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Estudios termodinámicos de adsorción en sólidos porosos mediante cromatografía supercrítica

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TFM REALIZADO EN PROGRAMA DE INTERCAMBIO

TÍTULO: **Thermodynamic studies of adsorption on porous solids by supercritical fluid chromatography**

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Resumen

Se ha expuesto que partículas de sílica aerogel son un potencial candidato como sistema de administración oral de fármacos, donde la carga del fármaco se realiza habitualmente mediante $scCO_2$. En este trabajo, las interacciones entre la matriz, CO_2, metanol como modificador y diferentes solutos son analizadas mediante SFC, utilizando partículas de aerogel como fase estacionaria.

También se evaluaron como fase estacionaria otros tres tipos de partículas se sílice comercial. El hold-up time se calculó mediante la inyección de N_2O como "pico no retenido", que mostró ser un buen marcador para todas las fases. Se comprobó la estabilidad de las columnas a diferentes temperaturas y concentraciones de metanol. La columna de aerogel mostró buena estabilidad en un tiempo de operación de 48 horas en todos los casos estudiados.

La importancia de diversos tipos de interacciones intermoleculares que reflejan las propiedades de las fases estacionarias se evalúa mediante regresiones LSER utilizando 15 solutos. Se mostró que LSER es capaz de generar valores aceptables de tiempos de retención no sólo para las columnas comerciales (R^2 mayor que 0.94), sino también para las columnas de partículas de aerogel (R^2 mayor que 0.88). Los coeficientes a y b, relacionados con la acidez y la basicidad, son los descriptores dominantes que afectaron a la retención. Los compuestos polares son más sensibles a los cambios de temperatura, presión y concentración.

Se empleó un modelo de retención mixta para racionalizar el comportamiento de retención del soluto en SFC en presencia de metanol, considerando dos mecanismos: la interacción con las moléculas de metanol adsorbidas en la fase estacionaria y con los grupos silanol. La influencia de ambas contribuciones se rige por la fracción de cobertura superficial, que se determina a partir del

modelo de Langmuir. Este modelo muestra un buen ajuste a los datos experimentales, pero se necesita una justificación adicional para poder extraer más información. Para tener en cuenta también el papel de la fase móvil, se necesita una mejora del modelo de retención mixta.

Palabras clave: Sílica aerogel, Cromatografía supercrítica, adsorción, regresiones LSER

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Abstract

Silica aerogel particles have been shown to be a potential candidate for oral drug delivery system, where the loading of the drug in the aerogel is usually performed with $scCO_2$. In this work, interactions between the carrier, CO_2, modifier and different solutes will be studied by SFC, using silica aerogel particles as stationary phase.

Three kind of commercial silica particles were also evaluated as stationary phase and the hold-up time of the columns was obtained by the injection of N_2O as unretained peak, which showed to be a good marker for all the stationary phases. The stability of the columns at different temperatures and modifier concentrations was tested. Aerogel column showed good stability in an operation time of 48 hours in all the studied cases.

The importance of various types of intermolecular interactions that reflect the properties of the stationary phases is evaluated by LSER regressions using 15 solutes. The results showed that LSER is capable of generating acceptable values of retention times not only for Kromasil packed columns (R^2 greater than 0.94) but also for the aerogel-particles packed column (R^2 greater than 0.88). The results showed that *a* and *b* coefficients, related to the H-bond acidity and basicity respectively, are the dominating solute descriptors that affected retention in all stationary phases. Polar compounds are more sensible to changes in temperature, pressure and concentration.

Mixed retention model was employed to rationalize the retention behavior of the solute in SFC in presence of a modifier (methanol) considering two mechanisms: interaction with the adsorbed modifier molecules on the stationary phase and with the silanol groups. The influence of both contributions is governed by the surface coverage fraction, which is determined from the Langmuir model. The model shows a good adjustment to the experimental data, but further justification is needed to extract more information from the fitting. In order to take into account also the role of the mobile phase, an improvement of the mixed retention model is needed to be done.

List of Symbols

Latin symbols

Symbol	Unit	Meaning
A	-	Descriptor related to the hydrogen bond donating ability (acidity)
A_s	$m^2 g^{-1}$	Specific surface Area
a	-	System coefficient related to hydrogen bond donating ability
B	-	Descriptor related to the hydrogen bond accepting ability (basicity)
b	-	System coefficient related to hydrogen bond accepting ability
C_m	mg mL^{-1}	Concentration of the solute in the mobile phase
C_s	mg mL^{-1}	Concentration of the solute in the stationary phase
c	-	Intercept of the LSER regression
E	-	Descriptor related to the solute excess molar refraction
e	-	System constant related to the excess molar refraction
G^0	J mol^{-1}	Gibbs free energy
H^0	J mol^{-1}	Enthalpy
I	-	Solute-solute interaction parameter in the Moreau adsorption model
K_D	-	Distribution coefficient
k	-	Numerical coefficient in the Jovanovic adsorptioin model
k′	-	Capacity factor
k_0	-	Capacity factor at zero concentration of modifier
k_{obs}	-	Observed capacity factor
k_c	-	Capacity factor at total coverage of active sites of the modifier

Latin symbols (cont.)

Symbol	Unit	Meaning
N	-	Efficiency
N_s	-	Number of modifier molecules adsorbed on the stationary phase
$N_{s,max}$	-	Maximum number of modifier molecules adsorbed on the stationary phase
P	bar	Pressure
R	J K^{-1} mol^{-1}	Idel gas constant
Rs	-	Resolution
S^0	J K^{-1} mol^{-1}	Entrophy
S	-	Descriptor related to the polarity and polarizability
s	-	System coefficient related to the polarity and polarizability
T	K or ^0C	Temperature
t_0	min	Hold-up time
t_R	min	Retention time
u	-	Solvation vector length
V	-	Descriptor related to the solute's characteristic volume
V_m	mL	Volume of the mobile phase
V_o	mL	Void volume
V_P	cm^3 g^{-1}	Pore volume
V_s	mL	Volume of the stationary phase
v	-	System constant related to dispersive interactions and cavity effects
v	-	Surface heterogenety measurement coefficient in the Tóth adsorption model
x_{mod}	v/v	Concentration of the modifier in the mobile phase

Greek symbols

Symbol	**Meaning**
Δ	Increment
A	Selectivity
B	Stationary/mobile phase ratio
Θ	Surface coverage fraction
Λ	Wavelength
M	Viscosity
π	Related to the π interactions

List of Abbreviations

Abbreviation	Meaning
API	Active Pharmaceutical Ingredient
CBP	Chemical Bonded Phase
DDS	Drug Delivery System
FITR	Fourier Transform Infrared
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
LC	Liquid Chromatography
LSER	Linear Solvation Energy Relationship
MRM	Mixed Retention Model
PDA	Photo-Diode Array
QSPR	Quantitative Structure Property Relationship
QSRR	Quantitative Structure Retention Relationship
$scCO_2$	Supercritical carbon dioxide
SEM	Scanning Electron Microscope
SFC	Supercritical Fluid Chromatography
SIL	Silica
SIL-60	Kromasil 60-5-SIL particles
SIL-100	Kromasil 100-5-SIL particles
SIL-300	Kromasil 300-5-SIL particles
SIL-Aerogel	Silica aerogel
TUHH	Technische Universität Hamburg-Harburg (Hamburg University of Technology)
UV	Ultraviolet

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1 Introduction

1.1 Research purpose

Inhaled medications are widely accepted for the treatment of lung diseases due to the direct delivery of active components to the diseased organs. Silica porous materials have been used as "drug delivery systems (DDS)" because they are not-harmful materials that allow the release of the drug at a controlled rate (Rimola, Costa, Sodupe, & Ugliengo, 2013). It is expected that silica aerogels, which have the same properties as amorphous silica but much larger specific surface area, can be excellent carriers for being used as pulmonary DDS.

The loading of the drug in the aerogel is performed by static adsorption and adsorptive precipitation experiments in $scCO_2$ and, sometimes, mixed with methanol in order to increase the solubility of the drug. The purpose of this work is to have a better understanding of the interactions between different drugs and silica aerogel, which would provide essential information to understand the drug loading mechanism of drugs in the silica aerogel matrix. In order to simulate the loading process and study the interactions between the drug and the carrier, in this work, silica aerogels would be used as stationary phase in Supercritical Fluid Chromatography (SFC).

The main characteristic property to study in SFC is the retention, which is described by the capacity factor. The capacity factor is proportional to the coefficient distribution. Unlike GC and HPLC, where various retention mechanisms have been extensively studied for years, SFC suffers from the lack of systematic studies on retention

mechanisms and useful models for solute retention prediction. Retention in SFC is more complex than in GC or HPLC, since it is a function of the temperature, pressure, mobile phase density, mobile phase composition and stationary phase. Therefore, this study has been carried out in order to get crucial information for the aerogel drug loading about the interactions between the carrier, CO_2, methanol as modifier and different solutes at different temperature, pressure and concentration of modifier.

1.2 Dissertation organization

This work consists of 5 chapters. This one, Chapter 1, gives a brief summary about aerogels as drug delivery system and SFC, and introduces the research topic.

Chapter 2 deals with literature review and it is divided in two sub-chapters. The first one gives a summary of silica aerogels and the second one describes the advantages of the use of a supercritical fluid as mobile phase in chromatography.

Chapter 3 is about the properties stationary and mobile phase substances used in the work as well as the different solutes. It also provides the description of the SFC system.

The purpose of Chapter 4 is to study the retention mechanisms in silica aerogels stationary phase and compare them to silica gel stationary phases, and it is divided in three subchapters. The first one studies the hold-up time and the stability of the columns under different temperatures and concentration of modifier. In the second one, the LSER methodology is applied to characterize the stationary phases. The estimated LSER parameters are compared and analyzed at various concentration of methanol, temperature and pressure levels. In the third subchapter, Mixed Retention Model was performed, which suggests that organic modifiers molecules of the mobile phase adsorbed on the surface of the stationary phase also contributes to the retention.

Finally, a summary of the research project and recommendations for further research are given in Chapter 5.

2 Basic Principles and State of the art

2.1 Aerogels

2.1.1 Aerogel: An overview

The term "aerogel" is used to encompass all materials with a specific geometrical structure. This structure is an extremely porous, solid foam, with high connectivity between branched structures of a few nanometers across.

It is technically a foam, but it can take many different shapes and forms. The majority of aerogel is composed of silica, but carbon, iron oxide, organic polymers, semiconductor nanostructures, gold and copper can also form aerogel. However, within the aerogel structure, very little is solid material, with up to 99.8% of the structure consisting of air. This unique composition gives aerogel an almost ghostly appearance; hence it is often referred to as "frozen smoke".

In general terms, aerogel is created by drying a gel, so that the liquid component is replaced by air. Aerogel was first created in 1931 by S. Kistler (Thomas, 2012). The properties of aerogels allow them to be used as host matrix for drugs (Guenther, Smirnova, & Neubert, 2008).

In this work, silica aerogels will be used as stationary phase for supercritical fluid chromatography (SFC) separations, as its use has been successfully demonstrated in literature (Gurikov et al., 2013). The main aim of this work is to study the interactions between the solutes and the stationary phase. This investigation would provide

essential information to understand the drug loading mechanism. Due to this, the rest of the section will be focused on silica aerogels and the possibility of their use as pulmonary drug delivery system.

2.1.2 Silica aerogels

Silica aerogels are low-density highly porous solids, consisting of silicon oxide. They have an open structure and its skeletal density is around 2 g cm^{-3}, close to that amorphous silica (2.2 g cm^{-3}).

Silica aerogels are prepared by means of the sol-gel process, which involves the hydrolysis and polycondensation of silicon alkoxides. First, the gel is created in a solution, and then the liquid component is removed slowly by supercritical drying, in order to maintain the structural shape. This process is detailed in literature (García-González, Alnaief, & Smirnova, 2011; Ulker & Erkey, 2014)

2.1.2.1 Physical properties and applications

Due to its unique structure, silica aerogels have found place in several applications in the fields of pharmacy/agriculture, electronic, chemistry, and so on. Some of its properties are shown in Table 2.1.

Table 2.1 Most important properties of silica aerogels. Extracted from (Schmidt & Schwertfeger, 1998)

Typical properties of silica aerogels	
Particle size	Up to some millimeters
Particle density	≈ 120 kg m^3
Bulk density	≈ 80 kg m^3
Specific surface area	600-1000 m^2 g^{-1}
Mean pore diameter	≈ 20 nm
Water resistance	Durably hydrophobic up to 250 ^{0}C (in air)
Temperature stability	Up to 500 ^{0}C
Coefficient of thermal expansion	2.0 – 4.0 x 10^{-6}

Some of the applications of silica aerogels are summarized in Figure 2.1.

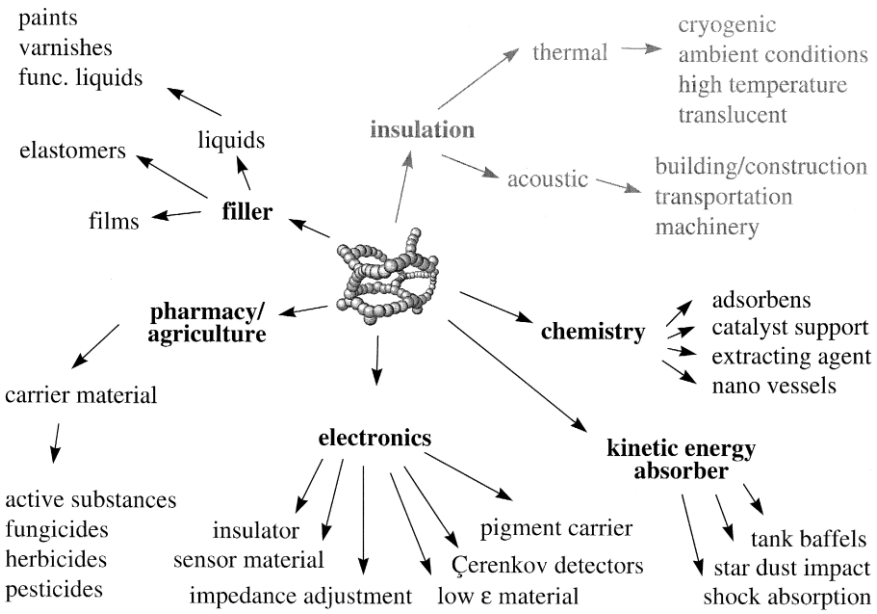


Figure 2.1 Application fields of silica aerogels. Extracted from (Schmidt & Schwertfeger, 1998)

Pore structure. They are usually largely mesoporous, with interconnected pore sizes typically ranging from 5 to 100 nm and an average pore diameter between 20 and 40 nm. Micropores (pore sizes <2 nm) become significant in aerogels synthesized under acid catalysis conditions or having undergone particular treatments. The associated specific surface area is rather high, typically from 250 to 800 $m^2\ g^{-1}$ and can exceed 1000 $m^2\ g^{-1}$. They can have a pore content as high as 99% of their whole monolith volume and some ultraporous and ultralight silica aerogels can have a density as low as 0.003 g cm^{-3} (Gurikov et al., 2013; Pierre & Rigacci, 2011).

Thermal conductivity. One of the major characteristics of silica aerogels is their very low thermal conductivity, typically of the order of 0.015 W $m^{-1}\ K^{-1}$ at ambient temperature, pressure and relative humidity. These values are significantly lower than the conductivity of air under the same conditions, e.g., 0.025 W $m^{-1}\ K^{-1}$. Thus and together with the non-flammability of the silica, these kinds of aerogels are among the best-known thermal insulating materials.

Optical properties. The transparency and visible light transmittance of silica aerogels can be high, although they tend to scatter the transmitted light to some extent, which reduces their optical quality. Due to this, they have applications when a transparent thermal insulation is targeted, such as in windows.

Acoustic properties. Silica aerogels are excellent acoustic insulators and their acoustic properties are closely related to their thermal insulation properties. The acoustic propagation in these kind of materials depends on the interstitial gas nature, the pressure and the aerogel density.

Mechanical properties. The compressive strength, tensile strength and elastic modulus of silica aerogels are very low and largely depend on the network connectivity and its density. Indeed, silica aerogels can easily be elastically compressed and the magnitude of the contraction can reach approx. 50% by length, although they are also very brittle.

Dielectric properties. The relative dielectric constant of silica aerogels can be as low as 1.1. Hence, thin film silica aerogels are being considered as super-low dielectric constant material for integrated circuits in computers. It is also possible to modify the surface of the silica aerogel in order to obtain good electret materials.

Entrapment, release, sorption and storage. The combination of a high specific pore volume with, in some specific cases, a relatively resistant solid SiO_2 network, can also be advantageously used to entrap a large variety of molecules or nanoparticles. Biomaterials and bacterias can also be successfully immobilized inside aerogels. The controllable pore size and high specific pore volume of silica aerogels make them also ideal candidates for releasing medical drugs or agriculture chemicals in a controlled fashion. Hydrophilic silica aerogels can be loaded with chemicals during the sol-gel synthesis process or by posttreatment of dried aerogels. Inversely, aerogels can be used to adsorb or extract some chemical compounds, for instance, to treat waste water, to confine radioactive waste or to filter gases. Aerogel particles can also be used as the

dispersed phase in composite materials, such as elastomers for tires or paints, and it will provide them with additional hardness, resistance to wear, and also exert a thickening effect on the mixture (Pierre & Rigacci, 2011).

2.1.3 Aerogels as pulmonary drug delivery system

Silica aerogels are chemically inert and non-harmful to the human body and have applications in the pharmaceutical industry and agriculture. Recently they were shown to be a potential candidate for oral drug delivery system (DDS) (Smirnova, Suttiruengwong, Seiler, & Arlt, 2005).

Inhaled medications are widely accepted for the treatment of lung diseases and they are considered the optimal route of administration of first-line therapy for local diseases, such as asthma and chronic obstructive pulmonary diseases. In recent years, the lung has been studied as a possible route of administration for the treatment of systemic diseases, such as diabetes mellitus (Labiris & Dolovich, 2003a). The advantages of pulmonary delivery of drugs to treat respiratory and systemic diseases are summarized in Table 2.2.

Table 2.2 Advantages of pulmonary delivery of drugs to treat respiratory and systemic disease. Extracted from (Labiris & Dolovich, 2003a).

Treatment of respiratory diseases	**Treatment of systemic diseases**
• Deliver high drug concentrations directly to the disease site • Minimizes risk of systemic side-effects • Bypass the barriers to therapeutic efficacy • Rapid clinical response • Achieve a similar or superior therapeutic effect at a fraction of the systemic dose	• Noninvasive delivery system • Suitable for a wide range of substances, from small molecules to very large proteins • Large molecules with very low absorption rates can be absorbed in significant quantities • Enormous absorptive surface area (100 m^2) and a highly permeable membrane (0.2-0.7 μm thickness) in the alveolar region • Reproducible absorption kinetics

Major advantages of the inhalation route of drug delivery in order to treat lung diseases include direct delivery of active components to the diseased organs and cells and prevention of potentially toxic therapeutics in the bloodstreamand, therefore limiting possible adverse effects upon other healthy organs (see Figure 2.2). However, some factors such as several technological challenges asociated with inhalation, can potentially limit the practical implementation of this approach in the clinic.

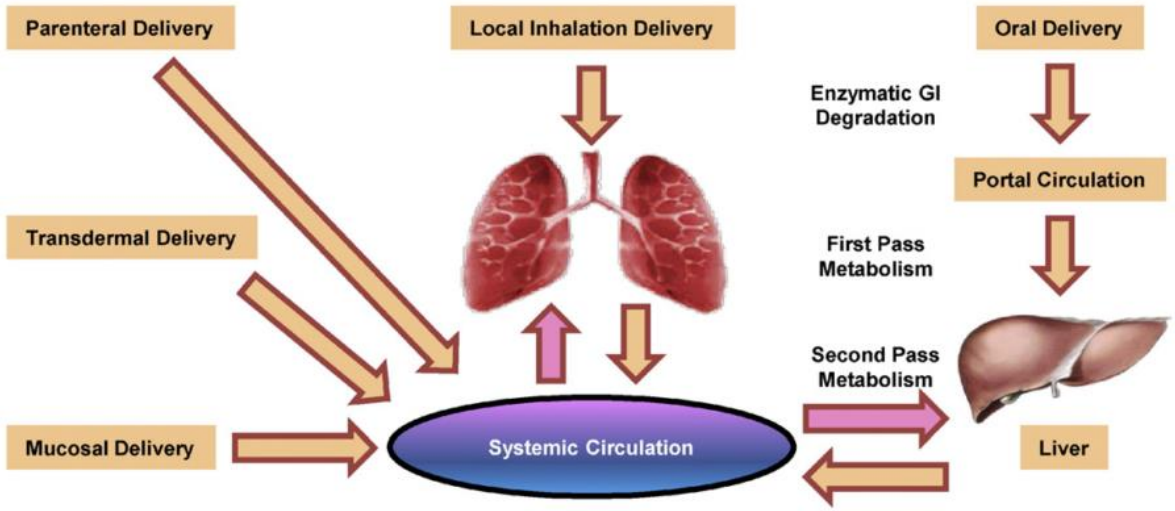


Figure 2.2 Advantages of pulmonary drug delivery (Kuzmov & Minko, 2015)

Physiological factors (e.g. mucocilliary clearance, alveolar macrophages, airway geometry and humidity, lung condition) have led to the development of different drug formulations and types of inhalation devices aiming for a higher effectiveness of inhaled drugs. In this context, porous particles present more advantages compared to other pulmonary delivery formulations in terms of drug stability, flow properties of the bulk powder, size and shape uniformity, drug dissolution and bioavailability (Labiris & Dolovich, 2003a).

It has been demonstrated in literature (Edwards et al., 1997) that the inhalation of large porous insulin particles resulted in elevated systemic levels of insulin and suppressed systemic glucose levels for 96 hours, whereas small nonporous insulin particles had

this effect for only 4 hours. The same effect has been demonstrated with aerosol formulations of large porous hollow particles (Labiris & Dolovich, 2003b).

On the other hand, amorphous silicon oxide or "fumed silica" as well as silicon dioxide aerosol have been used in the pharmaceutical industry for many years. They have passed all clinical tests and has been found to be non-toxic and non-harmful to the human body. Silica gels are the main example of "drug delivery systems" (DDSs) with two principal aims: the development of systemic delivery systems, able to release drug at a controlled rate by a degradation of the silica matrix, avoiding premature degradation of the active agents and reducing toxicological side effects; and implantable local-delivery devices, able to release drug as a response to an external stress (for instance, the application of a magnetic field) or a change in internal conditions (Rimola et al., 2013).

It is expected that silica aerogels, that have the same chemical composition and amorphous structure as fumed silica (surface area 300 m^2 g^{-1}), would have similar clinical characteristics. Furthermore, aerogels have much larger internal surface (500-1000 m^2/g), enabling them to exhibit superior properties in pulmonary drug delivery applications as it was said above (Edwards et al., 1997; Musante et al., 2002).

