

**UNIVERSITY OF PÉCS**  
**Doctoral School of Chemistry**

**Investigation of Modifier and Additive Adsorption in Supercritical Fluid  
Chromatography**

PhD thesis

**MUHAMAD YAHIA KAZMOUZ**

Department of Analytical and  
Environmental Chemistry

Supervisor

**Dr. ATTILA FELINGER**  
professor of chemistry



**PÉCS, 2022**

## 1. Introduction

The importance of chromatography as an analytical technique in industrial applications arises from the ability to separate a mixture into individual components as a result of different rates of distribution of the components between stationary and mobile phases. Supercritical fluid chromatography (SFC) utilizes a mobile phase in a supercritical phase, which is characterized by “gas-like” viscosity and “liquid-like” density. The supercritical phase is obtained when both temperature and pressure are above the critical values.

In general, SFC is considered an environment-friendly approach due to utilizing carbon dioxide as the main eluent, in turn less consuming of organic solvents in comparable to high performance liquid chromatography (HPLC), hence interest in SFC is growing under the increased care given to policies related to environmental issues (especially the use of solvents) and as a result of continuous rationalization efforts in every industrial area.

The applications of SFC using 100% CO<sub>2</sub> as the mobile phase are limited due to the low polarity of CO<sub>2</sub>, which is very close to that of liquid pentane or liquid hexane; hence, it can only dissolve nonpolar compounds. Therefore, the majority of SFC experiments are carried out with alcohol modified - CO<sub>2</sub>, such as methanol or isopropanol, to improve the solubility of the analytes in the mobile phase, which would give better chromatographic peaks.

Furthermore, for expanding the range of analytes polarity, which can be analyzed with SFC, a third component called an additive, which can be a base, acid, salt, or water, is highly recommended to be used in the mobile phase in a very small amount, typically less than 1% of the modifier. It is usually premixed with the modifier, and then both are transferred to the mobile phase, resulting in a better peak shape of the analytes. For instance, the basic analytes (e.g., nitrogen-containing molecules) are considered problematic, such as the dibenzylamine and benzylamine mixture, which was studied by Berger and his co-workers. They reported that the mixture does not elute with a good peak shape even using methanol-modified CO<sub>2</sub> but in the presence of isopropylamine as an additive, a good improvement in the peak shapes was obtained.

It is known in nonlinear chromatography that the separation process and the migration rate of sample components through a porous stationary phase are highly influenced by the adsorption isotherms of each component. For instance, in a ternary mobile phase (CO<sub>2</sub> + modifier + additive) where they are at a sufficient concentration (multicomponent system), their adsorption on the stationary phase will be influenced by each other; hence, determining the adsorption isotherm will be a useful tool to describe the adsorption behavior of each component on the stationary phase.

For determining the isotherms for a multicomponent system, the competitive form should be used, which is called 'competitive isotherms', whereas a single component isotherm is used to describe the adsorption for a single-component system.

## 2. Objectives

The main aims of this research were:

- To show how the reversed stationary phase would be affected in the presence of methanol as a modifier by calculating the excess adsorption isotherm of methanol.
- Comparing the excess adsorption isotherm of methanol under two conditions of temperature and back pressure, the subcritical (26 °C, 145 bar) and the supercritical (40 °C, 250 bar).
- To evaluate the influence of polar and non-polar sample solvents on the separation efficiency of alkylbenzenes mixture on the reversed stationary phases using 100% CO<sub>2</sub> as the mobile phase.
- To determine the surface heterogeneity of the used columns by fitting the surface excess adsorption amount of methanol to a theoretical model.
- To study the adsorption behavior of water, methanol, and their mixture on the polar stationary phase by calculating the corresponding adsorption isotherms.
- Evaluating the use of the inverse method for calculating both the single-component and competitive adsorption isotherms for binary and ternary systems, respectively.
- Predicting the elution profile of the methanol and water mixture sample based on the isotherm parameters obtained from (i) the bi-Langmuir isotherm of a single component of both methanol and water, and (ii) the competitive bi-Langmuir isotherm of the methanol and water mixture.
- Investigating the effect of water as an additive in the methanol-modified carbon dioxide as a mobile phase on the efficiency, peak shape, and retention time of polar solutes on the polar stationary phase.

### 3. Experimental and methods

The chromatographic experiments were performed on the Waters UPC<sup>2</sup> SFC system. Two end-capped reversed phase columns, Supelcosil ABZ+plus HPLC (embedded with amide groups) from Sigma-Aldrich, Symmetry C<sub>18</sub> from Waters, and Viridis BEH (hybrid silica) from Waters, have been used in this study. A Coriolis mass flow meter was used to measure the actual mass flow rate of the mobile phase. A digital pressure meter was used to measure pressure values at the inlet and outlet of the columns.

