), 3.0 μmol/m^2 and an average distance among the linked groups of 7.4 Å (based on sulphur percentage). The percentage of weight increase, experimentally determined by weighing silica after its derivatization, was 12%. According to the known Kiselev-Zhuravlev constant for bare silica [17,18] (i.e. 4.9 silanol sites distribute on a surface of 1 nm^2), the S-H group and residual silanol densities on the surface of MPSG are 1.8/nm^2 and 1.6÷3.1/nm^2 (depending if one or two silanols are engaged in linking the 3-mercaptopropyl-trimethoxysilane), respectively.

2.2. Synthesis of the MPSG-Ag stationary phase

MPSG-Ag stationary phase was prepared according to the synthetic procedure reported in Ref. [8]. In detail, 2.1 g of MPSG silica were dispersed in 25 mL of acetonitrile and maintained under continuous stirring in the dark. A solution of $AgNO_3$ in acetonitrile (0.493 g, corresponding to 2.9 mmol, in 5 mL) was quickly added to dispersion. The reaction mixture was maintained under stirring for 2 h at room temperature. The modified MPSG-Ag silica was isolated by filtration and then washed in five steps with acetonitrile, methanol, methanol/water 1/1, methanol, acetonitrile. After drying, a 5% weight increase of MPSG was found, corresponding to incorporation of 0.47 mmols of Ag into 1g of MPSG-Ag silica matrix. This allowed to assess that in the final MPSG-Ag SP, the residual silanols should amount to 1.6÷3.1/nm^2, the S-H groups to 0.9/nm^2 and the S-Ag groups to 0.9/nm^2, with an average distance between two adjacent S-Ag sites assessed to be about 10 Å (Figure S-1 of Supplementary Data, SD).

2.3. Experimental chromatographic runs

Argentation chromatography separations were performed on the MPSG-Ag (150 × 4.6 mm I.D.) SP, by using the mixture n-hexane/chloroform (stabilized by amylene) 90/10 (v/v) as the mobile phase. A void volume of 1.64 mL was measured by pycnometric determination, which corresponds to a void time of 1.64 min at the used flow rate of 1.0 mL/min.

Normal phase chromatographic runs on bare silica were performed on a Kromasil 5 μm (150 × 4.6 mm I.D.) SP, by using the mixture n-hexane/chloroform (stabilized by amylene) 90/10 (v/v) as the mobile phase. A void volume of 1.67 mL was measured by pycnometric determination, which corresponds to a void time of 1.67 min at the used flow rate of 1.0 mL/min.

All HPLC solvents were purchased from Aldrich (Italy) and used without further purification. The analytical HPLC apparatus was the Nexera instrument (Shimadzu), equipped with a 20-μL sample loop and a photodiode array (PDA) detector. All chromatographic runs were performed at the flow rate of 1.0 mL/min and recorded at the wavelength of 254 nm.

2.4. Theoretical DFT calculations

All calculations were performed with the software package SPARTAN 10, v. 1.1.0 (Wavefunction, Inc., Irvine, CA, USA), by using the B3LYP/6-31G* level of theory. The used 6-31G* basis set was imposed by the presence in the structures of the optimized adducts of the Ag atom, which is not supported by more extended basis sets.

The Ag-S-CH_2-CH_2-CH_3 framework was used as a simplified model of the SP. Selector-selectand complexes were built by hand by considering: i) all the non-equivalent carbons of the aromatic ring; ii) all the nuclephilic atoms belonging to the **G$_i$** substituent groups. Energy optimization were performed in gas phase and, among a total of 51 optimized complexes, only the adducts characterized by the strongest selector-selectand interaction of **SP-Ag:^{y}Ar-G$_i$** and **SP-Ag:G$_i$-Ar** type (see next section) were considered for each benzene derivative. (Table S-1 of SD). Afterwards, all the most stable **SP-Ag:^{y}Ar-G$_i$** and **SP-Ag:G$_i$-Ar** optimized complexes

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