Figure 2.3 Silica aerogel

2.2 Supercritical Fluid Chromatography

2.2.1 Background and Current Status in Supercritical Fluid Chromatography

Chromatography is by far the most widely used technique in analytical chemistry to separate groups of molecules or individual molecules, peptides or proteins from more or less complex mixtures (Enmark, 2015). Gas chromatography (GC) and (high-performance) liquid chromatography (HPLC) have gained acceptance in numerous applications areas as environmental chemistry, food and polymer chemistry and clinical and agricultural research. Supercritical Fluid Chromatography (SFC) was introduced in the early sixties and it is the third form of (column) chromatography (Caude & Thiâebaut, 1999).

The outcome of the liquid or supercritical fluid chromatography separation process is governed by the interactions between the solute and the mobile and stationary phase (Enmark, 2015). Supercritical Fluid Chromatography is a chromatographic technique that operates with a supercritical or subcritical fluid as main solvent, which has potential advantages in not only chromatographic efficiencies but also cost and ease of use. The technique was first demonstrated by Klesper et al in 1962 (Klesper, Corwin, & Turner, 1962). In that work, mono and dichlorodifluoromethane were used as mobile phase to separate porphyrins. In the early eighties, the introduction of the first commercial instrument by Hewlett Packard improved the attractiveness of the use of the SFC, together with the introduction of open-tubular columns by Novotny et al. Nowadays, CO_2 is the mainly used solvent and SFC instrumentation, which enables the analytical chemist to develop highly efficient chromatographic methods with fast re-equilibration (Enmark, 2015; Taylor, 2014).

2.2.1.1 Industrial applications of supercritical Fluids

As it was said above, the advantages of SFC are due to the use of a supercritical fluid as mobile phase. Supercritical fluids are gases at pressure and temperatures slightly above those of the vapor-liquid critical point. At this critical point, the compound shows intermediate properties between those of a pure liquid and a pure gas. Beyond that point, the difference between the coexisting liquid and vapor phase disappears and the one-phase fluid has an isothermal compressibility or infinity. A change of temperature or pressure in the supercritical region changes the phase properties of the compound. This phenomenon is plotted in the Figure 2.4 (Abdullah, 2007).

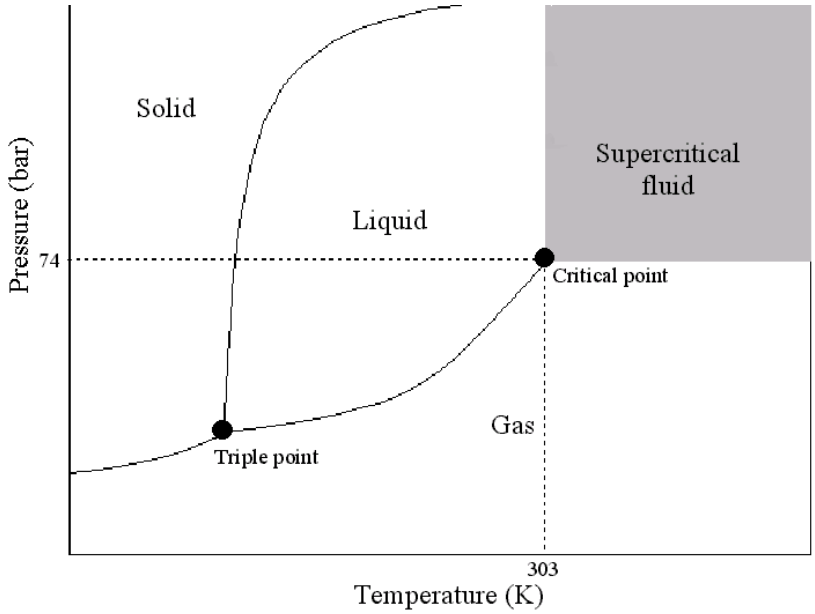


Figure 2.4 Pressure-Temperature phase diagram for carbon dioxide. Extracted from (Mendes, Nobre, Cardoso, Pereira, & Palavra, 2003)

Since the supercritical fluids have a special capability to dissolve solutes, they and specially supercritical CO_2 have been used in several industrial applications, as Supercritical Fluid Extraction (SFE), Particles from Gas Saturated Solutions (PGSS), Rapid Expansion of Supercritical Solution (RESS), Supercritical Fluid Reaction (SFR) and Supercritical Fluid Chromatography (SFC) (Martín & Cocero, 2008).

Regarding to the SFC, various single component mobile phases have been used in packed column SFC, as carbon dioxide, xenon, ammonia, nitrous oxide, various lower

alkanes and ether, among others. However, carbon dioxide is the most commonly used fluid in SFC due to several reasons: It is readily available at high purity, non-toxic, non-flammable and cheap; Its critical temperature is low, 304.1 K, so its use minimizes the thermal degradation of the stationary phase; Its critical pressure is moderate, 7.38 MPa, so it is liquefiable at reasonable pressures; It is environmentally favorable because it uses add no additional carbon dioxide to the atmosphere, It is miscible with a wide range of organic solvents; Its high diffusivity and low viscosity give better penetration into pores and matrices than liquids (Martín & Cocero, 2008; Peter J Schoenmakers & Frank, 1987).

2.2.1.2 Carbon dioxide as mobile phase in SFC

The main distinctive characteristic of the supercritical fluid condition is that the density of the fluid is very sensitive to small changes in pressure and temperature. Density is directly related to many other physical and chemical properties of a fluid as the solvent power, this is, the ability to dissolve other substances. In such a way, it can be adjusted by the control of pressure and temperature (Lesellier & West, 2015; Nunes da Ponte, 2003). For pure CO_2, it varies from 0,2 to 1,1 g/mL, thus from a little above the density of a gas to the density of a liquid.

On the other hand, the elution of the molecules in SFC is related to their adsorption on the stationary phase and also to their solubility of the solute in the mobile phase. Thus, SFC is basically a distribution process of solutes in the mobile phase and in the stationary phase. If the partition of the solute in the stationary phase is relatively low compared to that in the mobile phase, the solute is rapidly eluted. On the contrary, if the solute is adsorbed strongly on the stationary phase, while at the same time it is poorly distributed in the mobile phase, the solute will not elute at all or only after a very long time (Caude & Thiâebaut, 1999; Janssen, Schoenmakers, & Cramers, 1991).

As It was said above, SFC is an important chromatographic technique due to the intermolecular interactions in the mobile phase. Neat carbon dioxide or carbon dioxide

with added co-solvent at sub- or supercritical conditions has lower viscosity (μ), higher solute diffusion coefficients (K_D) and higher compressibility than comparable liquids used for liquid chromatography (LC). The practical consequences of lower viscosity and higher solute diffusion coefficients are the possibility of operating at higher linear velocities than LC or using longer columns to obtain high efficiency. Higher compressibility means that properties such as density and temperature of the mobile phase can be altered by changing the pressure, which in turn will affect the chromatographic separation processes. Furthermore, due to the presence of a pressure drop along the column, there will be gradients of these properties along and across it. In Figure 2.5, the most important components in a SFC system together with typical experienced gradients are schematic summarized.

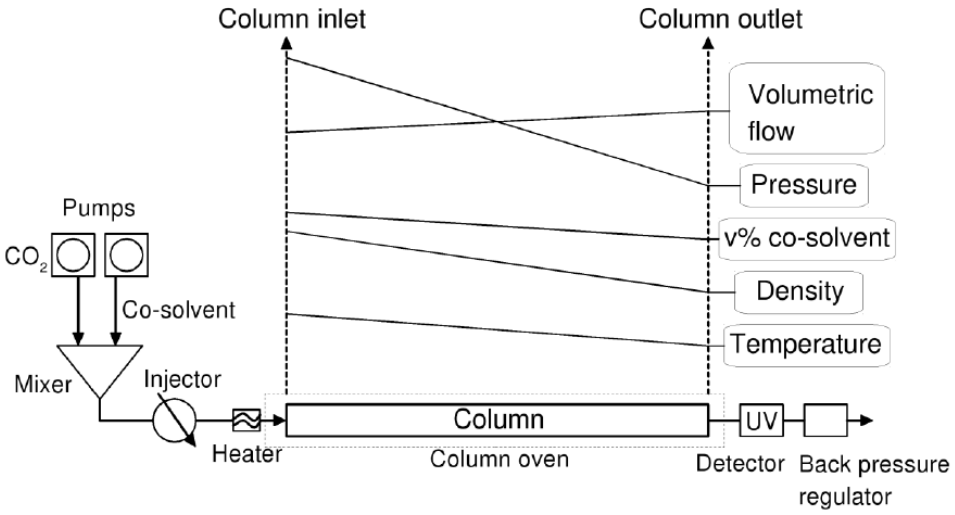


Figure 2.5 Schematic figure of the major components in a SFC system. Extracted from (Enmark, 2015)

2.2.1.3 Role of modifier and additive

Role of the modifier

Regardless of the stationary phase selected, in packed column SFC only apolar solutes can be eluted using pure carbon dioxide as the mobile phase, due to it is a nonpolar solvent. Even only mildly polar solutes may elute either as ill-shaped trailing peaks or

may not elute at all. It has been observed that relatively polar solutes can be eluted as nice, symmetrical peaks from highly inert open-tubular columns, what indicates that the problems observed with the elution of relatively polar species from packed SFC columns are not due to the mobile phase. It is now generally accepted that residual active sites on the surface of the packing materials in packed column SFC are the responsible for the poor peak shapes seen for such solutes (Caude & Thiâebaut, 1999).

The solvent strength of the mobile phase can be increased by adding volumes of polar organic solvents (CO_2-based mobile phase). The effects of adding even only minor concentrations of modifier on retention and peak shape can be stunning. This is especially true if silica based packaging materials are used, due to its polar surface. In this manner, the range of components that can be analyzed by packed column SFC can be significantly expanded by using modified fluids instead of pure CO_2 (Caude & Thiâebaut, 1999; Taylor, 2014).

Typical modifier concentrations range from a few percent to 20% and almost any organic solvent can be used as modifier. The most common organics are alcohols, such as methanol, ethanol, isopropanol, and acetonitrile (Terry A. Berger, 2015). Methanol is by far the most used modifier offering higher efficiencies and shorter retention time than ethanol and isopropanol. It is probably the most polar, common modifier completely miscible with supercritical fluid CO_2 over a wide range of temperatures and pressures. The critical temperature and critical pressure of the mixture increase with the increase of the modifier concentration (Taylor, 2014). Phase equilibria and critical properties of binary mixtures of carbon dioxide and methanol, ethanol, 1-propanol and 1-butanol are described in literature (Yeo, Park, Kim, & Kim, 2000).

Role of the additive

Even with polar organic solvents as modifier, SFC is still not always sufficient to facilitate elution in a reasonable time or with acceptable peak shapes for some ionic compounds and polar compounds. A third more polar component, an additive, added

into the mobile phase can help to mitigate this problem. Suggested roles for additives in the separation process are: enhance mobile phase solvating power; suppress sample ionization; ion pair with ionic analytes; and modify the stationary phase.

Typical additives are strong acids (e.g., formic acid, trifluoruroacetic acid, citric acid), bases (e.g., isopropylamine, trimethylamine), or salts (e.g., ammonium acetate). Water is also used as additive to help elute highly polar compounds, such as nucleobases and polypeptides where water introduces HILIC-like analyte beyond that they should be a stronger acid or base than the sample components as well as compatible with the choice of detector (Taylor, 2014).

2.2.1.4 Stationary phase in SFC

Supercritical-fluid chromatography (SFC) may be performed either in open (capillary) columns or in packed columns. Both approaches have been demonstrated numerous times in the literature (P. J. Schoenmakers, 1988). They do not compete between each other and choosing a column type sometimes is reduced to personal preference or to the type of instrumentation available.

Capillary columns are larger and they have lower internal diameter than packed columns. The stationary phase is coated with the inner wall of the column and they tend to work best as an extension of GC into higher molecular weights. In packed performance, the stationary phase is directly filled in the column. The most important area for packed column use involves modified mobile phases (T.A. Berger, 1995).

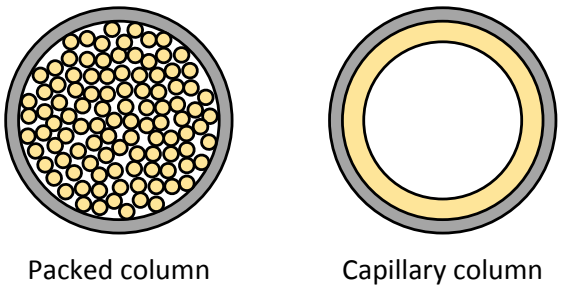


Figure 2.6 Packed and capillary columns

On the other hand, SFC is usually a normal phase technique because composition is programmed from low to high polarity (Terry A. Berger, 2015). In normal phase chromatography, the stationary phase is hydrophilic and therefore, the hydrophilic molecules in the mobile phase tend to adsorb to the column, while the hydrophobic molecules pass through the column and are eluted first. The introduction of alkyl chains covalently bonded to the solid support created a hydrophobic stationary phase, which has a stronger affinity for hydrophobic compounds. The use of a hydrophobic stationary phase can be considered the opposite, or "reverse", of normal phase chromatography, hence the term “reversed-phase chromatography” (Molnar & Horvath, 1976).

The most widely used separation mode in SFC is the partitioning of the solute over the mobile phase and the stationary phase, so that the selection of the mobile and stationary phases as well as the optimization in packed column SFC are based on partitioning (Caude & Thiâebaut, 1999).

For polar solutes, polar stationary phases are used. Classical polar phases are bare silica, cyano, diol and amino. In the last few years, a number of new stationary phases have been developed specifically for SFC, including several ethylpyridines and a number of proprietary phases. For low polarity solutes, reversed phase columns such as C18, C8, C4, and methyl are sometimes used. SFC is also useful for the separation of much less polar compounds such as many natural products, including fat soluble vitamins, carotenoids, and lipids. With such samples the stationary phase is usually C18 (Terry A. Berger, 2015). Options for mobile and stationary phase depending on the polarity of the solutes are represented in Figure 2.7.

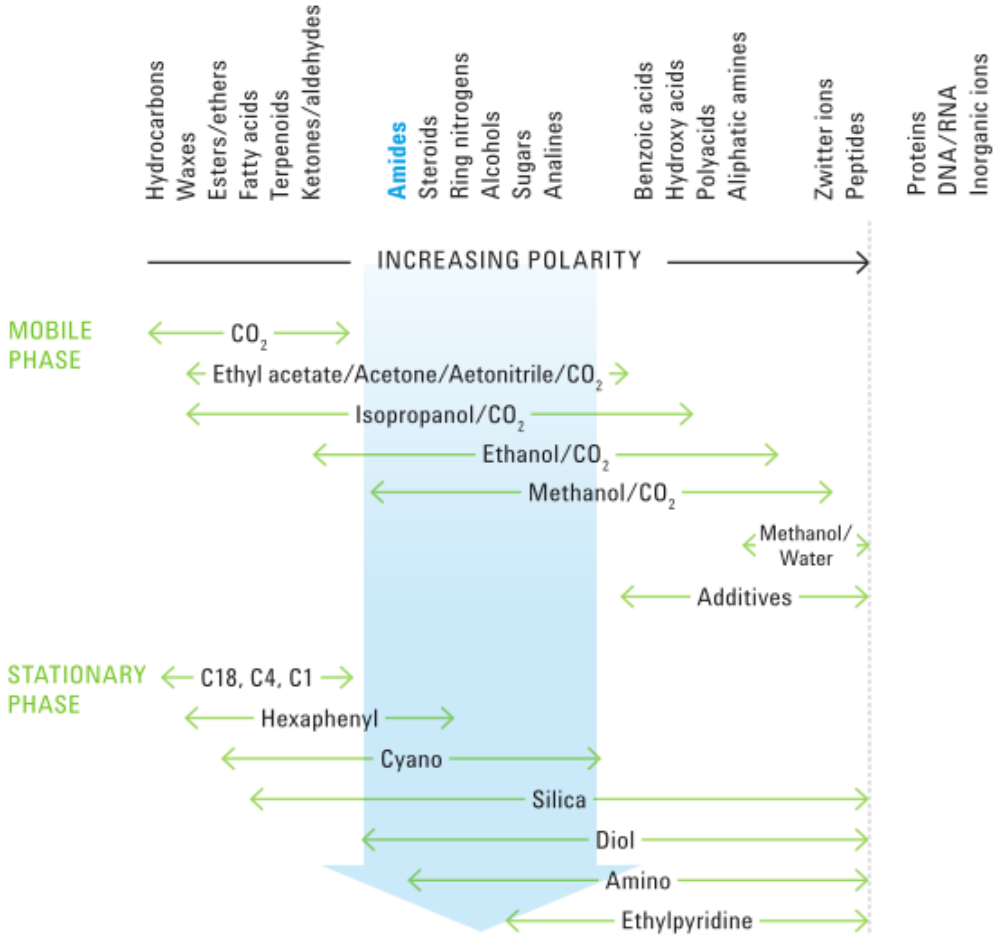


Figure 2.7 Options for mobile and stationary phase depending on the polarity of the solutes. Extracted from (Terry A. Berger, 2015).

Silica based stationary phases

Silica is by far the most widely used starting material for the preparation of packings for chromatography (Caude & Thiâebaut, 1999). It is used as stationary phase for liquid chromatography system in the pharmaceutical industry, in the analysis of contaminants, pesticides, bioanalytes, and drug residues in drinks and food samples, and in medical or environmental tests.

Silica is a solid material with a density between 2 and 3 g/cm^3 and high melting point (ca. 1700 °C), whose chemical formula is SiO_2. At the silica surface, the most common termination is given by two main functional groups: the siloxane links (Si–O–Si) and the silanol groups (Si–OH) (See figure 2.8) (Rimola et al., 2013).

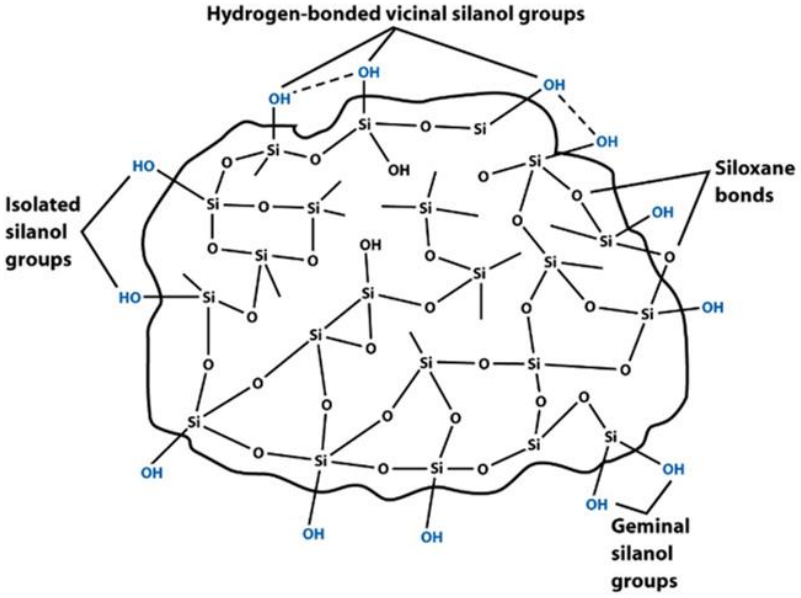


Figure 2.8 Schematic structure of silica gel. Extracted from (Caude & Thiâebaut, 1999)

The particles are made of totally porous, high surface area, hydrated silica. The more modern packings are spheres between 1 and 10 μm in diameter and have pore diameters between 60 and 300 Å. Commercial porous silica packing typically have a surface area of 100-500 m^2 g^{-1}. The original silanol concentration of this material is roughly 7 μmol m^{-2} (T.A. Berger, 1995; Caude & Thiâebaut, 1999).

Chemically bonded silica phases

In packed column SFC, chemically-bonded modified silica phases are quite popular. They cover the need of reversed-phase liquid chromatography with improved stability at extreme pH. They can be prepared in several ways giving materials with different chromatographic properties (Poole, 2012). The most common packings are of the monomeric type. These phases consist of a molecular layer of functional groups chemically bonded to the surface, this is, modifying surface silanols to give Si-O-M, being M the chemical group. The most popular ones are methyl, octyl, octadecyl, phenyl, cyanopropyl and aminopropyl. Because of the bulky size of the reagents used in this chemical bonding reactions, more than 50% of the silanol groups originally present on the silica particles remain present on the surface after reaction.

Important differences can exist in the activity of differently prepared silica based columns for SFC. Depending on the method used for preparation of the phase and the treatment of the column nominally identical phases can show large differences in activity (Caude & Thiâebaut, 1999).

FTIR analysis. Modifications on the surface

Infrared (IR) spectroscopy analysis is widely used to identify molecular species and determinate their concentration in the sample and it has been one of the most popular tools to investigate the microstructure of silica gel (residual porosity, Si-O-Si bonding rearrangements, etc.) (Innocenzi, 2003). The major advantage of IR over other techniques is that, apart from homonuclear diatomic molecules (e.g., N2, O2, H2, etc.), all compounds show IR adsorption and can thus be analyzed.

The introduction of the Fourier-Transform (FT) principle in Fourier Transform infrared (FTIR) spectrometers considerable improved the signal-to-noise ratio, resolution, speed and detection limits and it has been the dominant technique used for measuring the infrared (IR) adsorption and emission spectra of most materials (Bacsik, Mink, & Keresztury, 2005).

The FTIR technique has been used in analyses of the spectra of the solid phase remaining after organic thermal degradation or in the identification of the species desorbed by thermal treatment (Foschiera, Pizzolato, & Benvenutti, 2001). It has been applied to amorphous silica, in contact with either a gas phase or vacuum, for a long time. If silica is observed under air without any pretreatment, the contribution of the OH of adsorbed water molecules is dominant (Rimola et al., 2013).

In this work, FTIR analyses will be done in order to verify whether the surface of porous silica has been modified by organic groups of methanol used as modifier in the mobile phase during supercritical fluid chromatography experiments.

2.2.1.5 Applications of SFC

The application areas of SFC are compared to the application areas of the various forms of liquid chromatography in Figure 2.9. LC does cover the widest polarity range of analytes in terms of selectivity, but the technique had to be divided sharply into distinct operational classes: reversed-phase, normal-phase, HILIC, ion-chromatography, and so on. As shown, SFC with various mobile phase combinations covers nearly the same application space as HPLC in its various forms. The only area not significantly covered is ion chromatography (Terry A. Berger, 2015; Tarafder, 2016).