#### Estimation of the actual volumetric flow rate of the mobile phase inside the Viridis BEH column for calculating the single component and competitive adsorption isotherms

The actual volumetric flow rate of the mobile phase is different from the set value due to the compressibility of CO<sub>2</sub>. For determining the actual volumetric flow rate, we measured the actual mass flow rate using a Coriolis mass flow meter, which is installed after the CO<sub>2</sub> pump outlet and before the mobile phase mixer. We assumed that the actual and set flow rates of methanol were same. Also, the pressure at the inlet and outlet of the column was measured by an external pressure meter, the values were 184.57 and 154.3 bar, respectively. The density of the mobile phase inside the column was determined by the REFPROP software Ver. 8 from the National Institute of Standards and Technologies (NIST) at the set temperature of the column 26 °C and the measured pressure values at inlet and outlet of the column. By input, the values of the measured mass flow rate of the mobile phase  $F_m = 0.943$  g/min and the calculated density of the mobile phase  $\rho = 0.89891$  and  $0.87466$  g/cm<sup>3</sup> for the inlet and outlet of the column, respectively, in the formula:  $F_v = F_m / \rho$

we could obtain the average of the two volumetric flow rates as  $F_v = 1.065$  mL/min, which represents the actual volumetric flow rate of 100% CO<sub>2</sub> at a set value of 1 mL/min inside the Viridis BEH column. That will be used for calculating the single component and competitive adsorption isotherms.

#### The minor disturbance method for determining the surface excess adsorption of methanol on the Symmetry C<sub>18</sub> and ABZ+plus columns

The minor disturbance or perturbation method includes equilibrating the chromatographic column with different percentages of the organic modifier and carbon dioxide, covering the whole range between pure organic modifier and pure carbon dioxide. After reaching a stable baseline, which is called a concentration plateau, injecting a small amount 2 $\mu$ L of organic modifier (methanol) into the chromatographic column generates minor disturbance peaks, which were recorded at wavelength 210 nm. The retention volume of disturbance peaks was determined for calculating the amount of surface excess adsorption. The excess isotherm of methanol is represented by plotting the surface excess amount of methanol against the mole

fraction of methanol in the mixture. The experiments for measuring the surface excess of methanol on the reversed phases were performed under two conditions of temperature and back pressure, the subcritical (26 °C, 145 bar) and the supercritical (40 °C, 250 bar), and the mobile phase flow rate was set at 1 mL/min.

#### Elution of the alkylbenzenes sample from the reversed stationary phases

A mixture of nine alkylbenzenes was dissolved in two sample solvents, which have different polarities: methanol and heptane. 2 $\mu$ L of the mixture was injected into both the Supelcosil ABZ+plus and Symmetry C<sub>18</sub> columns and eluted using 100% CO<sub>2</sub> as the mobile phase. The elution was performed under subcritical and supercritical conditions. The chromatograms were recorded at the 210 nm wavelength of the detector.

#### Determination of the adsorption isotherm using the inverse method (IM)

The inverse method (IM) is one of the dynamic methods for adsorption isotherm determination. It is a fast and low-cost method compared to the plateau methods (frontal analysis or perturbation peak). It determines the isotherm parameters from overloaded profiles. This method has been widely applied to determine the liquid-solid equilibrium isotherms in HPLC.

The IM method comprises the following steps:

1. Recording the overloaded band profiles of each studied component.
2. Selecting a prior model for the adsorption isotherm, which is able to represent the distribution of a solute between the two phases accurately. The isotherm model is usually predictable (whether it is a convex, concave, or S-shaped) based on the shape of the recorded overloaded band profile.
3. Calculating the chromatograms using the initial estimated values of the isotherm parameters (the initial values were determined using the elution by characteristic point ECP method) and an appropriate model of the chromatography process. We used in the calculation the Equilibrium-Dispersive (ED) model.
4. Use the following formula to compare the experimental and calculated profiles:

$$\min \sum_i r_i^2 = \min \sum_i (C_i^{\text{sim}} - C_i^{\text{meas}})^2$$

where  $C_i^{\text{sim}}$  and  $C_i^{\text{meas}}$  are calculated and measured concentrations at point  $i$  and  $r_i$  is their difference.

5. Optimizing the isotherm parameter values by obtaining the minimum of the residual sum of squares (SSR) between the experimental and calculated curves using an optimization routine.

### Band profile simulation

The experimental elution profiles of methanol, water, and (10:90 v/v, methanol:water) mixture samples from the hybrid silica column were simulated by using an algorithm, which is a numerical solution of the partial differential equation of the Equilibrium-Dispersive (ED) model, which is the simplest model used to describe the chromatographic behavior of a solute within the column.