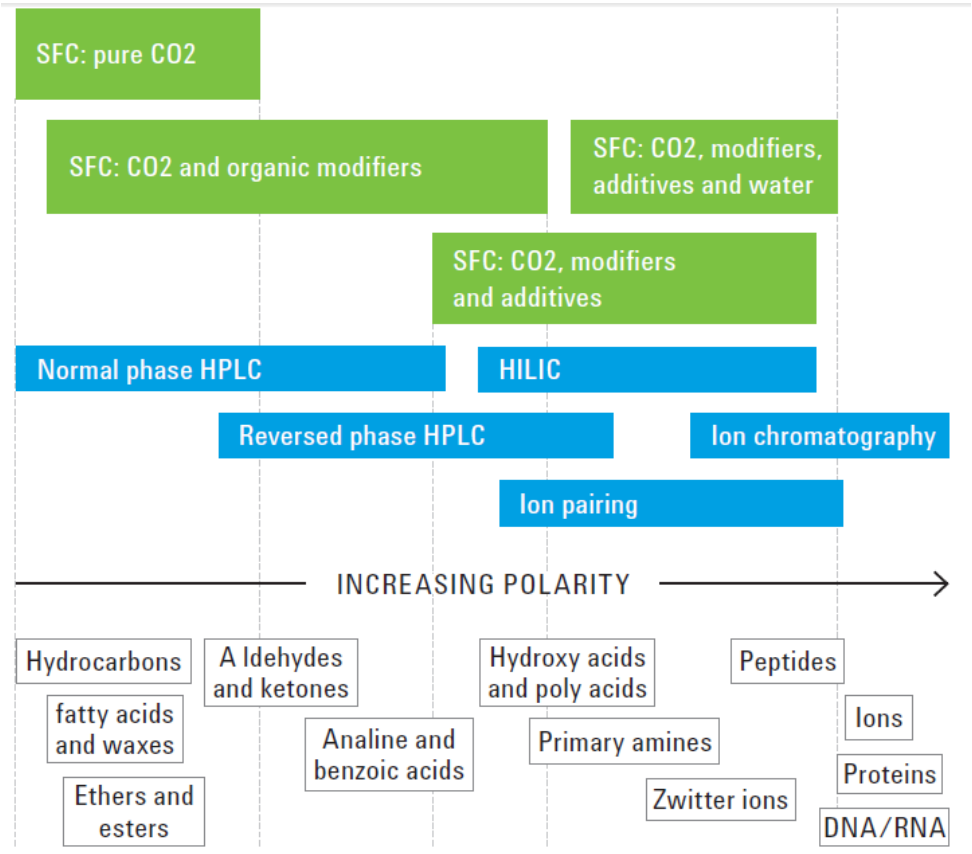


Figure 2.9 Different modes of liquid chromatography and the cases where SFC may be an alternative method. Extracted from (Terry A. Berger, 2015)

SFC is usually a normal phase technique because composition is programmed from low to high polarity. However, SFC has significant advantages compared to normal phase HPLC: equilibration is extremely fast, reproducibility is excellent, and even

small volume of aqueous solutions can be injected. Furthermore, CO_2 mixed with solvents of wide polarity range can simplify the analytical task considerably. Samples can be directly injected to the system, which in many other situations would need sample preparation or a derivatization step (Terry A. Berger, 2015).

Pharmaceutical applications

Over the last 15 years, SFC has been used largely in the pharmaceutical industry for the rapid elution of small drug-like molecules, particularly for chiral separations (Terry A. Berger, 2015).

The enantiomers of a drug can each show different properties, in terms of activity and/or toxicity. The need to test hundreds of compounds in search of a drug against a disease requires a high-throughput. HPLC has been the choice in the pharmaceutical laboratory for the separation of enantiomers using chiral columns, but due to its efficiency, speed, and success in chiral separations, SFC has been replacing HPLC in certain drug development projects (Abbott, Veenstra, & Issaq, 2008).

SFC has been used primarily for chiral purification and enantiomeric purity assessment for non-GMP (Good Manufacturing Practices) activities during drug discovery and development. However, due to the constant improvement in the sensitivity and the advancement of the instrumentation, nowadays SFC can also be used for GMP API (Active Pharmaceutical Ingredients) release and stability testing.

On the other hand, in-process testing of chemical synthesis schemes often requires testing samples in synthesis matrices that are not compatible with reversed-phase chromatography but they are with SFC mobile phases. In such a way, SFC has become a primary technique for pharmaceutical in-process testing (Taylor, 2014).

2.2.2 Important parameters in SFC

2.2.2.1 Retention

Retention in chromatography is governed by the partitioning of the solute over the mobile phase and the stationary phase. The retention time t_r of a solute is given by:

$$t_r = t_0(1 + K_D\beta) \tag{2.2}$$

where t_0 is the hold-up time, K_D is the distribution coefficient, β is the phase ratio and the product $K_D\beta$ is the capacity factor (k'). The distributtion coefficient is the ratio between the concentration of the solute in the stationary phase (C_s) and the mobile phase (C_m):

$$K_D = \frac{C_s}{C_m} \tag{2.3}$$

The phase ratio can be expressed as:

$$\beta = \frac{V_s}{V_m} \tag{2.4}$$

where V_s and V_m are the volumnes of the stationary and mobile phase. The influence of the nature and the pressure of the carrier gas on the capacity factors of the the solutes is usually negligible. The only way to change the capacity factor is to change the operating temperature. By (positive) temperature programming, components with increasingly high boiling points can be eluted. In liquid chromatography the effect of temperature on retention is much smaller.

Retention is a very strong function of the nature and the composition of the mobile phase. Mixed mobile phases are used almost exclusively. By varing the composition of the mobile phase, retention and selectivity can be varied (Janssen et al., 1991).

2.2.2.2 Capacity factor, k'

The capacity factor is a measure of the time the sample component resides in the stationary phase relative to the time it resides in the mobile phase. It expresses how much longer a sample component is retarded by the stationary phase than it would take to travel through the column with the velocity of the mobile phase. Mathematically, it is the ratio of the adjusted retention time (volume) and the hold-up time (volume):

$$k' = \frac{t_R - t_0}{t_0} = \frac{V_R - V_0}{V_0} \tag{2.5}$$

If the distribution constant is independent of sample component concentration, then the retention factor is also equal to the ratio of the amounts of a sample component in the stationary and mobile phases respectively, at equilibrium.

2.2.2.3 Hold-up

The hold-up volume, V_0, is defined as the volume of mobile phase that leaves the column during the passing of an unretained substance along it. Normally, this volume is equal to the total volume of mobile phase in the chromatographic phase system. It includes both the interparticle (exclusion) volume in packed columns and the mobile phase inside the pores of the packing material. The time corresponding to the retention of an unretained substance is the hold-up time, t_0.

It must be remembered that for a given chromatographic phase system at a defined temperature, retention volumes and the hold-up volume are independent of flow rate, but the corresponding times are not (Domínguez & Diez-Masa, 2001).

2.2.2.4 Other parameters

Efficiency

The efficiency of a packed column is expressed by the number of theoretical plates, N, and is a measure of the dispersion of the analyte band as it passes through the column.

It mainly depends on the physical properties of the chromatographic medium together with the chromatography column and system dimensions (Biosciences, 1999).

Selectivity

Selectivity, α, is the relative retention of the solute peaks and depends strongly on the chemical properties of the chromatography medium (T.A. Berger, 1995).

Resolution

Resolution, Rs, is generally defined as the distance between the centres of two eluting peaks as measured by retention time or volume divided by the average width of the respective peaks (T.A. Berger, 1995).

2.2.3 Retention mechanisms studied in general pSFC

Retention in SFC is a complex function of the operating temperature, the pressure (or the density) of the mobile phase and its composition, as well as the properties of the solutes and the stationary phase. Many of these variables are interrelated and do not change in a predictable way (Janssen et al., 1991).

2.2.3.1 Effects of modifiers in pcSFC

The effects of the modifier are not necessarily restricted to the mobile phase. Molecules of the modifier can partition into the stationary phase and interact with the free silanol groups. In this way, they can be adsorbed on the active sites of the packing materials thereby changing the properties of the stationary phase or giving rise to specific interactions, so there is a competitive adsorption between the modifier and the solute.

This means that the overall effect of adding a modifier to the supercritical mobile phase is a combination of mobile-phase modification effects and stationary-phase effects. Modifiers affect the density and the polarity of the mobile phase. Polarity effect includes all physico-chemical interactions between the solute and the mobile phase

components. Regarding to the stationary phase, modifier molecules can deactivate adsorptive sites present on the surface of the packing material or the column wall. Furthermore, the adsorption of the modifier molecules can lead to swelling, which at the same time leads to an increase of the volume and an increase or decrease of the polarity of the stationary phase (Janssen et al., 1991). The possible effects of a modifier on a chromatographic system are schematically shown in Figure 2.10.

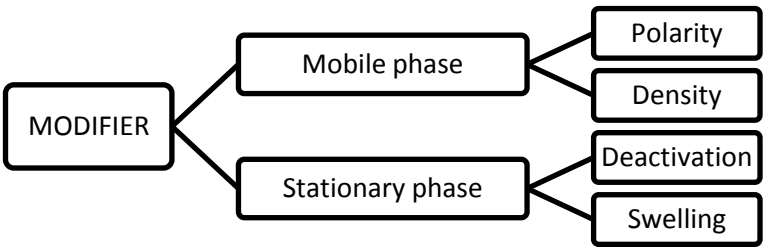


Figure 2.10 Schematic illustration of the effects of modifiers in SFC. Extracted from (Janssen et al., 1991)

Effects of modifiers on the mobile phase

The density of methanol/CO_2 mixtures increases with methanol concentration and with pressure, which leads to an increase in the solvation strength. Solvation or elution strength is a measure of the attraction and association of molecules of a solvent with molecules or ions of a solute. The more solvation strength of the mobile phase, the more able is to elute a solute that is adsorbed on the stationary phase (Terry A. Berger, 2015). Apart from this, polar modifier molecules can form clusters around polar solute molecules with different distribution properties (Poole, 2012).

On the other hand, dipolar, hydrogen bonding and dispersive interactions between the solute and the modifier can enhance the solubility of polar solutes in supercritical fluids significantly. The modifier-induced solubility enhancement could be understood qualitatively using dispersion, orientation and acid-base solubility parameters (Janssen et al., 1991).

Effects of modifiers on the stationary phase

Small amounts of modifiers are generally found to have a drastic effect on retention and efficiency in packed columns, but only a minor one in capillary columns. As it was said above, the difference between capillary and packed columns is the distribution of the particles in the column, so this statement could indicate that a major part of the modifier effects originates from stationary-phase effects (Janssen et al., 1991).

The adsorbed modifier can increase the volume of the stationary phase leading to a change in the column phase ratio (swelling) and it can also act as a component of the stationary phase (Caude & Thiâebaut, 1999; Taylor, 2014).

2.2.3.2 Effects of pressure in pSFC

Changing the pressure of modified fluids leads to an increase in the density, what at the same time increases the solvent strength. However, the magnitude of the changes due to variations in pressure is small compared with the changes due to modifier concentration. Furthermore, with most binary mixtures at low temperature, low densities cannot be made because the fluid tends to break up into two phases, making chromatography impossible. Thus, modifier concentration is the primary method for controlling retention in packed column SFC.

On the other hand, pressure changes tend to produce larger changes in selectivity than adjusting the modifier concentration when polar solutes are injected. At constant mass flow, the efficiency is not affected by changes in the pressure (T.A. Berger, 1995).

2.2.3.3 Effects of temperature in pSFC

Increasing the temperature of binary fluids, at constant pressure, decreases the density of the fluid, but may or may not increase k'. At high temperature and low pressure, greater care is required to avoid two-phase formation. At high temperature, the minimum pressure that must be maintained to avoid two-phase formation increases.

Increased temperature also causes desorption of MeOH from silica stationary phases. This tends to decrease both the volume and the polarity of the effective stationary phase, which tends to decrease k'. This opposes the effect of increasing temperature causing a decrease in mobile phase density which would increase k'.

2.2.3.4 Effects of flow rate in pSFC

Flow has no direct influence on selectivity. However, significant changes in flow through a packed column containing small particles generally cause changes in the pressure drop. This means that the inlet pressure and the average pressure both change. Despite the low viscosity of supercritical fluids, its flow creates significant pressure drops (5 µm packings of silica gel will cause a pressure drop of 10-30 bar over the length of a 20-25 cm long column) (T.A. Berger, 1995; Caude & Thiâebaut, 1999). Regarding to efficiency, there is a flow rate that produces a maximum value for a column. Either lower or higher flow rates cause a loss in efficiency (T.A. Berger, 1995).

2.2.3.5 Summary

A summary of the importance of the modifier, pressure, temperature and flow is shown in Table 2.3.

Table 2.3 Importance of physical parameters in adjusting performance. Extracted from (T.A. Berger, 1995).

Importance	Retention	Selectivity	Efficiency
↑	Percentage modifier	Temperature	Flow
	Pressure	Pressure	
	Temperature	Percentage modifier	Pressure
	Flow		Temperature
		Flow	Percentage modifier

2.3 Retention models in SFC

Unlike GC and HPLC, where various retention models have been extensively developed for years, SFC suffers from the lack of systematic studies of retention mechanisms and useful models for solute retention. Various models have been developed over the last two decades to systematically explain the retention behavior of different types of analytes in pSFC (Y. Wu, 2008).

Thermodynamic properties obtainable by SFC may be classified into the properties derived directly from solute retention and the properties derived from changes in solute retention with pressure (to obtain the difference between the partial molar volumes of the solute at infinite-dilution in the mobile and the stationary phase at constant temperature); temperature (to obtain the difference between the partial molar enthalpies of the solute at infinite-dilution in the mobile and the stationary phase at constant pressure); or composition of the (binary) mobile phase fluid (to obtain the composition derivative of the fugacity coefficient of the solute at infinite-dilution in the binary fluid at constant temperature and pressure) (Roth, 2004). The linear solvation energy relationship (LSER) methodology can be applied to correlate molecular interaction parameters with retention behavior in SFC.

2.3.1 Linear Solvation Energy Relationships (LSERs)

The most popular method for studying stationary phases in SFC is the Linear Solvation Energy Relationships (LSER) model. This model is known as a quantitative structure-property relationship (QSPR), in which the property is modelled as a function of molecular descriptors, that represent physico-chemical properties of the selected analytes. If the property considered is the retention, the QSPR is called QSRR, this is, quantitative structure-retention relationship. QSRRs are empirically derived relationships for a chromatographic system which can be used to predict the retention

of a new solute as well as to acquire a better understanding of the molecular mechanisms of separation operating in the system or to evaluate properties of stationary phases. Classify columns or compare them in different chromatographic systems using the LSER method is also possible (Galea, Mangelings, & Vander Heyden, 2015).

2.3.1.1 Overview of the model

The basis of the LSER is the cavity model of solvation. When a solute is transferred from one phase to another, a hole of a suitable size is formed in the acceptor phase to hold the solute. Simultaneously, the solvent molecules around the solute cavity are reorganized and solute-solvent intermolecular interactions are stablished. The opposite process takes place is the donor phase. The difference in cavity formation and solute-solvent interactions in each phase gives the free energy change that is characterized by an equilibrium constant. In LSER, the solvation process is described as a linear combination of several intermolecular interactions (Y. Wu, 2008) .

There are two LSER models: the solvatochromic model and the solvation parameter model. The solvatochromic LSER model was first developed by Kamlet and Taft et al. to describe solvation effects on physicochemical processes. The parameters in the model were later adapted to describe solute characteristics in investigating the solubility properties of various media.

Abraham et al. improved the correlation between retention in gas and liquid chromatography. The solvation parameter model allows obtain information about the stationary phase retention properties and it has also been used for retention prediction. The retention is calculated by multiple regression analysis of a linear combination of five different terms:

$$\ln SP = c + eE + sS + aA + bB + vV \qquad (2.9)$$

In the equation, SP is the dependent variable, here a measure related to solute partitioning (retention) (Studzińska & Buszewski, 2012).

The terms E, S, A, B and V are solute-dependent molecular descriptors, also called Abraham descriptors. Each descriptor is included in the LSER equation to account for a specific intermolecular interaction. Lower case letters represent the coefficients of the model or system constants and they are obtained through a multilinear regression of the retention data for a certain number of solutes with known descriptors. They represent the magnitude of difference for that interaction between the mobile and the stationary phase in a given chromatographic system, as it is shown in the following equation:

$$x = x_{Stationary\ phase} - x_{Mobile\ phase} \tag{2.10}$$

where x represents the system constant. A positive sign of a coefficient indicates that the respective molecular interaction is stronger in the stationary than in the mobile phase, what leads to an increase in the retention time. Opposite situation may be observed when the coefficients are negative. Consequently, the coefficients also reflect the system's relative selectivity towards that molecular interaction. The term c is the intercept of the equation, which when the retention factor is used as the dependent variable, is dominated by the phase ratio.

The different solute interactions incorporated in the LSER model for chromatographic retention are illustrated in Figure 2.11. E is the excess molar refraction and expresses polarizability contributions from n and π electrons; S is the solute dipolarity/polarizability; A and B are the solute overall hydrogen-bond acidity (donating ability) and basicity (accepting ability); and V is the McGowan characteristic volume in units of $cm^3\ mol^{-1}/100$ (Studzińska & Buszewski, 2012) (West & Lesellier, 2007).

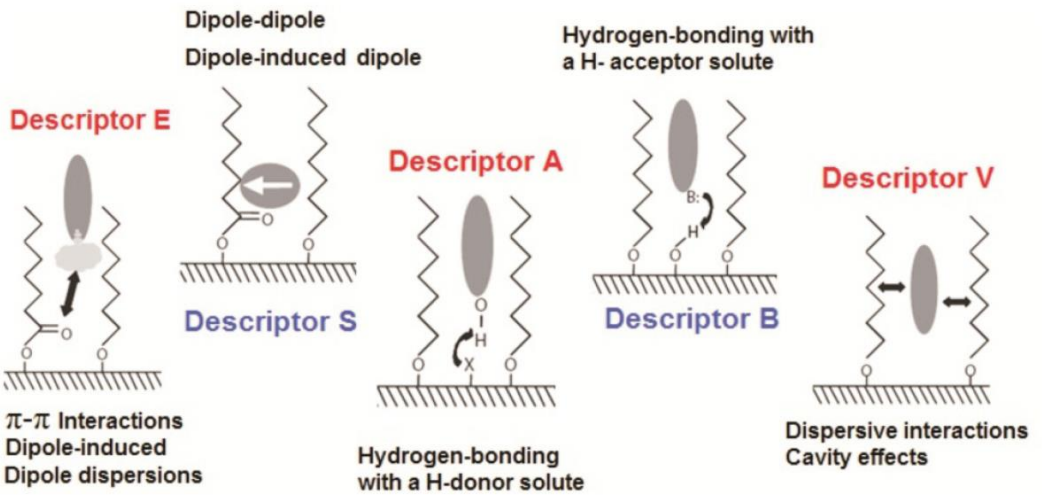


Figure 2.11 Representation of the different interactions estimated by the LSER model. Reproduced from Ref. (Grazieli & Collins, 2014).

2.3.1.2 Solute descriptors

The solute's characteristic volume (V) is calculated from its structure by summing the characteristic atomic volumes for each atom and subtracting a fixed volume of each bond of any type. The value is divided by 100 to match the scale of other solute descriptors.

The solute's excess molar refraction (E) models polarizability contributions from n- and π-electrons. It is calculated from the refractive index and characteristic volume as the difference in molar refraction of the solute and an n-alkane of identical volume. It is divided by 10 to match the scale of other solute descriptors.

The dipolarity and polarizability descriptor (S) models the ability of the solute to stabilize a neighboring dipole by its capacity for orientation and induction interactions.

The solute's effective hydrogen bond (acidity) (A) and accepting ability (basicity) (B) are measures of the solute's hydrogen bond donating and accepting abilities. These scales are unrelated to proton transfer acidity and basicity that are expressed by the

pK_a scale. The scale takes into consideration the propensity of a solute to interact with a large excess of surrounding solvent molecules.

S, A and B solute descriptors must be determined experimentally using chromatographic or liquid-liquid distribution systems or estimated using various parameters and computational approach (Y. Wu, 2008).

Solutes in LSER analysis

The procedure used for the characterization of stationary phases with the solvation parameter model involves the analysis of test solutes. It is essential to have enough solutes with varying descriptors so that the LSER model will have statistical and chemical validity. Although, mathematically, a minimum number of seven compounds is needed to perform multiple linear regression for the sis unknowns (five system coefficients and the intercept), 18 solutes (three varied values for each solute descriptor plus the intercept) is a reasonable minimum from the statistical point of view. However, since individual solutes express several interactions simultaneously and all interactions in the solvation parameter model contribute to retention, the minimum number of required solutes can be safely reduced from 18 to 9. Including more solutes than the minimum statistical requirements is a common practice, in order to decrease the error associated with individual measurements (West & Lesellier, 2007; Y. Wu, 2008).

Then, a minimum of 15-20 carefully solutes are needed to be included to have a relevant model. The main criteria of selection should be to collect both aromatic and aliphatic substances with a wide range of properties. Such collection is of great importance, especially when the significance of LSER equations is considered. Compounds should be chosen to avoid several effects commonly observed for Abraham model, e.g., high correlation of polarity with the solute size. In such a way, the analyte parameters (E, S, A, B, V) cannot covary in order to avoid problems with the variance during the multiple regression analysis. (Studzińska & Buszewski, 2012).

It is highly recommended for a complete characterization of a chromatographic system the choice of larger sets of test solutes in order to obtain more accurate results (West & Lesellier, 2007). In this work, only silica gel stationary phases will be compared, so that 17 solutes were carefully selected. More details of the selection of the solutes as well as its properties are described in Chapter 3.1.3.1 “Selection of solutes”.

Columns can be evaluated and compared through the five parameters of LSER model in a spider diagram, as it is simplified in Figure 2.12. As can be seen, all the classic polar phases such silica are closely together across the axis of proton donors. Diol has a tendency to also be a proton acceptor more than the other, while cyano tends toward dipole-polarizability characteristics (Terry A. Berger, 2015).

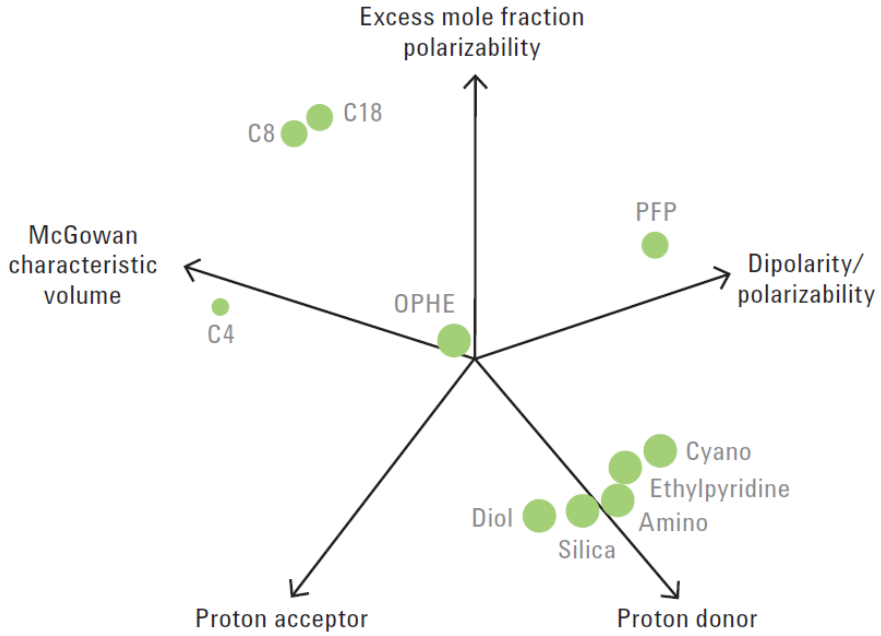


Figure 2.12 Column selectivity using LSER model. Extracted from (Terry A. Berger, 2015)

2.3.2 Mixed Retention Model

For packed-column SFC, chemically bonded silica phases are typically used. They are prepared by the reaction of the proper silylating reagent with hydroxyl groups on the surface of small silica particles. However, the reaction can never be completed, and a

fraction of silanol groups will remain even after subsequent "end-capping" treatments (Janssen et al., 1991).

For this kind of silica phase, Janssen et al. have developed a model in which the retention of a solute is due to a mixed retention mechanism, the adsorption of the solute on the silanol groups that are accessible and the partitioning of the solute on the chemical bonded phase (CBP). The observed capacity factor can be written as:

$$k_{obs} = k_{sil} + k_{CBP} \tag{2.11}$$

where k_{obs} is the experimentally observed capacity factor, k_{sil} is the capacity factor due to the interaction with the silanol groups and k_{CBP} is the capacity factor due to interaction with the chemically bonded phase.

The contribution of the silanols may cause long retention times as well as poor peak shapes due to non-linear distribution isotherms. On the other hand, when the mobile phase is modified by an organic modifier, its molecules will adsorb on the silanol groups due to its acidity and its hydrogen-accepting properties. If the interaction of modifier molecules with the silanol groups is much stronger than the solutes with the silanol groups or if the modifier is present in a much higher concentration, the effects of the silanol groups can be suppressed. If a site occupied by a modifier is assumed that it will no longer contributes to retention, the Equation 2.11 can be written as:

$$k_{obs} = k_{sil}^{0}(1 - \theta) + k_{CBP} \tag{2.12}$$

where k_{sil}^{0} is the contribution of the silanol groups at zero modifier concentration. The fractional occupancy of the adsorption sites can be defined as the relation between the number of modifier molecules adsorbed on the stationary phase and the maximum molecules that can be adsorbed (Janssen et al., 1991).

An adaptation of the mixed retention model proposed by Janssen et al. will be described in Section 4.3.1 "Overview of the Mixed Retention Model", considering that

a site occupied by a molecule of a modifier can contributed to the retention of a solute on silica non-bonded phases.

Retention is considered as a partitioning mechanism and it can be described using an adsorption isotherm model, this is, a plot of the concentration of a solute on a surface as a function of its concentration in the mobile phase. Frontal analysis (FA) is a reference method for adsorption isotherm determination in LC. It is usually carried out by injecting a series of increasing concentration pulses, but it will not be analyzed in this work (Enmark, Forssén, Samuelsson, & Fornstedt, 2013). More details about the description of the retention by an adsorption isotherm model are described in Section 4.3 "Adaptation of the Mixed Retention Model".

2.3.2.1 Van't Hoff Plot

In chromatography, the free energy of transfer of a solute from the stationary phase to the mobile phase (ΔG_T^0) is proportional to the logarithm of the solute distribution coefficient (K_D) (Equation 2.14). On the other hand, enthalpy and entropy of transfer can be obtained based on their thermodynamic relationship to the Gibbs free energy (Equation 2.15).

$$\Delta G_T^0 = -RTlnK_D \tag{2.14}$$

$$\Delta G_T^0 = \Delta H_T^0 - T\Delta S_T^0 \tag{2.15}$$

As it was said in Section 2.2.2.1 "Retention", the distribution coefficient is related to the capacity factor, k', and the phase ratio, β, as:

$$K_D = \frac{k'}{\beta} \tag{2.16}$$

In such a way, it is possible to write the Equation 2.14 as:

$$\ln k' = -\frac{\Delta H_T^0}{RT} + \frac{\Delta S_T^0}{R} - ln\beta \tag{2.17}$$

which represents a linear correlation of the capacity factor to 1/T at constant density, assuming that β and ΔS_T^0 are independent of temperature (V, Yonker, & Smith, 1986).

3 Materials and Methods

3.1 Chemical and Reagents

3.1.1 Stationary phases

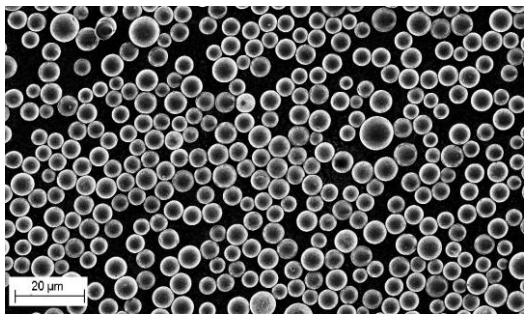
In this work, silica aerogel as well as silica gel non-bonded particles are used as stationary phase. The columns used are HPLC columns with a length of 50 mm and an inner diameter of 4.6 mm. In the following sections, the properties of the particles will be described.

3.1.1.1 Kromasil® particles

Silica gel stationary phases consist of Kromasil® particles, obtained from Akzo Nobel and whose properties are described in Table 3.1.

Table 3.1 Characteristics of the silica gel phases (Kromasil®, 2016)

Column	**Kromasil® 60**	**Kromasil® 100**	**Kromasil® 300**
Abbreviation	SIL-60	SIL-100	SIL-300
Trade name	Kromasil® 60-5-SIL	Kromasil® 100-5-SIL	Kromasil® 300-5-SIL
Manufacturer	Kromasil	Kromasil	Kromasil
Particle size, μm	5	5	5
Mean pore size, nm	6	10	30
Specific surface area, A_s, $m^2\ g^{-1}$	540	320	110
Pore volume, V_P, $cm^3\ g^{-1}$	1.12	0.9	0.9
Nature of the stationary phase	Silica gel	Silica gel	Silica gel



Kromasil® particles are claimed to be perfectly spherical and to have a smooth surface, as it is seen in Figure 3.1.

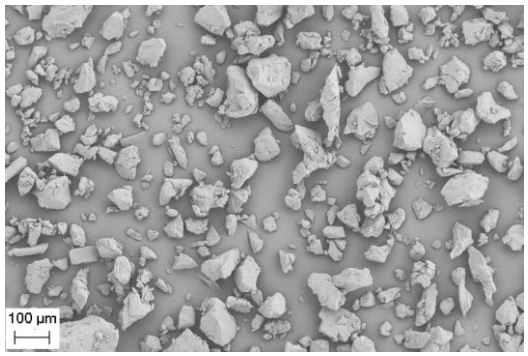
Figure 3.1 Silica gel particles. Picture obtained from SEM

3.1.1.2 Silica aerogels particles

Silica aerogel beads produced by the two-step sol-gel method are exposed under shear stress and are dried in the autoclave. Afterwards, gels were crushed by using a conventional mortar till achieving the desired particle size. The properties of the powder are shown in Table 3.2.

Table 3.2 Characteristics of the silica aerogel phase

Column	Silica Aerogel
Abbreviation	SIL-Aerogel
Mean pore radio, nm	13.3
Specific surface area, A_s, $m^2\ g^{-1}$	858
Pore volume, V_P, $cm^3\ g^{-1}$	5.2



The aerogel particles had a completely irregular form. They possessed a very high porosity consisting mainly of mesopores.

Figure 3.2 Aerogel particles. Picture obtained from SEM

3.1.2 Mobile phase

As a mobile phase, supercritical CO_2 is used together with an organic modifier (mainly methanol) in order to increase the polarity of the mobile phase. CO_2 with purity level of 99.995% is used (CO_2 4.5), this is, food grade, and it was obtained from Westfalen Austria GmbH. The cylinder capacity is 50 L. Regarding to organic modifiers, high-quality chromatography grade solvents are used. Methanol will be used as modifier during the whole and it is obtained from Roth. For the Section 4.1.1 "Hold-up time", also hexane and isopropanol from Roth and Ethanol from Sigma Aldrich will be used.

3.1.3 Solutes

3.1.3.1 Selection of solutes

A total of 17 solutes were carefully selected. They were obtained from several suppliers: Benzene, Caffeine and Pyridine from Merck; Hexane and p-Nitrophenol from Honeywell; Vanillin and Benzoic Acid from Roth; Nicotinamide, Ethyl benzoate, Anisole, p-Cresol, Nitrobenzene, p-Nitrotoluene, Butyl benzoate and Anthracene from Sigma Aldrich; and Naphthalene and Phenol from Fluka.

Key solutes

A preliminary selection of nine solutes were chosen according to West and Lesellier, towards decrease the time required for the use of this model in SFC. The aim of this work was to stablish a rapid testing procedure and to obtain equivalent information when operating parameters are varied. The nine solutes were selected among more than one hundred compounds by taken two by two to establish new equations, allow the calculation of the model coefficients. This methodology was correctly evaluated in 24 stationary phases and validated in 13 new SFC systems (West & Lesellier, 2007). Key solutes are summarized in Table 3.3. The solute descriptors used in the solvation parameter model were extracted from a database stablished from several sources by West and Lesellier (West & Lesellier, 2008).

Table 3.3 Key solutes selected for a rapid evaluation (West & Lesellier, 2007, 2008)

SOLUTES	E	S	A	B	V
Toluene	0.601	0.52	0	0.14	0.8573
p-Nitrotoluene	0.87	1.11	0	0.28	1.032
Nitrobenzene	0.871	1.11	0	0.28	0.8906
Anisole	0.708	0.75	0	0.29	0.916
p-Cresol	0.82	0.87	0.57	0.31	0.916
o-Nitrophenol	1.045	1.05	0.05	0.37	0.949
Nicotinamide	1.01	1.09	0.63	1	0.9317
Butyl benzoate	0.689	0.85	0	0.46	1.214
Ethyl Benzoate	0.668	0.8	0	0.46	1.4953

E: Excess moral refraction; S: Dipolarity/Polarizability; A: Hydrogen bond acidity; B: Hydrogen bond basicity; V: McGowan´s characteristic volume

Additional solutes

In order to get a more consistent model, eight more solutes were added to the previous ones, thus obtaining a total of 17 solutes. The solute descriptors were also extracted from a database stablished by West and Lesellier (West & Lesellier, 2008).

Table 3.4 Extra solutes selected for a rapid evaluation (West & Lesellier, 2008)

SOLUTES	E	S	A	B	V
Benzene	0.61	0.52	0	0.14	0.7164
Pyridine	0.631	0.84	0	0.52	0.6753
Caffeine	1.5	1.6	0	1.35	1.363
Benzoic Acid	0.73	0.9	0.59	0.4	0.9317
Phenol	0.805	0.89	0.6	0.3	0.7751
Vanillin	1.04	1.33	0.32	0.67	1.1313
Naphthalene	1.34	0.92	0	0.2	1.0854
Anthracene	2.29	1.34	0	0.26	1.454

E: Excess moral refraction; S: Dipolarity/Polarizability; A: Hydrogen bond acidity; B: Hydrogen bond basicity; V: McGowan´s characteristic volume

As it was said in Chapter 2.3.1.2 “Solute descriptors”, significant cross-correlation among the descriptor values (E, S, A, B, V) of chosen solutes should be avoided in order to prevent the multicollinearity problem, which reduces the capability of the multiple linear regressions models to distinguish the correlated descriptors. Therefore, the correlation coefficient matrix is shown in Table 3.5. The cross-correlations among various descriptor values ranged from 0.041 to 0.715 which are acceptable for LSER analysis (Studzińska & Buszewski, 2012).

Table 3.5 Correlation Coefficient Matrix of solute descriptors

	E	**S**	**A**	**B**	**V**
E	1				
S	0,715	1			
A	-0,131	0,041	1		
B	0,228	0,659	0,190	1	
V	0,592	0,542	-0,278	0,336	1

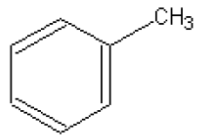
3.1.3.2 Properties of each solute

A summary of their properties is shown in Table 3.6. The signal of the solutes was recorded from 200 to 250nm, and the wavelength was selected from the absorption spectrum regarding to the absorbance and the peak shape. Their structure is shown in Table 3.7.

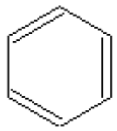
Table 3.6 Properties of solutes. Extracted from (ACD/I-Lab, 2017)

Solute	Molecular weight, g mol^{-1}	Density, g cm^{-3}	No. of hydrogen bond donors/acceptors	
Toluene	92.14	0.871	D: 0	A: 0
Benzene	78.11	0.873	D: 0	A: 0
Naphthalene	128.17	1.037	D: 0	A: 0
Anthracene	178.23	1.130	D: 0	A: 0
Pyridine	79.1	0.956	D: 0	A: 1
Anisole	104.14	0.953	D: 0	A: 1
Butyl benzoate	178.23	1.007	D: 0	A: 2
Ethyl benzoate	150.17	1.044	D: 0	A: 2
p-Nitrotoluene	137.14	1.166	D: 0	A: 3
Nitrobenzene	123.11	1.215	D: 0	A: 3
Caffeine	194.19	1.45	D: 0	A: 6
p-Cresol	108.14	1.038	D: 1	A: 1
Phenol	94.11	1.071	D: 1	A: 1
Benzoic Acid	122.12	1.197	D: 1	A: 2
Vanillin	152.15	1.231	D: 1	A: 3
o-Nitrophenol	139.11	1.395	D: 1	A: 4
Nicotinamide	122.12	1.204	D: 2	A: 3

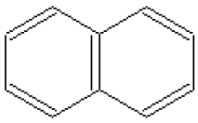
Table 3.7 Structure of solutes



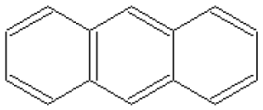
Toluene
$C_6H_5CH_3$



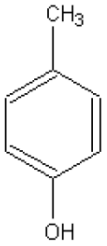
Benzene
C_6H_6



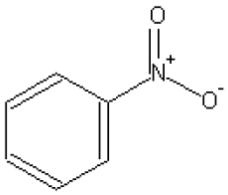
Naphthalene
$C_{10}H_8$



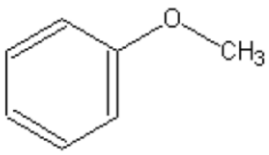
Anthracene
$(C_6H_4CH)_2$



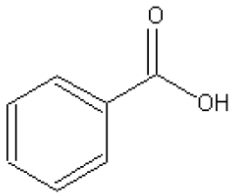
p-Cresol
$CH_3C_6H_4OH$



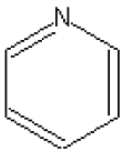
Nitrobenzene
$C_6H_5NO_2$



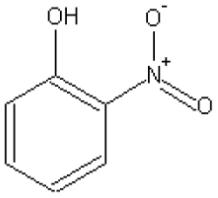
Anisole
$CH_3OC_6H_5$



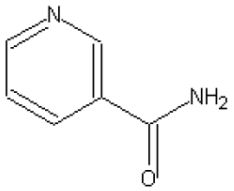
Benzoic Acid
C_6H_5COOH



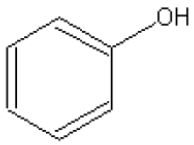
Pyridine
C_5H_5N



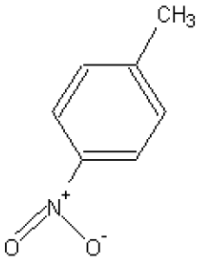
***o*-Nitrophenol**
$C_6H_5NO_3$



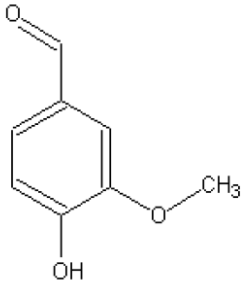
Nicotinamide
$C_6H_6N_2O$



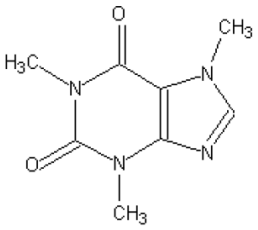
Phenol
C_6H_6O



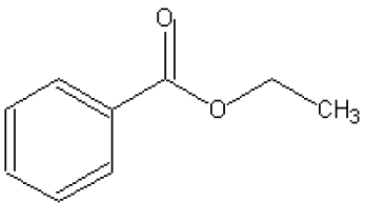
p-Nitrotoluene
$CH_3C_6H_4NO_2$



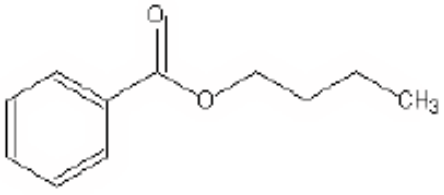
Vanillin
$C_6H_3(OH)(OCH_3)CHO$



Caffeine
$C_8H_{10}N_4O_2$



Ethyl benzoate
$C_6H_5COOC_2H_5$



Butyl Benzoate
$C_6H_5COOC_4H_9$

3.1.3.3 Hold-up time

The methods used to determine the hold-up volumes of chromatographic columns without affecting their structural properties can be divided into two main groups: Static methods and dynamic methods. Static methods measure the hold-up time volumes of columns after disconnecting them from the chromatograph, while dynamic methods measure the hold-up volumes of columns connected to the system. The most common methods use a hold-up volume marker, the minor disturbance method and inverse size exclusion chromatography. The use of a proper hold up time marker affords the opportunity to estimate changes of the adsorbent volume due to the adsorption of mobile phase molecules (Vajda & Guiochon, 2013).

The hold-up times of the columns were determined by using nitrous oxide dissolved in methanol as the hold-up time marker. Nitrous oxide was successfully used as hold-up time marker before (Vajda & Guiochon, 2013), because it is not or to a very small degree adsorbed on the interface and it can be detected by the detectors used in SFC. Nitrous oxide was obtained from Sigma Aldrich in a can of 1L with a purity of 99%.

Samples were prepared by bubbling the gas in methanol, so that a stream of nitrous oxide was directed into pure methanol for approximately one minute. Although it is relatively well soluble in alcohols, the solution is not stable for a long time and new samples are needed to be done after one week. The signal of nitrous oxide was recorded at 195 nm.

3.2 Instrumentation

3.2.1 Supercritical Fluid Chromatographic system

3.2.1.1 Column packing

Silica gel columns are packed with a slurry method, while silica aerogel particles are packed by using a dry vibration method.

Silica gel columns. Slurry method

The slurry is prepared with enough particles to fill the column completely plus about 20% excess material. For packing, only the column outlet is closed with the "sandwich technique" filter-metal sieve-filter and the fitting, and it is completely filled with silica gel particles mixed with the packing solvent, hexane. The column inlet is connected to a reservoir that contains the rest of the slurry and fresh solvent is pumped into it for several minutes, achieving a pressure of 400 bar. The column is then disconnected from the reservoir; excess particles are gently scraped from the top of the packed bed with a sharp object and the column inlet is closed with the "sandwich technique".

Figure 3.3 Slurry packing for silica gel columns

Silica aerogel columns. Vibration method

The capillary forces generated by immersing the aerogel particles into a liquid would lead to collapse their structure. Thus, the employment of a dry method was necessary to pack the chromatographic columns. The method applied in this work consists in successive filling of the column with the silica aerogel particles helped by a funnel, followed by mechanical vibration in a shaker. One side of the column was left open, so the repacking of the column after shaking it for 15 minutes was done several times. The other side is closed by a filter, a metal sieve and a ring followed by the fitting part. Once the column was full, it was placed in the SFC system and it was compress and dried with supercritical CO_2 at a temperature of 40 ºC for 24 hours. The filling process was repeat until the column was filled completely.

Figure 3.4 Dry packing for silica aerogel columns

3.2.1.2 Samples preparation and measurements

Samples are prepared in 2 mL vials and they are dissolved previously in methanol in order to achieve a concentration of 0.1 mg mL^{-1}. Methanol was obtained from Roth at a HPLC gradient. Every sample is identified by the name of the solute and a number with the date and the sample number (for instance, in the sample number Ben

010217_1, "Ben" means benzene, 01 is the day, 02 is the month, and the 17 is the year of the sample preparation, and 1 is the sample number that was done in that day. The signal of the different solutes was recorded between 195 and 250 nm. The retention time of every injection was the corresponding time of the emergence of the peak maximum of the solute.

3.2.1.3 SFC instrumentation

The measurements were carried out using a Waters Acquity UPC² supercritical fluid chromatograph. It is formed by 5 chambers, as it is possible to see in Figure 3.5: The PDA detector, column manager, convergence manager, sample manager and binary solvent manager.

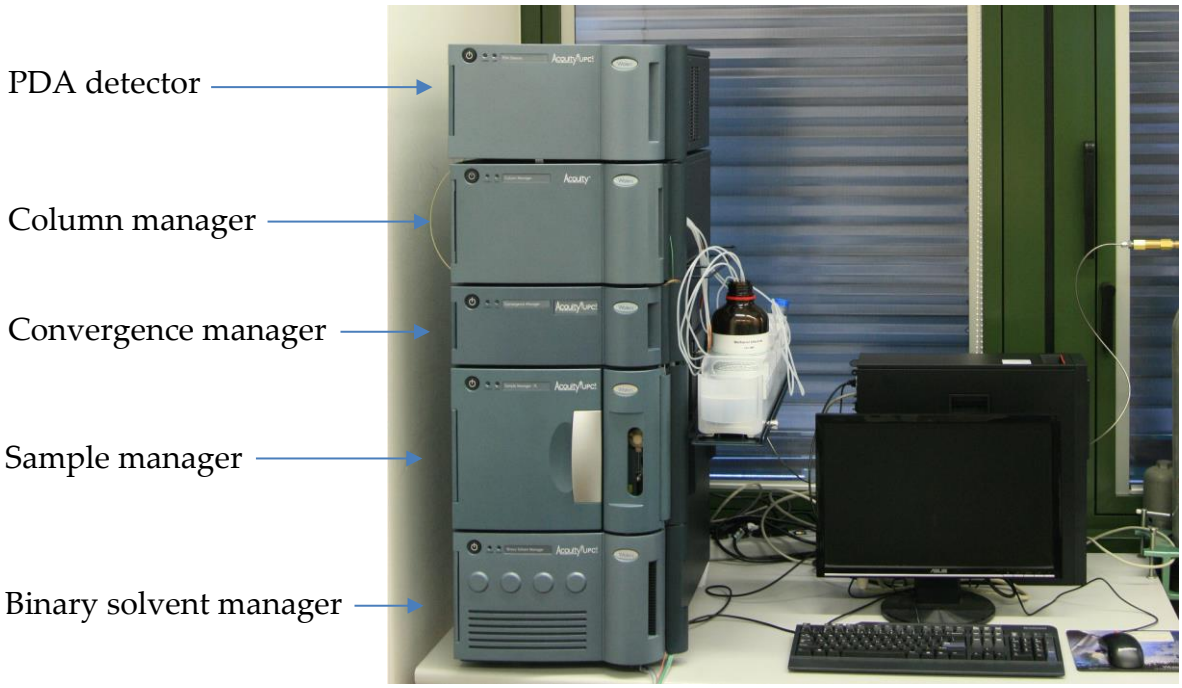


Figure 3.5 SFC System

PDA detector. The photodiode array detector (PDA) is the most commonly used to record the ultraviolet and visible (US-vis) absorption spectra of samples that are passing through a supercritical fluid chromatograph. It has a high-strength silica lens that compensates differences in refractive index between the CO_2 and co-solvents,

resulting in a reduction of noise. The wavelength range available is from 190 to 800 nm and the light source is a deuterium lamp. It has a pressure limit of 6000 psi (413 bar).

Column manager. It has a capacity of two columns with a length from 50 to 200 mm and internal diameters from 2.1 mm up to 8.0 mm. The column holding plates have independent active preheated incorporated, what allows to achieve temperatures up to 90 ºC in 0.1 ºC increments.

Convergence manager. It has an automated back pressure regulator (ABPR) in order to improve the density control of the mobile phase. The control precision is <±0.5 bar.