### Chromatographic experiments for the polar solutes elution from the hybrid silica column

The chromatographic experiments for the following solutes: aniline, caffeine, uracil, propranolol HCl, sulfamethazine, and sulfamethizole were carried out at set values of 26 °C, 150 bar, 2 mL/min of column temperature, back pressure, and flow rate, respectively. The detector signal was recorded at 204 nm. All the solute samples were prepared with a concentration of 0.5 mg/1.5 mL and aniline of 1 µL/1.5 mL. Methanol was used as a sample solvent. The sample injection volume was 1 µL.

#### 4. Results

The surface excess isotherms of methanol on both reversed stationary phases ABZ+plus and Symmetry C<sub>18</sub> columns under sub- and supercritical conditions show that a large amount of carbon dioxide adsorbs onto hydrophobic bonded ligands of the stationary phase, which is represented by a wide negative part of the excess isotherm. While methanol adsorption appears much less, which is represented by a positive small part at a low concentration of methanol in the mobile phase. It also indicates that a small amount of methanol could adsorb on the accessible silanols and other polar groups, if they are present in the structure of the stationary phase (such as the amide group in the ABZ+plus column); consequently, the maximum amount of surface excess of methanol for Symmetry C<sub>18</sub> is smaller than for the ABZ+plus column.

The chromatograms of alkylbenzenes separation on the reversed stationary phases ABZ+plus and Symmetry C<sub>18</sub> columns for both cases, methanol and heptane as sample solvents, indicate that baseline resolution was achieved for all the components of the mixture under the subcritical conditions, while under the supercritical conditions, the first three peaks of the alkylbenzenes are not completely resolved and all components elute from the column faster (shorter retention times are achieved), which may be due to the increased mixture solvation by higher dense CO<sub>2</sub> compared to the subcritical conditions. The elution order of the compounds is from the short alkyl chain compound to the long alkyl chain; the longer the alkyl chain the higher the retention time.

The results of the separation efficiency of alkylbenzenes demonstrate a similar trend of increasing values starting from the component with a short alkyl chain to the long one under the studied experimental conditions and both columns, except for the case of the methanol solvent on the ABZ+plus column under the subcritical conditions, where there is an abrupt drop in the curve after the decylbenzene peak. This may arise from the influence of the adsorbed methanol on the surface of the stationary phase, where a competition between the abundant methanol (sample solvent) and alkylbenzenes for adsorption introduces a combined displacement and tag-along effects for the various alkylbenzenes, in turn reducing the *N* values of these components.

By comparing the retention times of alkylbenzenes for both columns and both sample solvents, we found out that the analytes elute with the use of methanol faster than with heptane, which is explained by a higher amount of the adsorbed methanol on the ABZ+plus column compared to Symmetry C<sub>18</sub>, which in turn modifies the polarity of the stationary phase surface, hence forcing the hydrophobic analytes to elute faster from the column.



The IM has been used to calculate the adsorption isotherm for both methanol and water from the binary mixtures (methanol/CO<sub>2</sub>) and (water/CO<sub>2</sub>), respectively, on the hybrid silica column with 100% CO<sub>2</sub> as the mobile phase. The bi-Langmuir model was used to describe the adsorption of compounds on the column, which assumes the stationary phase is nonhomogeneous and covered with two different adsorption energy sites. The results point out that there is very good agreement between the elution and the simulated profiles, which confirms that the bi-Langmuir model was able to describe the adsorption process. The results indicate that one of the sites has a large saturation capacity for methanol, and the other one also has a large capacity for water.

Also, we used the IM for calculating the adsorption isotherm of the binary mixture of methanol and water from a ternary system (methanol, water, and CO<sub>2</sub>) on the hybrid silica column with 100% CO<sub>2</sub> as the mobile phase. The competitive bi-Langmuir model was used to describe the adsorption process.

The results indicate that the tendency of parameter values in the competitive isotherm is similar to that obtained with the single component isotherm, but the saturation capacities for adsorption of both methanol and water from the mixture are decreased compared to the single component sample, emphasizing the competition between methanol and water to adsorb on the same site.

The simulated band profile obtained by the parameter values of the competitive bi-Langmuir isotherm remarkably gave better agreement with the experimental profile than those obtained by the single component isotherms. Despite the good agreement between the measured and simulated profiles, the rear diffuse part of the calculated band profile has less agreement with the measured profile than the front part of the profile.