Sample manager. It has two sample plates with 48 position for 2 mL vials each. The injection volume range is from 0.1 to 50 µL in 0.1 µL increments and it has an automated injector with a 10 µL sample loop.

Binary solvent manager. Separate pumping systems are used for the CO_2 and the co-solvents. The pumping system of CO_2 is modified and features two-stage Peltier cooling. The operating flow rate is from 0.01 to 4 mL min^{-1} in 0.001 mL increments and the maximum operating pressure is 6000 psi (413 bar), up to 3 mL min^{-1} and 4250 (293 bar), up to 4 mL min^{-1}.

3.2.1.4 Flow diagram

The instrumentation of SFC is almost identical to that used in high performance liquid chromatography (HPLC). Complex mixtures can be separated and sometimes the individual components in the mixture can be identified. In Figure 3.6, the flow diagram of the SFC is represented. The following description will be according to it.

Four solvents labeled B1, B2, B3 and B4 can be selected by the Solvent Select Valve (SSV). The CO_2 and the solvent are pumped independently (A: CO_2 and B: Organic solvent) and they are mixed in a mixer. A solution of the sample is prepared in vials up to 2 mL and is injected into the high-pressure flow stream composed by pure CO_2 or a mixture CO_2-organic solvent. The sample passes into a column filled with fine

particles. The individual components of every sample interact differently with the surface of the particles and they emerge from the column at different times and pass through a PDA detector. As CO_2 is a compressed gas, a backpressure regulator is required on the system outlet to ensure the mobile phase remains a single dense phase throughout the chromatograph. The flow diagram is shown in Figure 3.6 and more details can be found in literature (Waters. Acquity UPC2 System, 2013, 2015).

The most significant difference from HPLC is the replacement of most of the liquid mobile phase with a dense compressed gas, almost always carbon dioxide (CO_2). At high pressures, such as greater than 80 bar, CO_2 acts as a solvent. Because it is a compressed gas, a backpressure regulator is required on the system outlet to ensure the mobile phase remains a single dense phase throughout the chromatograph. This, in turn, requires some detectors, such as an ultraviolet (UV) detector, to be operated at elevated pressures (Caude & Thiâebaut, 1999).

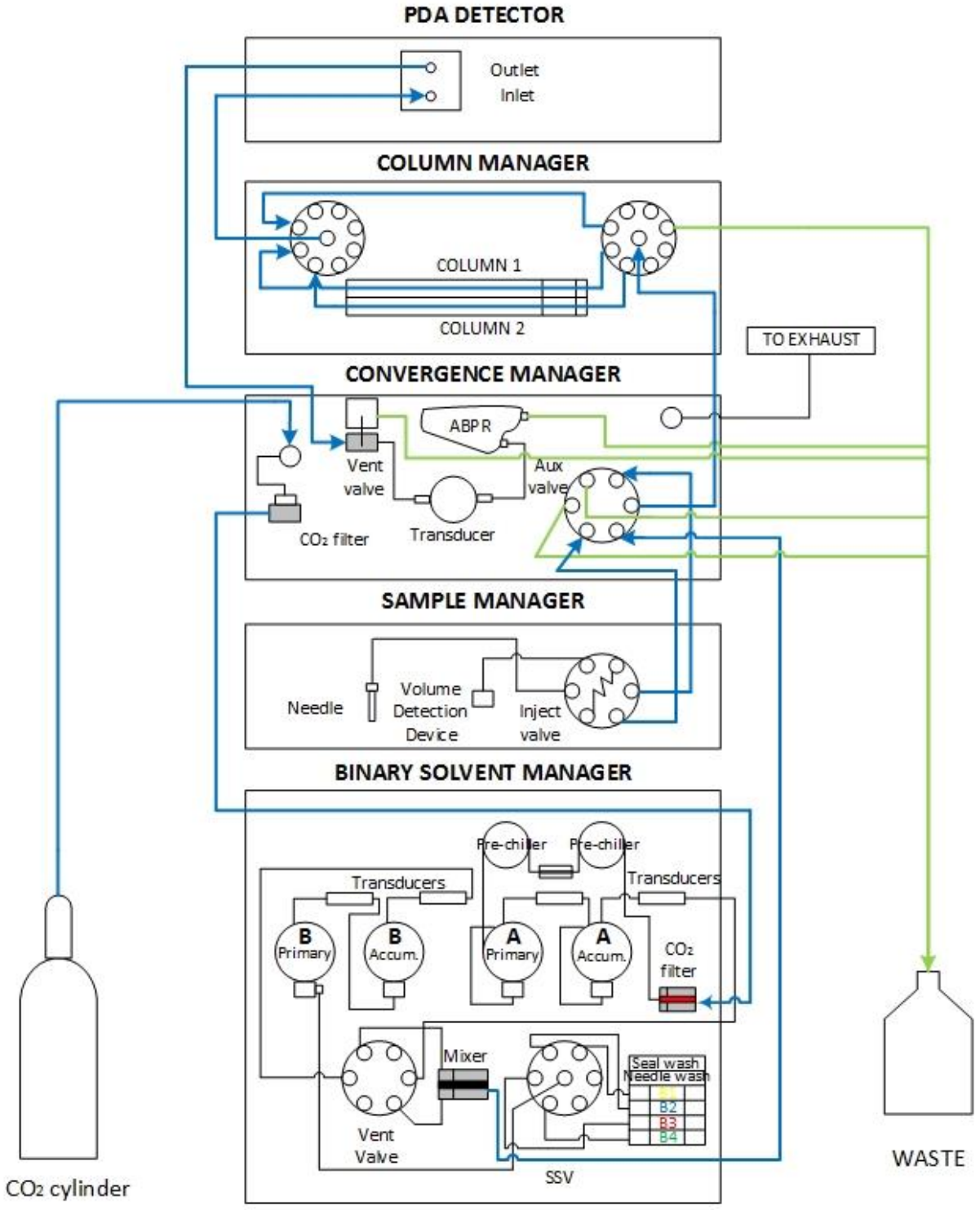


Figure 3.6 Supercritical Fluid Chromatography diagram. Blue line: principal connections; green line: to waste. Adapted from the graphical navigator view of Waters' webpage (Waters, 2017).

3.2.2 FTIR Analysis

The FTIR spectral studies were performed using Thermo scientific™ Nicolet™ iS ™ 10 FTIR spectrometer. Its spectral range is from 350 to 7800 cm^{-1}. It has incorporated a deuterated triglycine sulfate (DTGS) detector and a liquid-nitrogen-cooled mercury cadmium telluride (MCT) detector. More product specifications are shown in literature (Thermo Scientific, 2013)

Figure 3.7 Thermo Scientific™ Nicolet™ iS™ 10 FTIR spectrometer. Extracted from (Thermo Scientific, 2013)

A small amount of the sample is placed in the module and it is compress by the integrating sphere. First, a measure of the background is needed and afterwards, the measurement of the sample is performed directly through vials.

4 Results and discussion

4.1 Chromatographic conditions

4.1.1 Hold-up time

As it was said previously in Chapter 3.1.3.4 "Hold-up time", the hold-up time was determined by the injection of a solute that is not retained by the stationary phase. In order to select the best unretained solute, the injection of nitrous oxide dissolved in methanol was compared with several organic solutes under different conditions. These solutes are hexane, acetone, methanol and ethanol, and they have been seen previously in literature as hold-up time markers (Gurdale, Lesellier, & Tchapla, 2000; Pyo, Li, Lee, Weckwerth, & Carr, 1996; Vajda & Guiochon, 2013).

First, the possible enrichment in organic modifier on the silica surface was measured by equilibrating the column with mobile phases containing 0.1, 0.2, 0.5, 1, 2, 3, 4 and 5 (v/v%) methanol in carbon dioxide-methanol mobile phase (Figure 4.1). This analysis has been developed using Kromasil-100-5-SIL as stationary phase. The injections were carried out when the column was stabilized at 40 ºC and at 200 bar and the injection volume of the sample was 2 μL. The injections were done twice.

Because of the stationary phase is polar, polar organic solutes as ethanol, methanol and acetone are attracted by it so that their retention time is larger compared to the retention time of N_2O and hexane.

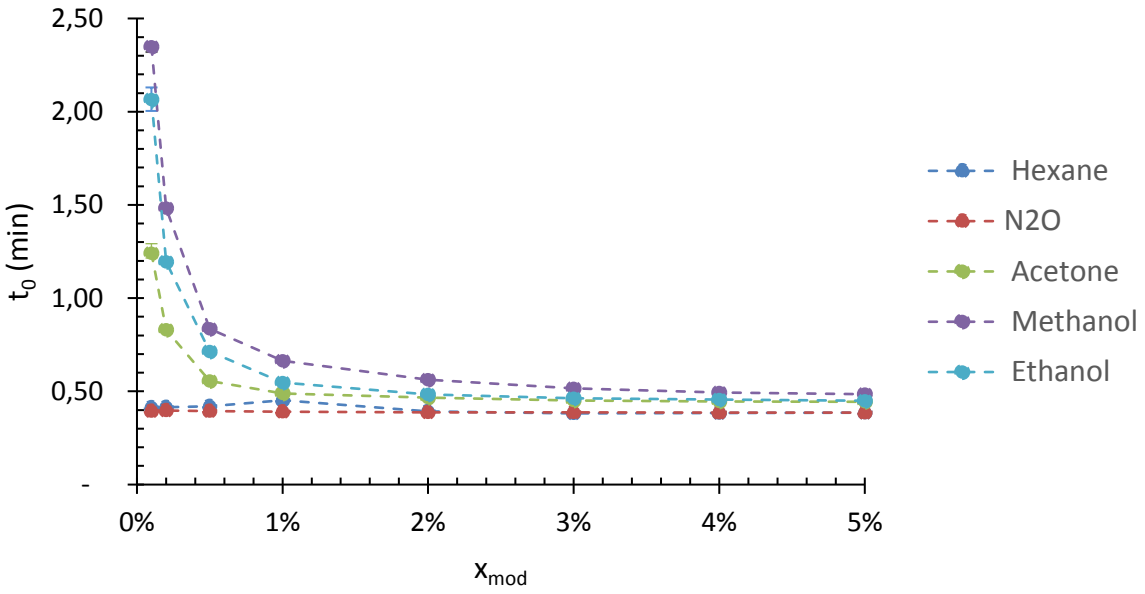


Figure 4.1 Retention times of several organic modifiers regarding to the concentration of methanol in the mobile phase

This result can be explained from the point of view of the stationary phase. At larger concentrations of methanol in the mobile phase, the surface coverage of active sites in the stationary phase is higher. As it was said before, polar modifier molecules compete with molecules of solutes for the adsorption on active sites. The more surface coverage of active sites by the modifier, the less interactions between the solutes and stationary phase, which result to lower retention time. However, for nonpolar compounds, hexane and N2O, their interaction to stationary phase is already very low at even zero surface coverage, so their retention time is not influenced by modifier concentration.

As N_2O and hexane are solutes that present less interaction with the mobile phase at any concentration of methanol in the mobile phase, they are chosen for further analyses.

The column will be equilibrated with different modifiers in the mobile phase, so that the retention time of nitrous oxide and hexane will be compared in methanol, hexane, isopropanol and ethanol-carbon dioxide mobile phases at 5% volume concentration

(Figure 4.2). Temperature was 40 ºC and the back pressure, 200 bar. Kromasil-100-5-SIL was used as stationary phase. Two injections were carried out at every condition.

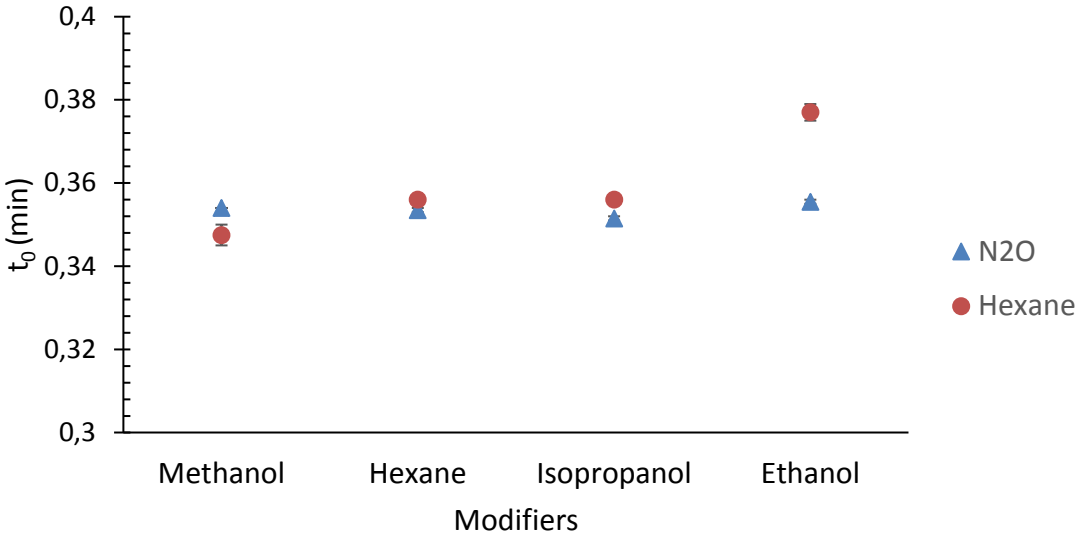
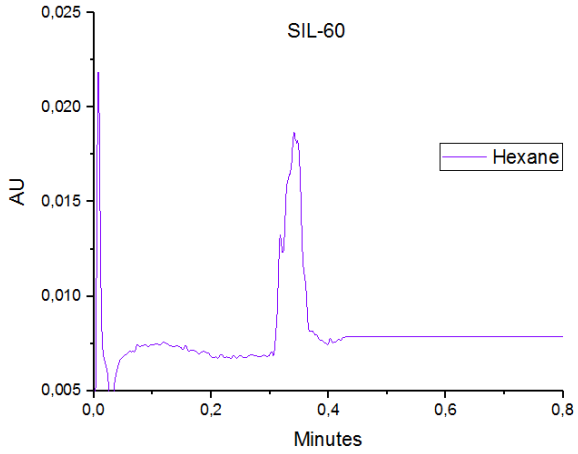


Figure 4.2 Retention times of N_2O and Hexane with different modifiers in the mobile phase

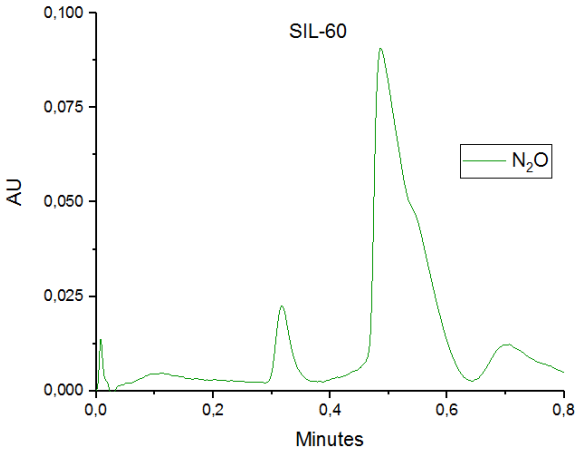
In Figure 4.2, it is shown that the retention time of hexane depends more on the nature of the modifier than N_2O. Because of the hold-up time is a characteristic of the stationary phase and shall not affected by the nature of the mobile phase, N_2O was concluded to be the better marker for hold-up time measurements. Moreover, the peak shape of both solutes was compared in Figure 4.3.

As it was said in Chapter 3.2.1.2 "Samples preparation and retention", the retention time was the corresponding time of the emergence of the peak maximum of the solute. Due to this reason, the peak shape is an important characteristic for the considered analysis. In Figure 4.3, peak shapes of hexane and N_2O were compared in Kromasil 100-5-SIL and Kromasil 300-5-SIL stationary phases. In N_2O chromatograms, two peaks are observed, an early and symmetrical peak and a retained, asymmetrical and tailing peak. The symmetrical one corresponds to the elution of nitrous-oxide and marks the hold-up time of the chromatographic system. The tailing peak is the overloaded elution band of methanol.

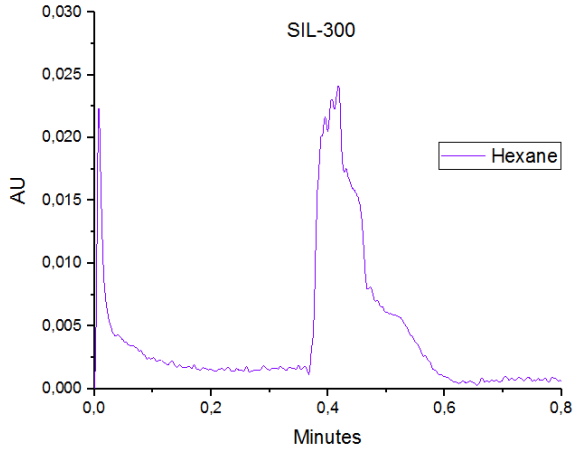
a) Hexane in SIL-60



b) N_2O in SIL-60



c) Hexane in SIL-300



d) N_2O in SIL-300

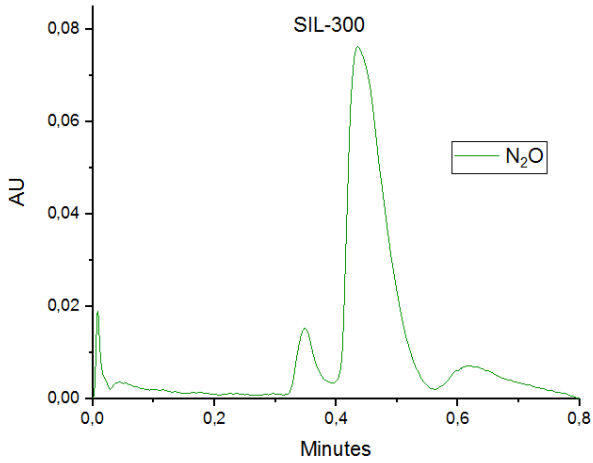


Figure 4.3 Chromatograms of Hexane and N2O at 5% concentration of modifier, 40 °C and 200 bar: a) Peak of hexane in SIL-100; b) Peak of N_2O in SIL-100; c) Peak of hexane in SIL-300; d) Peak of N_2O in SIL-300.

In SIL-100 phases, both solutes present acceptable peak shapes. However, in SIL-300 stationary phases, the hexane peak is clearly more asymmetrical and tailing than the N_2O peak. Therefore, N_2O was considered to be a better marker and it will be used for further analysis.

4.1.2 Stability of the columns

At relative high temperatures and at concentrations of methanol in the mobile phase up to 5%, some incongruences were found when the column temperature was decreased back to 30 ºC. An example is shown in Figure 4.4, where injections of solute, phenol, at different temperatures were carried out in two sets, concentration of modifier was 5% and the back pressure was 200 bar. The first set of temperatures was from 25 ºC to 85 ºC in increments of 10 ºC, and it is represented by blue. Then, the column temperature was decreased till 30 ºC and the second set was carried out up to 80 ºC in increments of 10 ºC, which is represented by the color red in the graph. Since the operation conditions of the two sets of experiments are the same, the capacity factor k' shall also concur. However, it is observed that, in the second set, the capacity factor has decreased regarding to the first set.

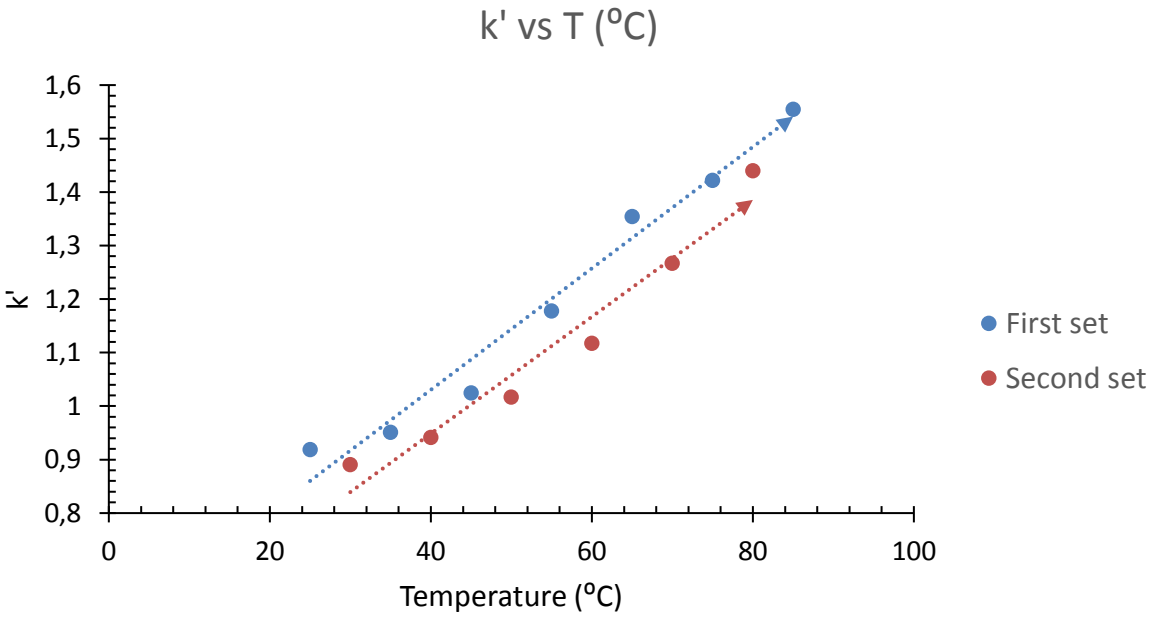


Figure 4.4 Injections organized in two sets. Blue: First set (25 ºC to 85 ºC, increments 10 ºC). Red: Second set (30 ºC to 80 ºC, increments 10 ºC). Solute: Phenol

This result suggests that high temperatures in presence of a modifier in the mobile phase have a permanent effect on the properties of the stationary phase. In order to test the mechanical and chemical stability of the columns, some analyses were carried

out and the results are discussed respectively in the next two sub-chapters. First, column packed with Kromasil particles and column packed with silica aerogel were stabilized at different intervals of temperature with or without no modifier in the mobile phase. The hold-up time was measured and the results are discussed in the sub-chapter 4.1.2.1. Then, FTIR analysis were performed to check the possible alteration in the chemical structure of the particles after stabilizing the column at high temperatures and at different concentrations of modifier in the mobile phase. The results of the FTIR analysis are given in the sub-chapter 4.1.2.2.

4.1.2.1 Stability of the columns with T

Column packed with Kromasil particles and a column packed with aerogel particles were stabilized at every temperature for 6 hours and, within this time, three injections were performed. The medium value of the three injections and the error bars were plotted. In order to prove that high temperatures do not affect the silica gel particles and the hold-up time was constant at a certain temperature, the columns were stabilized again at 40 °C in between of every temperature before reaching a higher one. Injections were made at 200 bar, at a flow rate of 2 mL min^{-1} and the injection volume was 2 µL. At least a flow of 2 mL min^{-1} was needed to obtain a legible peak of N_2O due to wider peaks on the aerogel-packed column. The same procedure was repeated with a concentration of 10% of methanol as modifier in the stationary phase, so that the effects of modifiers at high temperature can also be studied.

Kromasil particles

In Figure 4.5, the hold-up time is plotted against the operation time in hours, according to the temperature treatment explained above for Kromasil packed column at zero concentration of modifier, and in Figure 4.6, at 10% concentration of modifier. The red line represents the medium value of the hold-up time at 40 °C from the beginning to the temperature treatment till 70 °C, in order to check possible deviations at high temperatures.

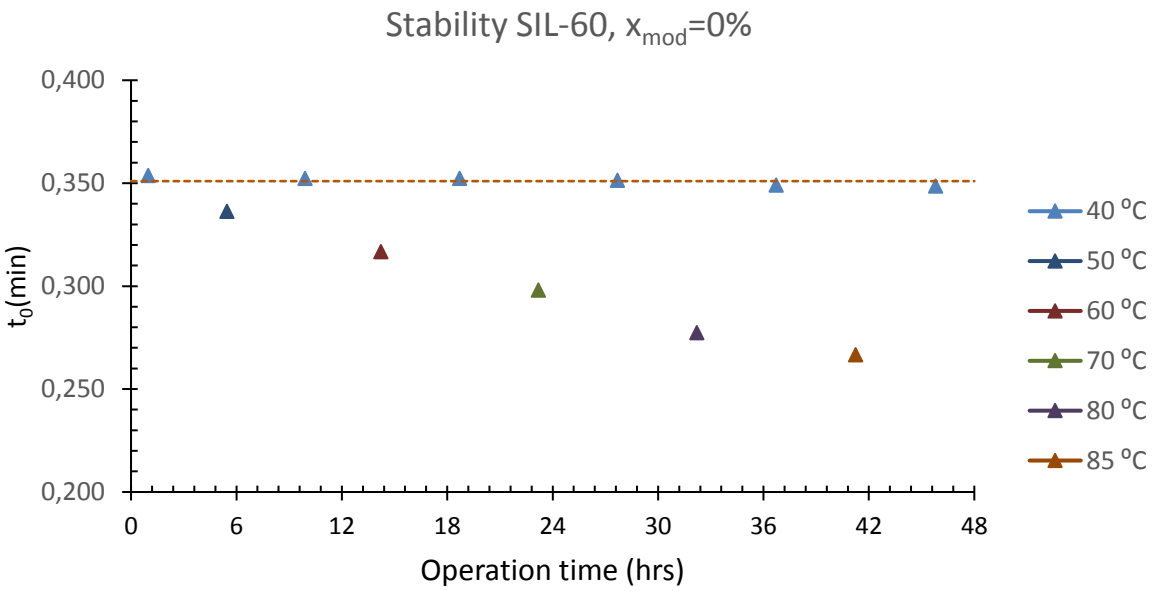


Figure 4.5 Stability of SIL-60 at different temperature treatments at 0% concentration of modifier. P=200 bar, Flow rate=2mL min^{-1}.

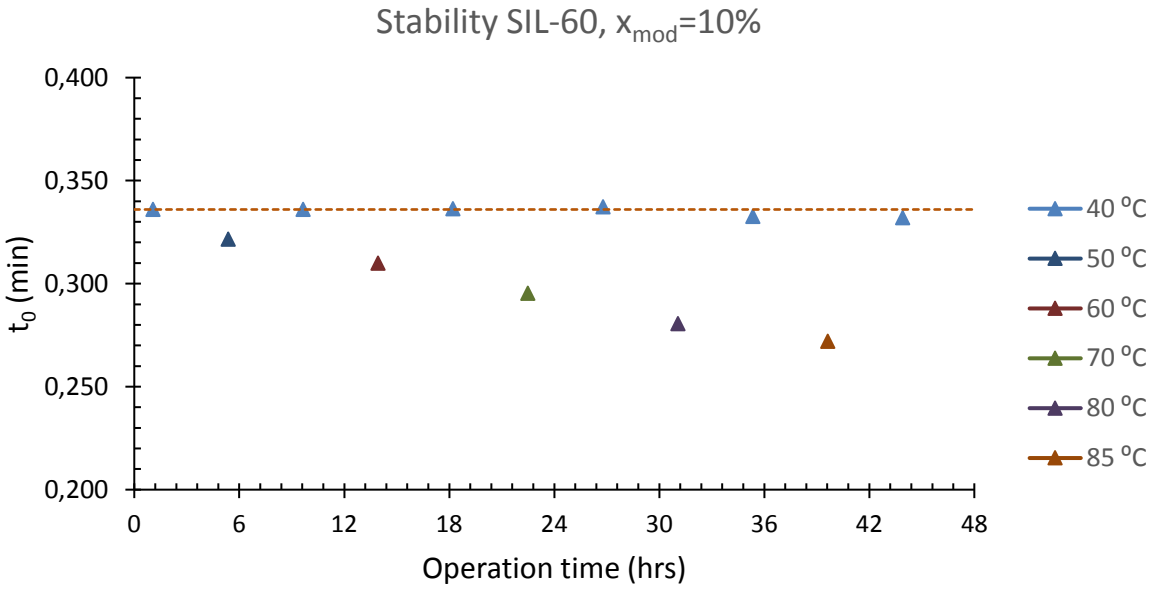


Figure 4.6 Stability of SIL-100 at different temperature treatments at 10% concentration of modifier. P=200 bar, Flow rate=2mL min^{-1}.

According to Figure 4.5, the hold-up time at 40 °C is not altered by temperature treatments, although a negligible deviation is possible to be seen after the column was

stabilized at 80 ºC. However, a bit higher deviation can be appreciated in Figure 4.6, when the mobile phase is composed by 10% methanol.

The statistical test "one sample t-test" by setting a significance level of 0.05 was used in order to test if the hold-up time measurements at 40 ºC after the 80 ºC belong to the same population as those before the 80 ºC temperature treatment. In the case of the stability test at 0% modifier concentration, the P value was 0.0005, whereas the P value obtained at 10% modifier concentration was equal to 0.0003. Both values are lower than the significance level and the difference is considered to be statistically significant to be included in the same population.

Due to this reason, the effect of the temperature in the hold-up time is considered negligible till 70 ºC, but the FTIR analysis is needed to be done in order to get a better understanding of the effect of the temperature and methanol as modifier in the mobile phase.

Silica aerogel

Since the silica aerogel-packed column needed more time to get stable than the Kromasil columns, the column was stabilized between 30 minutes and one hour before the injections after changing the column temperature. As before, in Figure 4.7, the hold-up time is plotted against the operation time in hours at zero concentration of modifier and in Figure 4.8, at 10% concentration of modifier. The red line represents the medium value of the hold-up time at 40 ºC from the beginning to the temperature treatment till 70 ºC, in order to check possible deviations from 80 ºC.

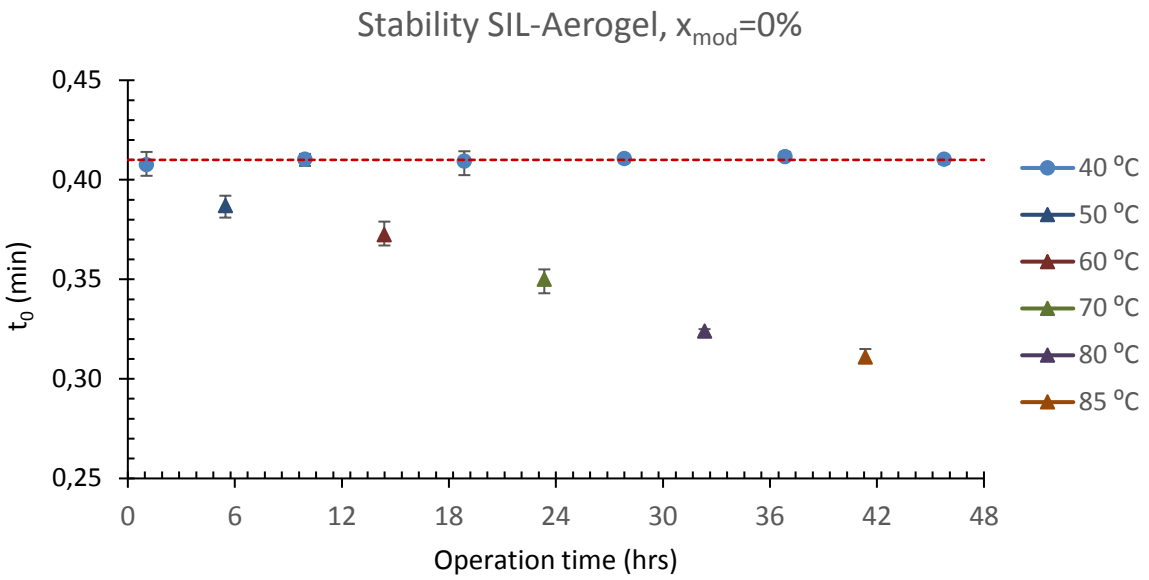


Figure 4.7 Stability of SIL-Aerogel at different temperature treatments at 0% concentration of modifier. P=200 bar, Flow rate=2mL min^{-1}.

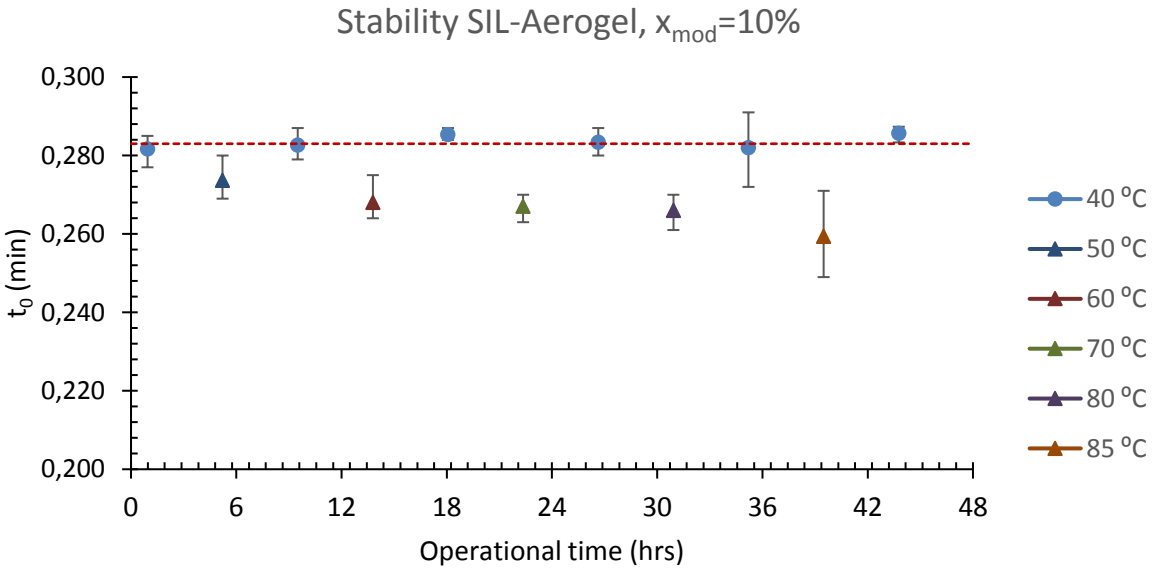


Figure 4.8 Stability of SIL-Aerogel at different temperature treatments at 10% concentration of modifier. P=200 bar, Flow rate=2mL min^{-1}.

Figures 4.7 and 4.8 show that, at a continuous running of 48 hours, the hold-up volume is also very stable in aerogel packed columns. Apart from this, the one sample t-test confirms that the hold-up time at 40 °C is not altered by high temperatures at 0% and at 10% concentration of methanol in the mobile phase (P values 0.275 and 0.766

respectively). However, to avoid a misunderstanding of the results, temperatures higher than 70 ºC will be avoided in the rest of the experiments. The FTIR analysis is not considered for the aerogel particles due to high temperatures seem not to have significant secondary effects.

On the other hand, the fact that the hold-up time changes with temperature in both Kromasil and silica aerogel particles can be due to the adsorption of the mobile phase on the stationary phase, as it was said in Chapter 3.1.3.3 "Hold-up time". During the first stabilization, only CO_2 was used as mobile phase. CO_2 adsorption on silica phases was already measured in literature (Strubinger, Song, & Parcher, 1991), resulting in an increase in adsorption when the temperature is increasing and the pressure is higher than 100 bar. As the hold-up time is a property of the stationary phase, it is possible to assume that adsorption of CO_2 can accumulate on the surface of the stationary phase in multi-layers, and behave as a part of the stationary phase, leading to a decrease of the hold-up time.

Another possible explanation would be the variations of the density due to changes in the temperature of the mobile phase. The flow rate is fixed at the pumps before the inlet of the column at 3 ºC, at which at 200 bar, the CO_2 has a density value of 1.008 g cm^{-3}. The temperature of the columns have been set from 40 ºC to 90 ºC, which means that the density changes till 0.839 g cm^{-3} at 40 ºC and till 0.537 g cm^{-3} at 90 ºC (R.B. Gupta, 2007). The change in density due to the change in temperature would lead to a change in the volumetric flow through the column, which at the same time, leads to a decrease in the hold-up time.

4.1.2.2 FTIR Analysis

Once checked that temperature does not affect the mechanical stability of the columns, possible surface silica modification with organic groups due to the addition of methanol as modifier in SFC at high temperatures were studied by the FTIR analysis.

Alcohols present an X–OH moiety. It has been reported that methanol molecules can be physisorbed at room temperature on silica by H-bonding, but they can also open strained siloxane rings, yielding methoxy groups as is schematized in Figure 4.9 (Rimola et al., 2013). This is an endothermic reaction, so that increases in temperature favor the formation of products.

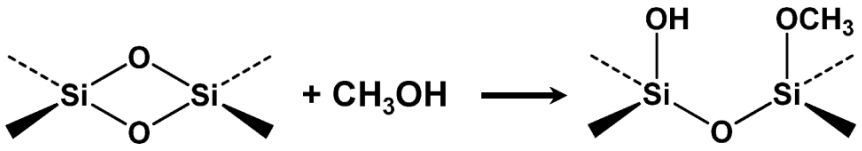


Figure 4.9 Grafting reaction of silica surfaces and methanol. Extracted from (Rimola et al., 2013)

For possible surface silica modification measurements, Kromasil 60-5-SIL was used as stationary phase in Supercritical Fluid Chromatography at high temperatures (60, 70, 80 and 90 ^{0}C). Different concentrations of modifier in the mobile phase, 10% and 20%, were also tested in order to check if higher concentrations of methanol as reactant favor the formation of the products in the equation from the Figure 4.9. A total of 8 samples were taken from the stationary phase every 30 minutes after stabilized the column at the desired temperature and concentration of modifier, at a pressure of 200 bar and a flow rate of 2 mL min^{-1}. After this, they were characterized by FTIR spectroscopy, this is, a non-destructive method which requires a minimal quantity of the sample.

In Figure 4.10, FTIR spectra of silica gel after temperature treatments is shown at two different conditions, 60 ºC at 10% and 90 ºC at 20%, in order to clarify discrepancies; whereas, the spectra of all temperatures (60, 70, 80 and 90 ^{0}C) and at various concentrations of methanol as modifier in the mobile phase can be found in Appendix.

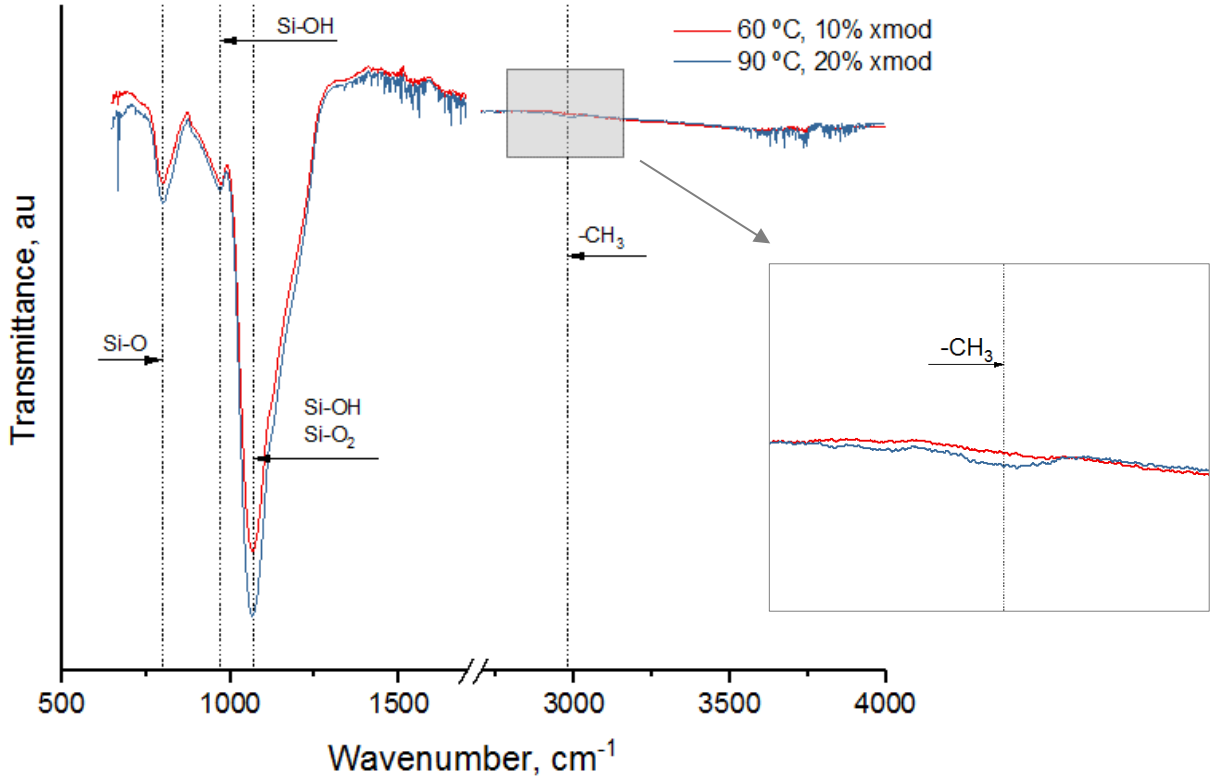


Figure 4.10 FTIR measurements at temperature treatments of 60 °C and 90 °C

According to literature, the peak at 800 cm^{-1} is ascribed to the asymmetric vibration of Si-O and the peak at 968 cm^{-1}, to the Si-OH stretching vibration. The adsorption band from 1000 to 1200 cm^{-1} have been attributed to various SiO_2 peaks and Si-OH bonding.In the spectra according to 90 ^{0}C, a small peak at 2980 cm^{-1} ascribed to -CH_3 bonds is possible to be observed, and it is associated to the Si-O-CH_3 vibrating bond (Alessi, Agnello, Buscarino, & Gelardi, 2013; Vijayalakshmi, 2005; Wörmeyer, Alnaief, & Smirnova, 2012).

As mentioned before, the modification process is favored by higher temperatures and higher methanol concentration. This is consistent with the observation from the Figure 4.10, in which the spectra of 90°C have a more obvious peak at 2980 cm^{-1} compared to the spectra of 60°C. It can be supposed that this peak is a result of a modification of the silica surface due to the use of methanol as modifier in the mobile phase. However, further investigations are need to be done in order to confirm this assumption.

4.2 LSER model

Linear Solvation Energy Relationships is applied to correlate molecular interaction parameters with retention behavior in Supercritical Fluid Chromatography. The primary goal of these studies was to provide an understanding of the role of solvent modifiers on retention for polar compounds when carbon dioxide is used as the main mobile phase component. Furthermore, it is possible to compare different stationary phases by the values of the system coefficients, which represents the contribution of different interactions to retention. Apart from this, LSER model allows to predict the retention time of other solutes that have not been evaluated yet, what can save time.

As it was said in Chapter 2.1.1 "Linear Solvation Energy Relationship's model", the retention of the solute is modelled as a function of the linear combination of intermolecular interactions, such as dispersive (v), dipole-dipole (s), π and e electrons (e) and hydrogen bonding (a and b). By modelling the retention characteristics of a varied group of solutes with a known capacity for specific intermolecular interactions, it is possible to identify the different intermolecular interactions that contribute to retention behavior in SFC.

The general equation for LSER is:

$$\ln k' = c + eE + sS + aA + bB + vV \qquad (4.1)$$

The lower letters in Equation 4.1 are the system constants used to characterize the contribution of the defined intermolecular interactions to the retention of neutral compounds. They are calculated by multiple linear regression analysis from the experimental retention factors determined for a varied group of compounds with known descriptor values (capital letters) that meet a set of chemical and statistical requirements for modeling (Poole, 2012).

4.2.1 Comparison of LSER coefficients across columns

As a preliminary study, the system constants of the four stationary phases (Kromasil 60, 100 and 300 and Silica aerogel) were compared. Injections were performed at 200 bar and 40 ºC, at a flow rate of 2 mL min^{-1}, 10% of methanol as modifier in the mobile phase and injection volume of 2 µL Pyridine and nicotinamide were not eluted by the silica aerogel, so that they are not considered in the analysis. The mass of particles packed in the columns is shown in Table 4.1, and it differs for every stationary phase:

Table 4.1 Mass of the particles of the stationary phases.

Stationary phase	Mass of particles (g)
SIL-60	0.352
SIL-100	0.389
SIL-300	0.401
SIL-Aerogel	0.094

4.2.1.1 Goodness of fit

The statistics of the regressions are shown in Table 4.2. The R^2 value of the Kromasil stationary phases regressions is larger than 0.94 and the standard error is less than 0.3. For the silica aerogel stationary phase, the R^2 value is 0.878 and the standard error is 0.35. In all cases, the regressions are considered to be good enough for the specific purpose and they demonstrated the applicability of LSER methodology.

Table 4.2 Statistics of the regressions at 40 ºC, 200 bar, 10%mod, 2mL min^{-1}

Stationary phase	**Statistics of the regressions**			
	Std dev	**R^2**	**Adj R^2**	**Multiple correlation coefficient**
SIL-60	0.236	0.969	0.952	0.984
SIL-100	0.297	0.947	0.918	0.973
SIL-300	0.174	0.974	0.960	0.987
SIL-Aerogel	0.357	0.878	0.811	0.937

In Figure 4.11, predicted ln k' obtained from the regression coefficients and the solute descriptors as in Equation 4.1 is plotted against experimental ln k' for the four stationary phases.