In regard to the use of water as an additive in the mobile phase for eluting polar compounds from the hybrid silica column, the results show that the more hydrogen bonding acceptor/donor sites in the analyte molecule, the more favorable the use of water as an additive for improving the chromatographic results. Moreover, the best chromatographic results of the analytes have been obtained at a water percentage of 0.03 ~ 0.06% in the total mobile phase, while adding a higher amount of water has a negative impact on the efficiency, the retention time, and the peak symmetry. On the other hand, it is found out that the use of water alone as an additive is ineffective for eluting such a strong basic compound as propranolol HCl with a good peak quality from the hybrid silica column (it requires adding a basic additive, e.g., diethylamine, for an ion suppression purpose). Additionally, incorporating water in the mobile phase containing a basic additive for eluting propranolol may result in propitious and unpropitious behaviors in accordance with the mobile phase components. It was noticed that in the case of increasing the modifier

percentage to higher than 3% in the mobile phase, the role of water in improving the chromatographic results won't be obvious.

## 5. Thesis statement

1. I determined the surface heterogeneity of two end-capped reversed stationary phases by calculating the excess adsorption isotherm for methanol from the methanol-CO<sub>2</sub> mixture under two sets of temperature and back pressure values using the minor disturbance method. The results obtained from the excess adsorption isotherms are very similar under the studied conditions for each column. Additionally, the surface excess adsorption amount of methanol for the embedded polar function stationary phase (amide group) is higher than the amount for the C<sub>18</sub> alkyl-bonded stationary phase.
2. I studied the influence of methanol and heptane as sample solvents to elute the alkylbenzenes mixture from the reversed stationary phases with 100% CO<sub>2</sub> as the mobile phase. I concluded that the analyte peaks are negatively influenced as a result of the tag-along effect of methanol on the ABZ+plus (alkylamide) column.
3. I investigated the adsorption behavior of both methanol and water on the hybrid silica column by determining the single component adsorption isotherms by the inverse method and using the bi-Langmuir adsorption model, which was a suitable choice to account for this adsorption.
4. I used the inverse method to determine the competition between methanol and water as components in the CO<sub>2</sub> mobile phase by calculating the competitive bi-Langmuir adsorption isotherms. The competitive bi-Langmuir adsorption isotherm parameters showed a decrease in the saturation capacity for both methanol and water compared to the saturation capacity values obtained from the single component bi-Langmuir adsorption parameters, emphasizing the competition between methanol and water to adsorb on the stationary phase.
5. I performed a simulation for the band profile of a 10:90 v/v methanol:water mixture sample by using the equilibrium-dispersive model. It was found that the simulated band profile calculated by using the competitive bi-Langmuir isotherm parameters gives a better fit with the experimental band profile than those obtained from single component isotherms.
6. I evaluated the influence of employing water in the methanol-modified mobile phase to elute polar compounds from the hybrid silica column. The results showed different behaviors for the use of water as an additive in the mobile phase, which depends on the target analyte structure. Some of the studied polar analytes exhibited improvements in the symmetry factor and efficiency when the water was added within the range of 0.03 ~ 0.06% in the total mobile phase. On the other hand, an excess amount of water has a negative impact on the separation properties.

## **PUBLICATIONS**

### Publications related to the thesis

1. M. Y. Kazmouz, Cs. Rédei, A. Felinger, The adsorption of methanol on reversed phase stationary phases in supercritical fluid chromatography, *Journal of Chromatography A*, 1653 (2021) 462386.
2. M. Y. Kazmouz, A. Felinger, Competitive Adsorption of Water and Methanol on a Hybrid Silica Stationary Phase in Supercritical Fluid Chromatography (submitted for publication).
3. M. Y. Kazmouz, A. Felinger, Impact of Water Use as Additive on Elution Some Basic Compounds in Supercritical Fluid Chromatography (submitted for publication).

### Posters

1. M. Y. Kazmouz, A. Felinger, The Competitive Adsorption of Water and Methanol on a Hybrid Silica Stationary Phase in Supercritical Fluid Chromatography; METT25, Egerszalók, Hungary (2021).
2. M. Y. Kazmouz, A. Felinger, Improve peak shape of basic analytes analyzed by supercritical fluid chromatography; 12 th Balaton symposium, Siófok, Hungary (2019).
3. M. Y. Kazmouz, A. Felinger, Competitive adsorption of the mobile phase modifiers in supercritical fluid chromatography; HPLC conference, Milan, Italy (2019).
4. M. Y. Kazmouz , Cs. Rédei, A. Felinger, use of Methanol as organic modifier with carbon dioxide in Supercritical Fluid Chromatography; separation science conference, Tapolca, Hungary (2018).
5. M. Y. Kazmouz, Sz. László, Cs. Rédei, I. Bacskay, A. Felinger, Surface excess isotherms of organic modifier and carbon dioxide mixture in sub- or supercritical fluid chromatography; Applications of supercritical fluids 2018 conference, Budapest University of Technology and Economics, Hungary (2018).