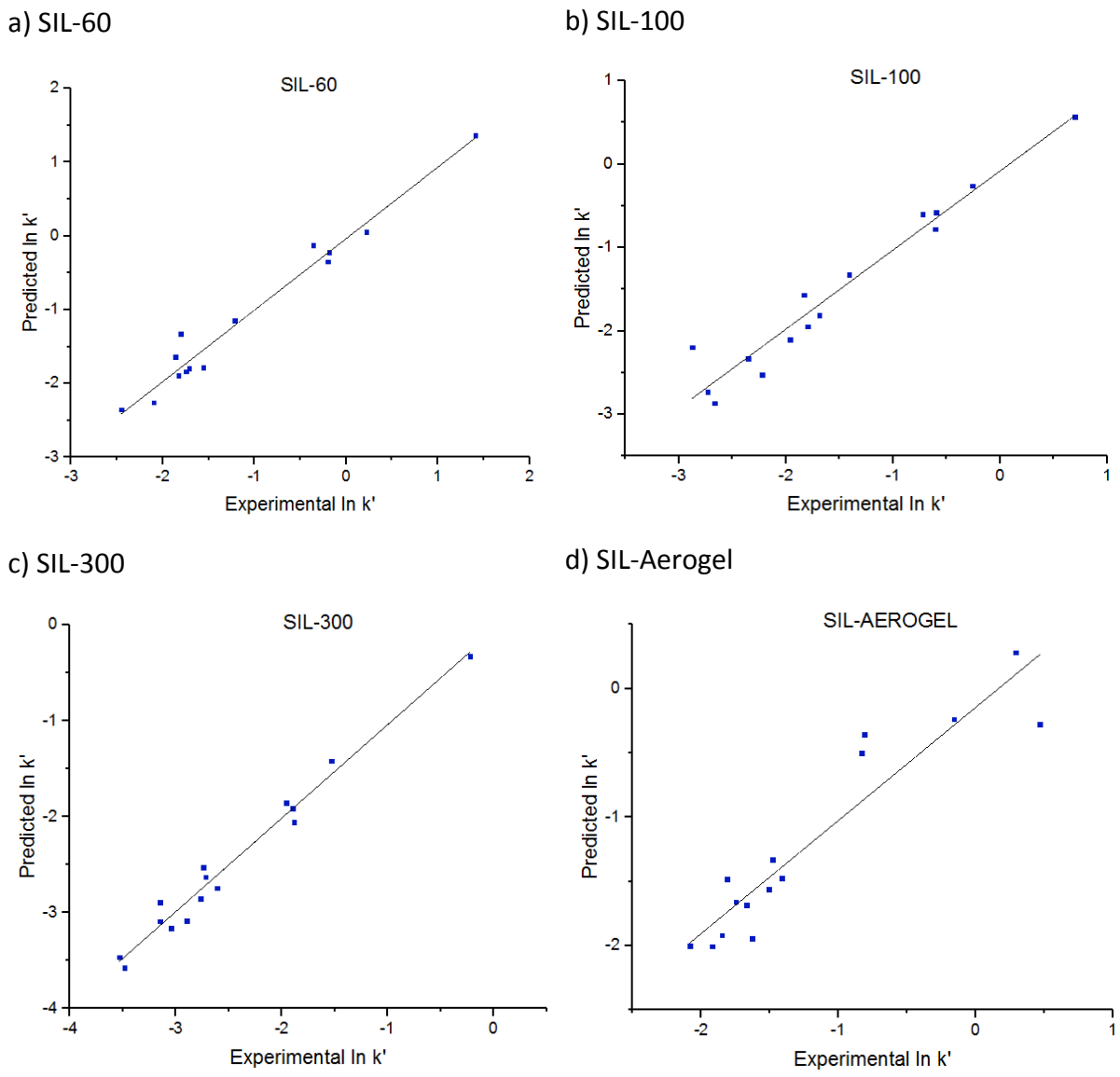


Figure 4.11 Predicted ln k' vs experimental ln k' for the following stationary phases: a) Kromasil 60-5-SIL; b) Kromasil 100-5-SIL; c) Kromasil 300-5-SIL; d) Silica Aerogel. Conditions: 40 ºC, 200 bar, 10%mod, 2mL min^{-1}

On the other hand, as it was said above, pyridine and nicotinamide were not eluted from the aerogel column during the selected experimental condition. Nicotinamide was the strongest H-bond donor/acceptor analyte, so perhaps its interactions with the stationary phase were so high that the mobile phase was not strong enough to elute it.

The fact that these two solutes are not considered makes the analyses less comprehensive, due to less situations are evaluated. However, the prediction of their retention time represented the highest error for the Kromasil particle-packed columns, so that the regression coefficient using 17 solutes instead of 15 in the predicted ln k' against experimental ln k' plot was degraded in all columns. In figure 4.12, an example is represented for SIL-60, in which the regression coefficient changed from 0.954 considering 17 to 0.985 considering 15 solutes.

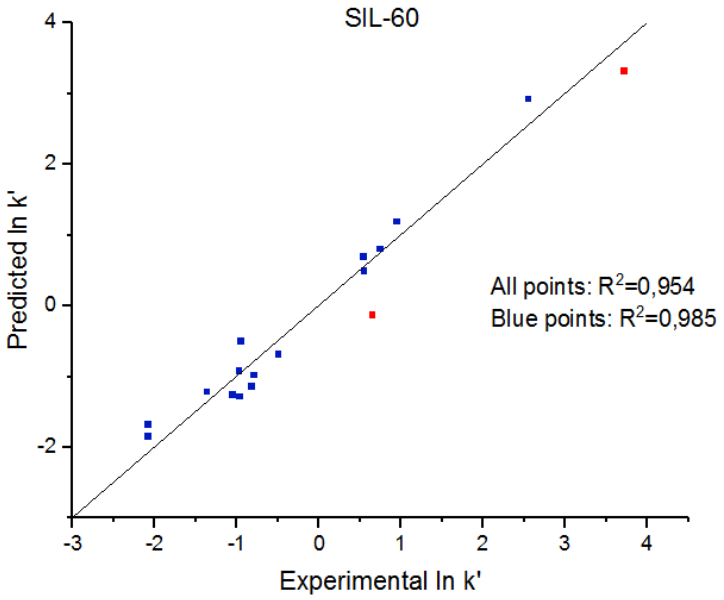


Figure 4.12 Comparison of the regression coefficients in SIL-60 using 17 solutes and 15 solutes. Conditions: 200 bar, 40 °C, 2 mL min^{-1}, 10% mod. All points: 17 solutes; Blue points: 15 solutes

4.2.1.2 LSER coefficients across columns

The systems constants obtained from the regressions are summarized in Table 4.3 and plotted in Figure 4.13. System coefficients of Kromasil particles are in accordance to literature (West & Lesellier, 2008). The error bars were taken from the standard error of the regression.

Table 4.3 Comparison of LSER coefficients across columns at 40 ºC, 200 bar, 10%mod, 2mL min^{-1}

Stationary phase	System constants					c
	e	S	a	b	v	
SIL-60	0.770	-0.036	2.477	2.813	-0.666	-2.623
SIL-100	0.550	1.166	2.303	1.775	-0.929	-3.255
SIL-300	0.282	0.837	1.507	2.050	-0.775	-3.802
SIL-Aerogel	-0.198	0.608	1.881	1.752	-0.624	-1.911

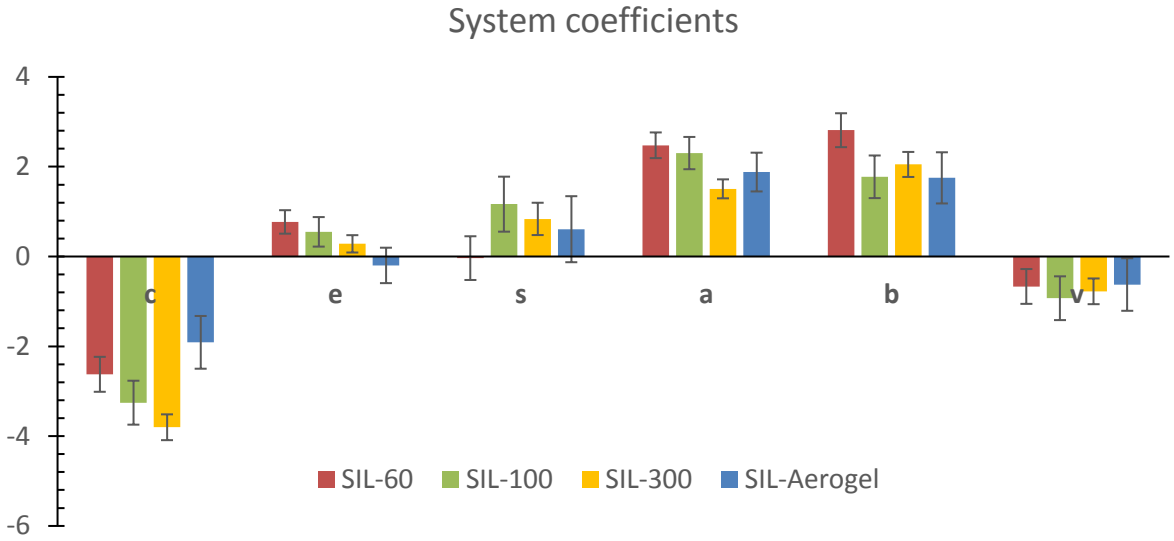


Figure 4.13 Comparison of system coefficients across different stationary silica phases. Red: SIL-60; Green: SIL-100; Yellow: SIL-300; Blue: SIL-Aerogel

The results showed that *a* and *b* coefficients are the dominating solute descriptors that affected retention. The *a* coefficient measure the difference in hydrogen bond accepting ability of the stationary phase and the mobile phase, whereas the *b* coefficient, the difference in hydrogen bond donating ability of the two phases. This can suggest that silica columns demonstrated high hydrogen bond donating and accepting ability, due to the silanol groups on the surface, which can act as H donating as well as H accepting.

$$\mathrm{a} = a_{stationary\ phase} - a_{mobile\ phase} \quad (4.2)$$

$$\mathrm{b} = b_{stationary\ phase} - b_{mobile\ phase} \quad (4.3)$$

This result can suggest that the chromatographic system, with large a and b coefficients, is highly retentive toward acidic and basic solutes, meanwhile is also highly selective for compounds with little difference in their acidic character.

All of the coefficients are mainly positive except v and c. The coefficient c is the model intercept and it is assumed to be a constant essentially related to the phase ratio contribution retention (V_s/V_m) (West & Lesellier, 2005). It differs from one stationary phase to the other, which maybe is related to the different weight of particles in the stationary phases.

The possible iterations or effects described by v are the van der Waals interaction of London type (dispersive), cavity effect or hydrophobic effect and the steric resistance to insertion in chiral cavities. The fact that this coefficient is negative means the mobile phase is dominant over the stationary phase with respect to this property, as it is defined in Equation 4.4. It also suggests that, as the v coefficient is related to the hydrophobic volume, hydrophobic moieties of compounds favor fast elution (Khater, West, & Lesellier, 2013).

$$\mathrm{v} = v_{stationary\ phase} - v_{mobile\ phase} \tag{4.4}$$

The system constants e and s are small in comparison with the others, what could mean that the interaction between the stationary phase and the mobile phase are countered. The coefficient e represents the Van der Waals interactions of London and Debye type (dispersive and dipole – induced dipole) and the π-π interactions. The coefficient s represents the van der Waals interactions of Debye and Keesom type (dipole – induced dipole and dipole – dipole).

$$\mathrm{e} = e_{stationary\ phase} - e_{mobile\ phase} \tag{4.5}$$

$$\mathrm{s} = s_{stationary\ phase} - s_{mobile\ phase} \tag{4.6}$$

4.2.1.3 Classification of solutes

As it was said above, *a* and *b* are the system coefficients with the largest value, what means that they have more influence in the retention time, followed by *v*. According to the corresponding descriptors A and B, it is possible to classify the selected solutes in "families", in which each solute will behave similarly. In this work, the solutes were classified into 4 categories as Weak H-bond acceptor analytes, strong H-bond acceptor analytes, strong H-bond donor/acceptor analytes and caffeine, which was classified as the strongest H-bond acceptor analyte. A similar classification has been already done in literature (Blackwell, Stringham, & Weckwerth, 1997).

Table 4.4 Solutes classified according to its H- bond donor/acceptor properties

No.	SOLUTES	E	S	A	B	V
		Weak Hydrogen Bond Acceptor analytes				
1	Toluene	0.601	0.52	0	0.14	0.8573
2	Benzene	0.61	0.52	0	0.14	0.7164
3	Naphthalene	1.34	0.92	0	0.2	1.0854
		Strong Hydrogen Bond Acceptor analytes				
4	Anthracene	2.29	1.34	0	0.26	1.454
5	*p*-Nitrotoluene	0.87	1.11	0	0.28	1.032
6	Nitrobenzene	0.871	1.11	0	0.28	0.8906
7	*o*-Nitrophenol	1.045	1.05	0.05	0.37	0.949
8	Anisole	0.708	0.75	0	0.29	0.916
9	Butyl benzoate	0.689	0.85	0	0.46	1.214
10	Ethyl Benzoate	0.668	0.8	0	0.46	1.4953
		Strong Hydrogen Bond Donor/Acceptor analytes				
11	*p*-Cresol	0.82	0.87	0.57	0.31	0.916
12	Benzoic Acid	0.73	0.9	0.59	0.4	0.9317
13	Phenol	0.805	0.89	0.6	0.3	0.7751
14	Vanillin	1.04	1.33	0.32	0.67	1.1313
		Strongest Hydrogen Bond Acceptor analyte				
15	Caffeine	1.5	1.6	0	1.35	1.363

Figure 4.14 identifies the solutes according to the classification mentioned above. It underlines that the general retention behavior in silica stationary phases has a similar tendency, which is, increasing polarity causing increased retention. The groups of weak and strong hydrogen bond acceptor analytes do not differ that much, which suggests that, at low polarity of the solutes, the descriptor V acquires further significance.

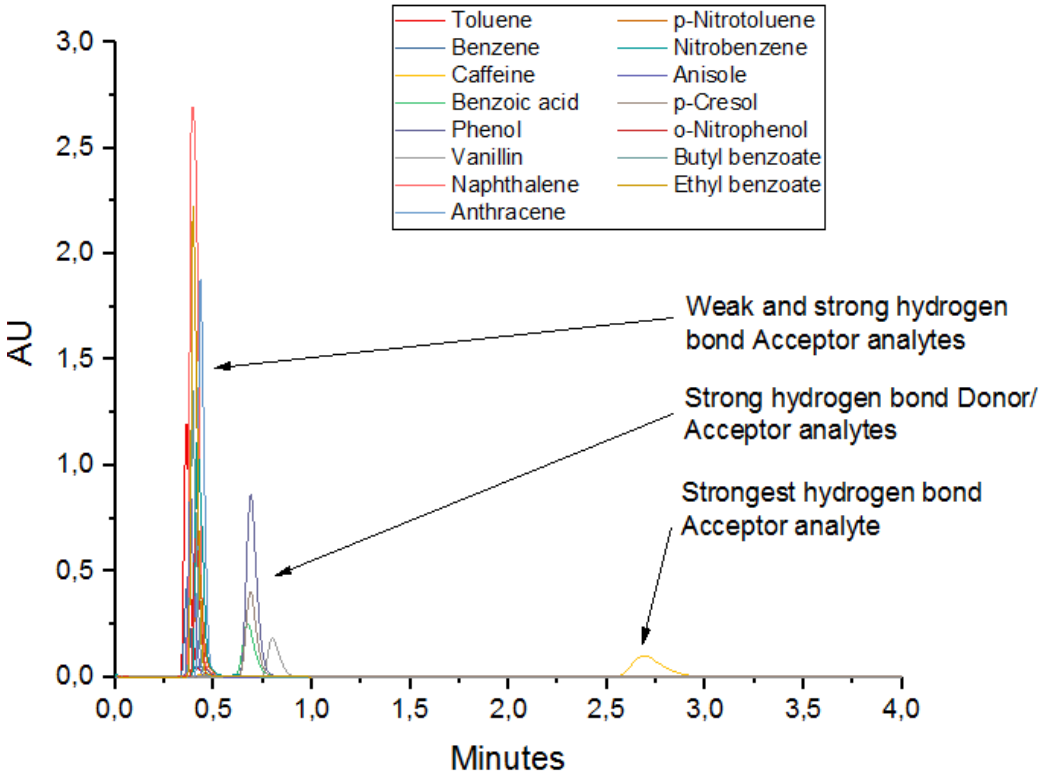


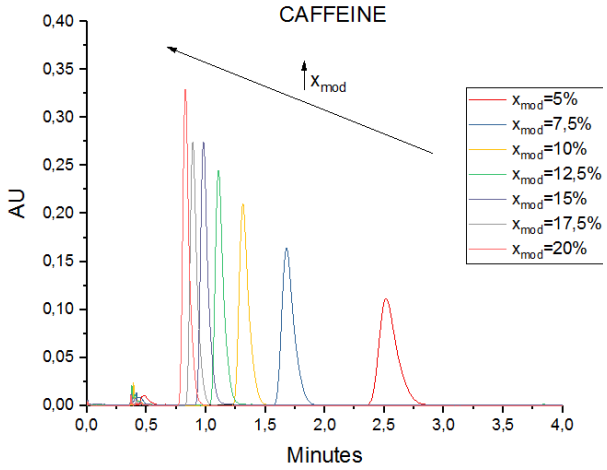
Figure 4.14 Identification of the 4 groups of solutes in the chromatogram. P=200 bar, T=45 ºC, xmod=10%, flow rate=2 mL min^{-1}. Stationary phase: SIL-60

In the following figures, how the classified solutes have a different response regarding to changes in concentration of modifier, temperature and pressure are discussed. To this aim, one solute of each class was selected: Toluene as the representative compound of the weak H-bond acceptor analytes class; Anisole, of the strong H-bond acceptor analytes class; Phenol, of the strong H-bond donor/acceptor analytes class; and caffeine as the strongest H-bond acceptor analyte. SIL-100 was the stationary phase used in all cases.

Dependence of concentration of modifier

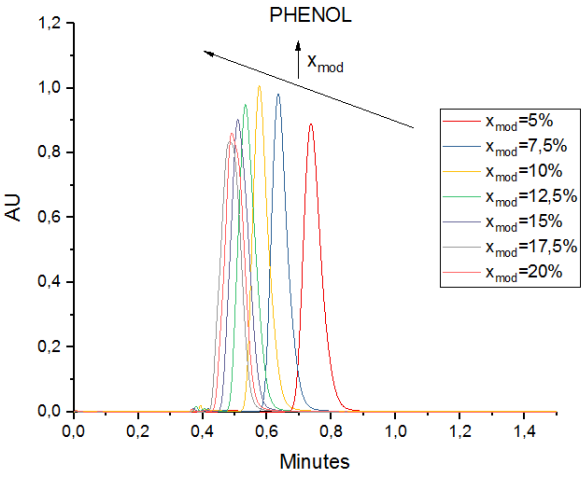
Strongest H Bond Accept. analyte

a) Caffeine



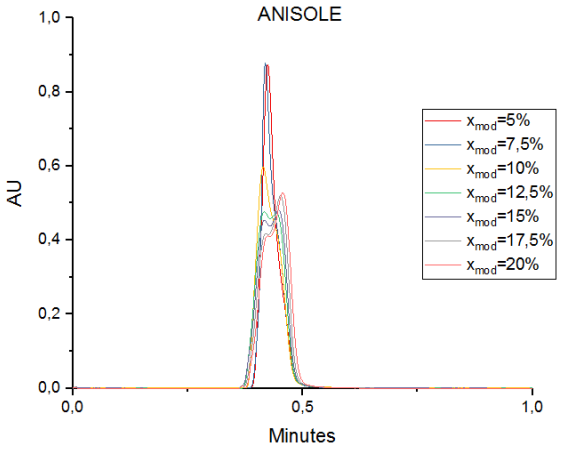
Strong H-Bond Donor/Accept. analytes

b) Phenol



Strong H-Bond Accept. analytes

c) Anisole



Weak H-Bond Accept. analytes

d) Toluene

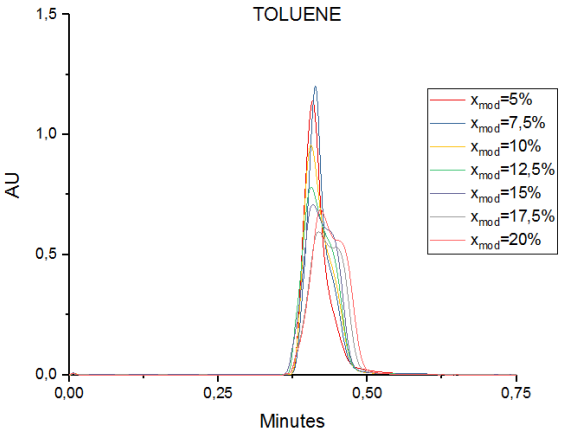
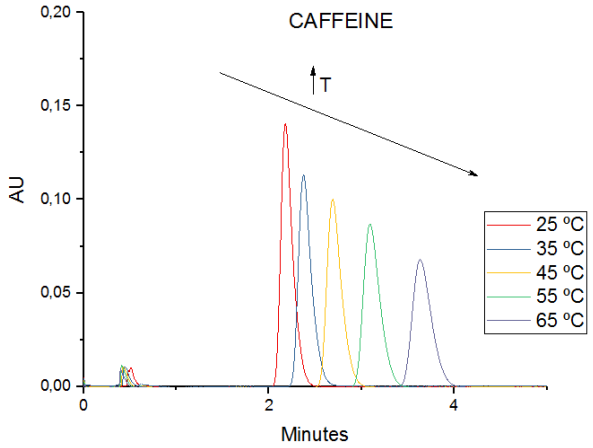


Figure 4.15 Influence of the concentration of modifier in the retention time and in the peak shape of the representative solutes of four categories: a) Caffeine as the strongest H-bond acceptor analyte; b) Phenol as strong H-bond donor/acceptor analyte; c) Anisole as strong H-bond acceptor analyte; d) Toluene as weak H-bond acceptor analyte. Stationary phase: SIL-100. Conditions: 35 ^{0}C, 200 bar and 2mL min^{-1}.

Dependence of Temperature

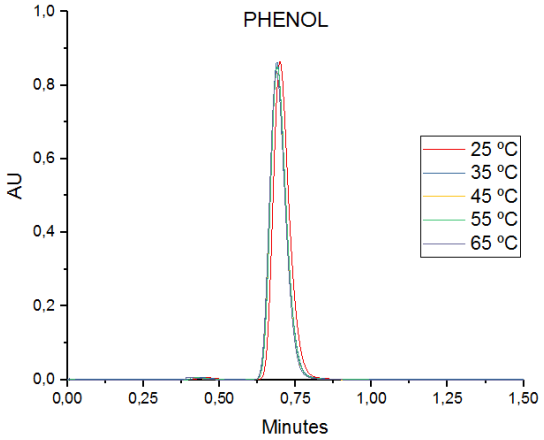
Strongest H Bond Accept. analyte

a) Caffeine



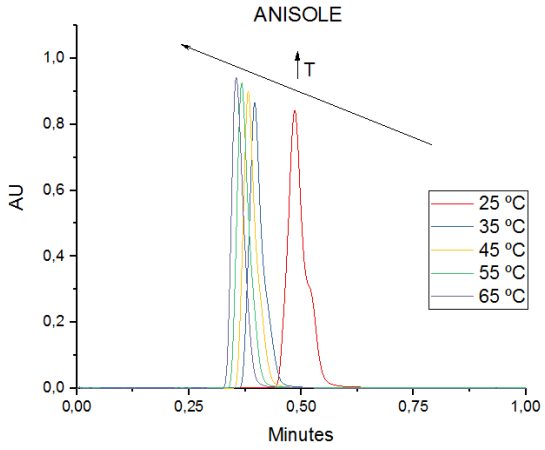
Strong H-Bond Donor/Accept. analytes

b) Phenol



Strong H-Bond Accept. analytes

c) Anisole



Weak H-Bond Accept. analytes

d) Toluene

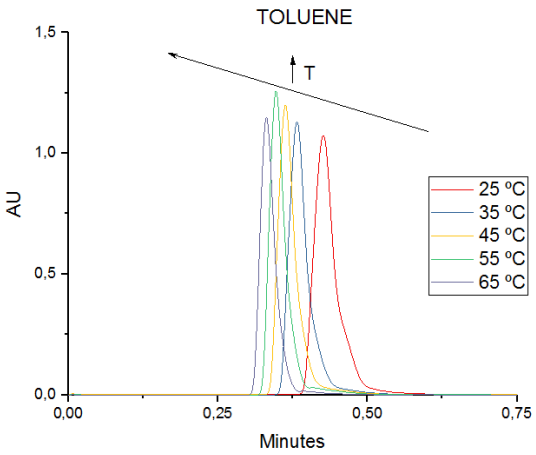
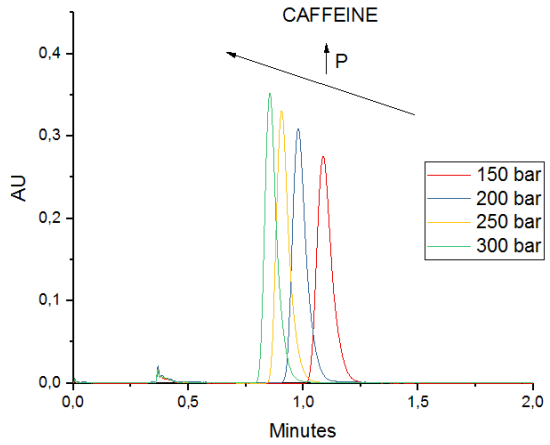


Figure 4.16 Influence of the temperature in the retention time and in the peak shape of the representative solutes of four categories: a) Caffeine as the strongest H-bond acceptor analyte; b) Phenol as strong H-bond donor/acceptor analyte; c) Anisole as strong H-bond acceptor analyte; d) Toluene as weak H-bond acceptor analyte. Stationary phase: SIL-100. Conditions: 200 bar, 5% x_{mod} and 2mL min^{-1}.

Dependence of Pressure

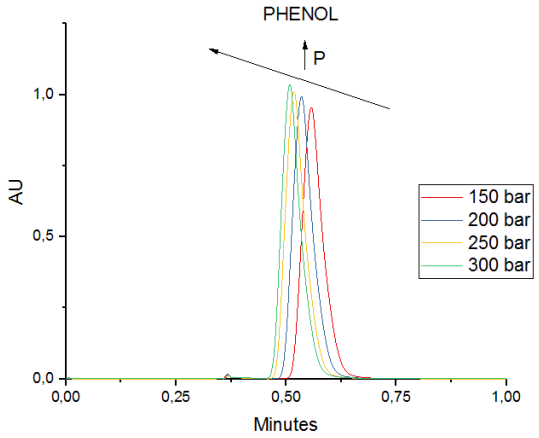
Strongest H Bond Accept. analyte

a) Caffeine



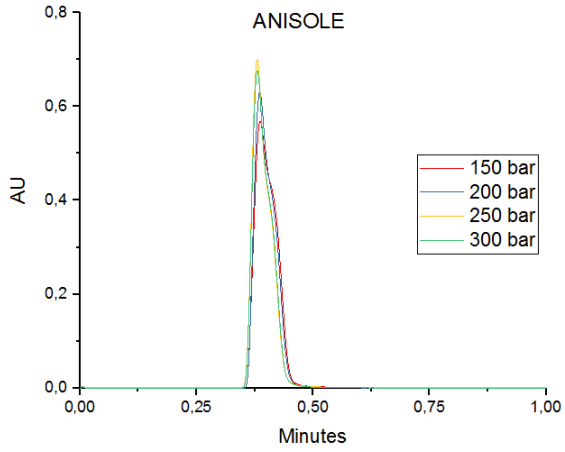
Strong H-Bond Donor/Accept. analytes

b) Phenol



Strong H-Bond Accept. analytes

c) Anisole



Weak H-Bond Accept. analytes

d) Toluene

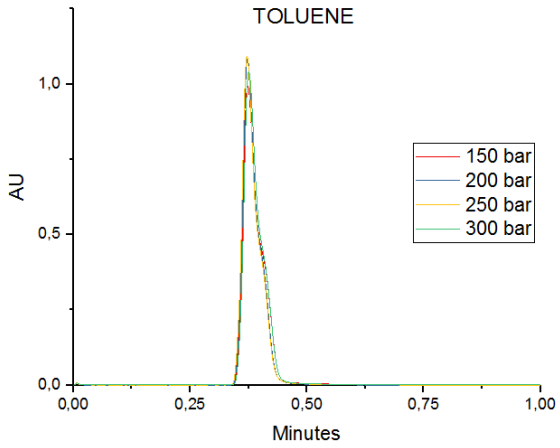


Figure 4.17 Influence of the pressure in the retention time and in the peak shape of the representative solutes of four categories: a) Caffeine as the strongest H-bond acceptor analyte; b) Phenol as strong H-bond donor/acceptor analyte; c) Anisole as strong H-bond acceptor analyte; d) Toluene as weak H-bond acceptor analyte. Stationary phase: SIL-100. Conditions: 35 ^{0}C, 10% x_{mod}, and a flow rate of 2mL min^{-1}.

Concentration of modifier was evaluated in Figures 4.15 a), b), c) and d) at 35 ^{0}C, 200 bar and flow rate of 2mL min^{-1}. Temperature is evaluated in Figures 4.16 a), b), c) and

d) at 200 bar, 5% concentration of modifier and 2mL min^{-1}. Pressure is evaluated in Figures 4.17 a), b), c) and d) at 35 ^{0}C, 10% concentration of modifier, and a flow rate of 2mL min^{-1}.

In Figure 4.15 a), b), c) and d), the influence of the concentration of modifier in the stationary phase of the representative solutes mentioned above is represented. It is shown that the strongest H-bond acceptor analyte, caffeine, is the solute most influenced by the concentration of modifier and increasing in the concentration leads to a shorter retention time and narrower peak shapes. Phenol is also affected by the concentration of modifier in the same way than caffeine but in less proportion. The retention times of anisole and toluene, the solutes that do not have H-bond donor acceptor properties, are not influenced by the concentration of modifier, but it has negative effects in their peak shape. In fact, two peaks appeared at high concentrations. This could be explained due to the adsorption of methanol in the stationary phase, which is higher at high concentrations of modifier, resulting in an unexpected retention.

In Figure 4.16 a), b), c) and d), the influence of the temperature in the stationary phase of the representative solutes mentioned above is represented. The retention time and the peak shape of caffeine is favored by lower temperatures. As it will be described in Chapter 4.3.1 "Overview of the mixed retention model", it is considered that the modifier is adsorbed on the surface of the stationary phase and it plays a role as another kind of active site. Its adsorption is less at high temperatures. As caffeine is a polar compound, it is easily retained by the polar stationary phase, and the adsorption of the modifier on the mobile phase decrease its retention. The response of phenol is almost not affected by changes in temperature, whereas the response of anisole and toluene is the opposite as the one of caffeine. As they are nonpolar solutes, they do not have affinity for the stationary phase, so that their adsorption is not favor by high temperatures. Also, the peak shape is influenced by the temperature, which could be due to, at lower temperature, the adsorption of modifier on the stationary phase is

higher what leads to a retention of the methanol. This means that the adsorption of non polar compounds and molecules of modifier is not competitive, which is also consistent with the result obtained from the Figure 4.15.

In Figure 4.17 a), b), c) and d), the influence of the pressure in the stationary phase of the representative solutes mentioned above is represented. Caffeine is more retained by the stationary phase at low pressure than at high pressures, and the peak shape improves a bit. This can be explained by the solvation power of the mobile phase. The more the pressure, the more density of the mobile phase, so that the solvation power increases and the solute elutes faster. The same response of phenol can be appreciated, whereas anisole and toluene are almost not affected by changes in pressure.

The main purpose of this classification was to show how the retention time is influenced by the properties of a concrete solute. In such a way, it can provide a better understanding about the retention and what would be the most influencing parameters depending on the properties of the solute, in order to get a good selectivity, appropriate retention times and a proper peak shape. It is also a valuable result in order to develop a proper model like the Mixed Retention Model explained below in Chapter 4.3.

This classification would be used as a guide as well if the aim is to predict the retention time of a solute in a silica stationary phase. If the solute is quite polar, the low temperatures, high pressures and a high concentration of modifier favors the elution. On the other hand, if the solute is not polar, high temperatures would favor the elution whereas pressure does not affect it and high concentrations of modifier would lead to a distortion in the peak shape.

4.2.1.4 Classification of stationary phases

In this section, stationary phases were classified according to the retention time and the peak shape of the solutes under the same conditions. To this aim, the same representative solutes of each class were tested: Caffeine as the strongest H-bond

acceptor analyte (Figure 4.18), phenol as strong H-bond donor/acceptor analyte (Figure 4.19) and toluene as weak H-bond acceptor analyte (Figure 4.20). Since in the previous section it was checked that anisole has almost the same response as toluene, it was not evaluated in this analysis. The injections of the solutes were carried out at 200 bar, 40 ºC, 10% concentration of modifier and 2 mL min^{-1}.

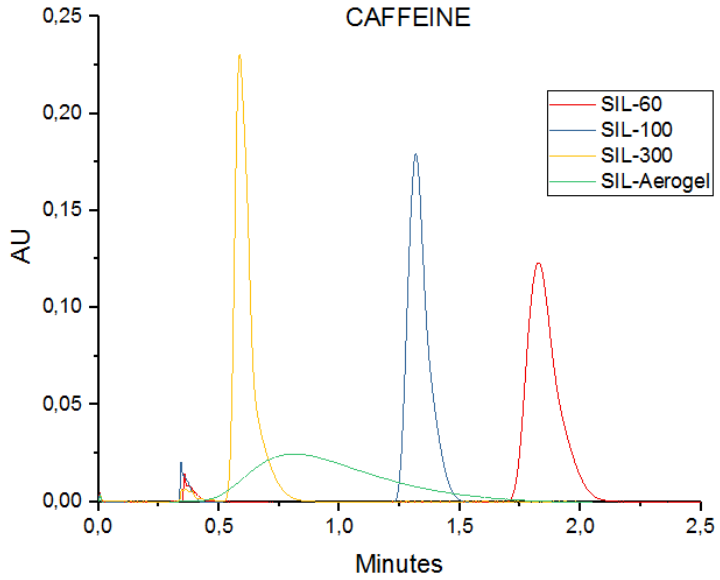


Figure 4.18 Chromatogram of Caffeine in the different stationary phases. Conditions: 40 ºC, 200 bar, x_{mod}=10%, 2mL min^{-1}

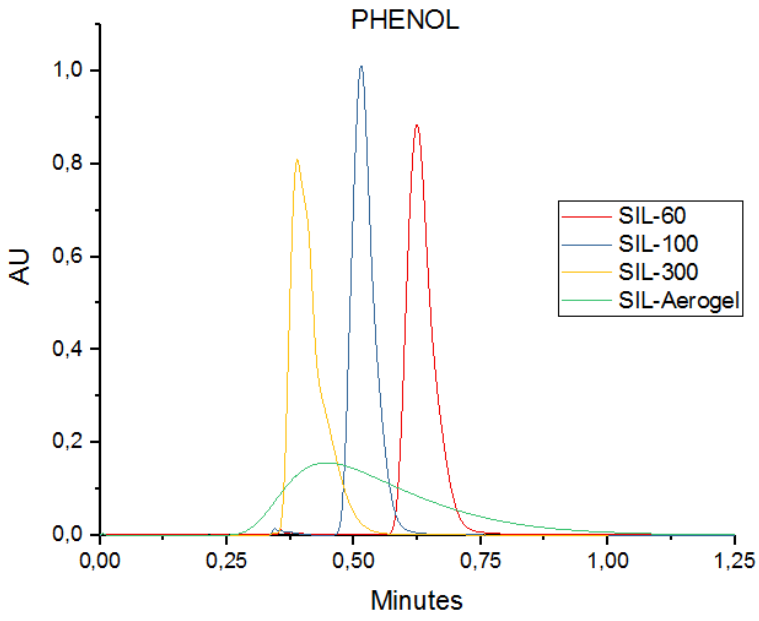


Figure 4.19 Chromatogram of Phenol in the different stationary phases. Conditions: 40 ºC, 200 bar, x_{mod}=10%, 2mL min^{-1}

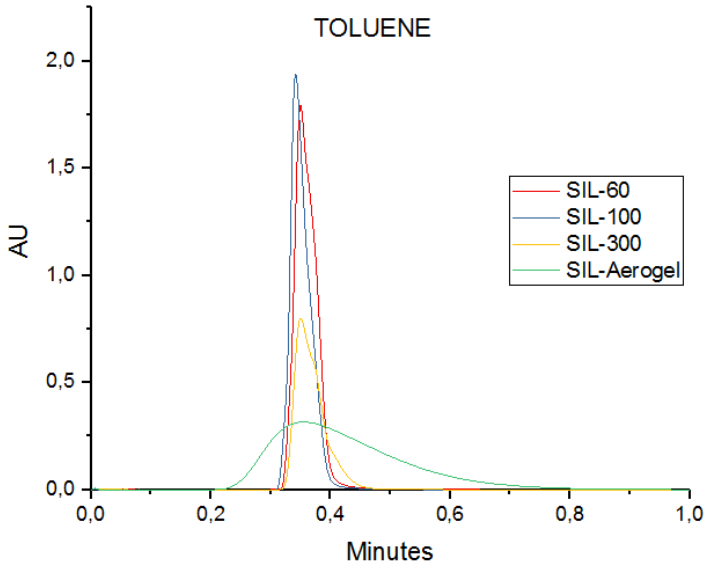


Figure 4.20 Chromatogram of Toluene in the different stationary phases. Conditions: 40 °C, 200 bar, x_{mod}=10%, 2mL min^{-1}

It is shown that the different solutes have different response regarding to the stationary phase. Caffeine is the solute that present the major difference in retention time among the stationary phases, the retention time of toluene is almost not affected by the stationary phase, whereas the response of phenol is in between of both. Respecting to the Kromasil stationary phases, all the peaks have a proper peak shape for all solutes. The peak shape of the solutes in the aerogel column is wider and tailing, but it is considered acceptable for the analysis. The tailing can be due to the irregular shape of the aerogel powder.

In Figure 4.18 and as it was said above, remarkable differences can be observed in the retention time of caffeine between the three Kromasil stationary phases, which only differ in the porous size and, in consequence, in the specific surface area. Kromasil 60-5-SIL has the highest value of specific surface area, 540 m^2 g^{-1}, and it is the stationary phase in which caffeine is more retained. On the other hand, the surface area of Kromasil 300-5-SIL is 110 m^2 g^{-1}, and in this case, caffeine is eluted faster. In such a way, it is reasonable to think that the surface area gives an idea of the amount of active sites on the stationary phase. The mass of particles in the aerogel column differs greatly

from the mass of particles of the Kromasil stationary phases. In order to compare them, the total surface area was calculated by the following equation:

$$\text{Active sites} \propto \text{total surface area } (\text{m}^2) = particles\ (g) \cdot As\ (m^2 g^{-1}) \qquad (4.7)$$

The values of the mass of particles, surface area and active sites are summarized in Table 4.5 for the different stationary phases.

Table 4.5 Estimation of the active sites volume of each stationary phase by means of the mass of the particles and the surface area, As

Stationary phase	Mass particles, g	As, $m^2\ g^{-1}$	Actives sites, m^2
SIL-60	0.3515	540	189.81
SIL-100	0.3885	320	124.32
SIL-300	0.4046	110	44.51
SIL-Aerogel	0.0939	858	80.56

The active sites are in concordance with the retention time described above: SIL-60 presents the highest volume of active sites and the largest retention time, SIL-300 presents the lowest value of active sites and the shortest retention time, whereas the value of active sites of SIL-Aerogel is in between SIL-100 and SIL-300, so that the retention time.

4.2.2 LSER coefficients regarding to concentration of modifier, temperature and pressure

As it was said, modifiers can influence the qualities of a separation in several ways: (1) the modifier can alter the density and solvating power of the mobile phase; (2) the modifier can block active sites on the stationary phase and inhibit adsorption; (3) adsorbed modifier can act as a component of the stationary phase; (4) adsorbed modifier can increase the volume of the stationary phase leading to a change in the column phase ratio; and (5) the modifier may selectively solvate polar compounds in the mobile phase with the formation of clusters with different distribution properties.

The combination of these factors can change retention in an unpredictable manner while three of these factors result in a change in stationary phase properties with a strong dependence on mobile phase composition, column temperature, and density drop along the column, related to the pressure (Poole, 2012).

In this section, The LSER parameters are compared and analyzed for different columns at methanol modifier concentration levels from 5% to 20%, in increments of 2.5%, at temperature levels from 25 to 60 ºC in increments of 5 ºC and at pressure levels from 150 to 300 bar in increments of 50 bar. The aim of this analysis is to have a better understanding about how the pressure, temperature and concentration of modifier affect the interactions between the stationary phase, solute and mobile phase.

4.2.2.1 Concentration of modifier

As it was said in Chapter 2.2.2 "Importance of the mobile phase", the elution strength of carbon dioxide is generally too weak to elute polar compounds and, in order to increase its solvent strength, methanol is added. In this section, LSER regression coefficients are evaluated in the different stationary phases when amounts of methanol from 5% to 20% (v/v) are added in increments of 2.5%.

In Figure 4.21, the system coefficients of SIL-60 stationary phase are represented at concentrations of modifiers from. The temperature was 40 ºC, the back pressure was 200 bar and the flow rate of the mobile phase was 2 mL min^{-1}.

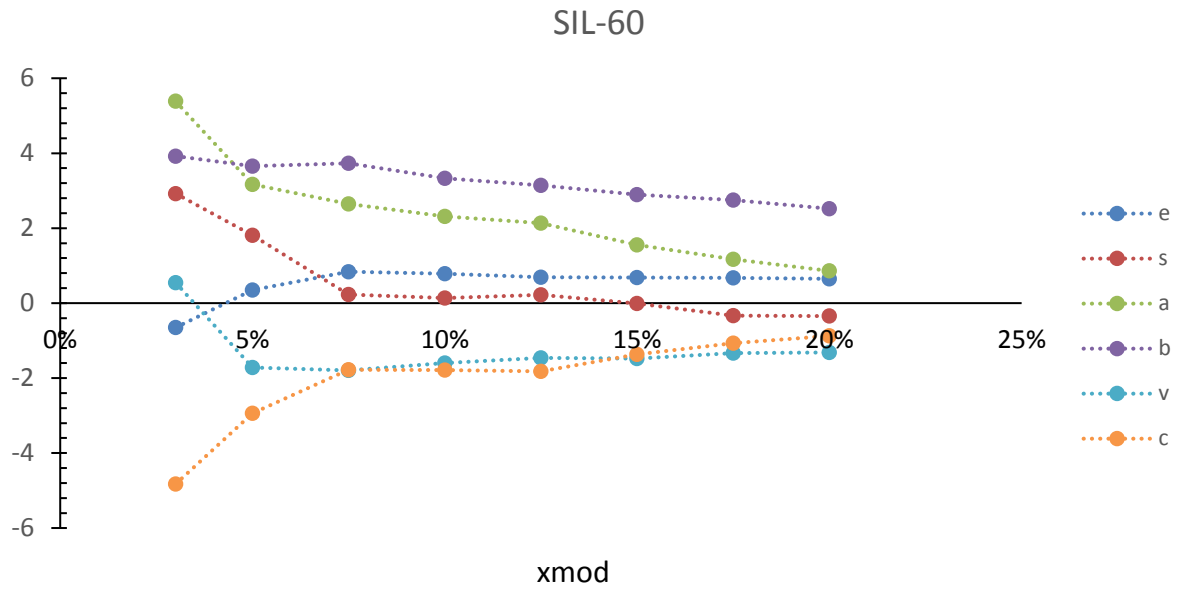


Figure 4.21 System coefficients of LSER regression in SIL-60 at 200 bar, 40 °C and 2mL min^{-1}.

Figure 4.22 Medium value of the regression coefficients from 5% to 20% evaluated in SIL-60, SIL-100, SIL-300 and SIL-Aerogel. Conditions: 40 °C, 200 bar, 2mL min^{-1}.

In Figure 4.22, the medium value of the regression coefficients from 5% to 20% was taken and they were evaluated across columns. The error bars represent the difference

in the values of the regression coefficients from 5% to 20%. It is shown that a and b are the strongest interaction that affect most the retention time in all cases and they will be evaluated separately in Figures 2.23 and 2.24.

a term

The a term is related to the H bond donating ability (HBD) of the solute. It describes the difference in H-bond accepting ability (HBA) between the mobile and stationary phase. a = $a_{stationary} - a_{mobile}$

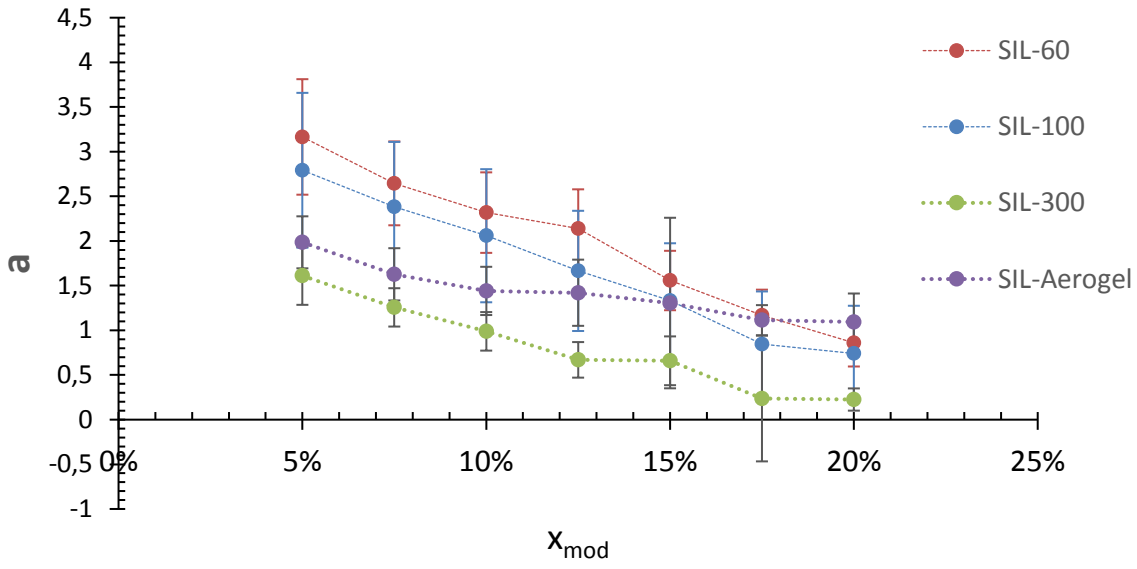


Figure 4.23 Comparison of the a system coefficient among columns at different concentrations of modifier in the stationary phase

It shows higher values than s or e but varies strongly with the modifier percentage in all stationary phases, as it is shown in Figure 4.23. When increasing the percentage of modifier in the mobile phase, a_{mobile} (representing the basic character of the mobile phase) increases, leading to a decrease of a.

b term

It describes the difference in H-bond donor ability (HDA) between the mobile and stationary phase. b = $b_{stationary} - b_{mobile}$

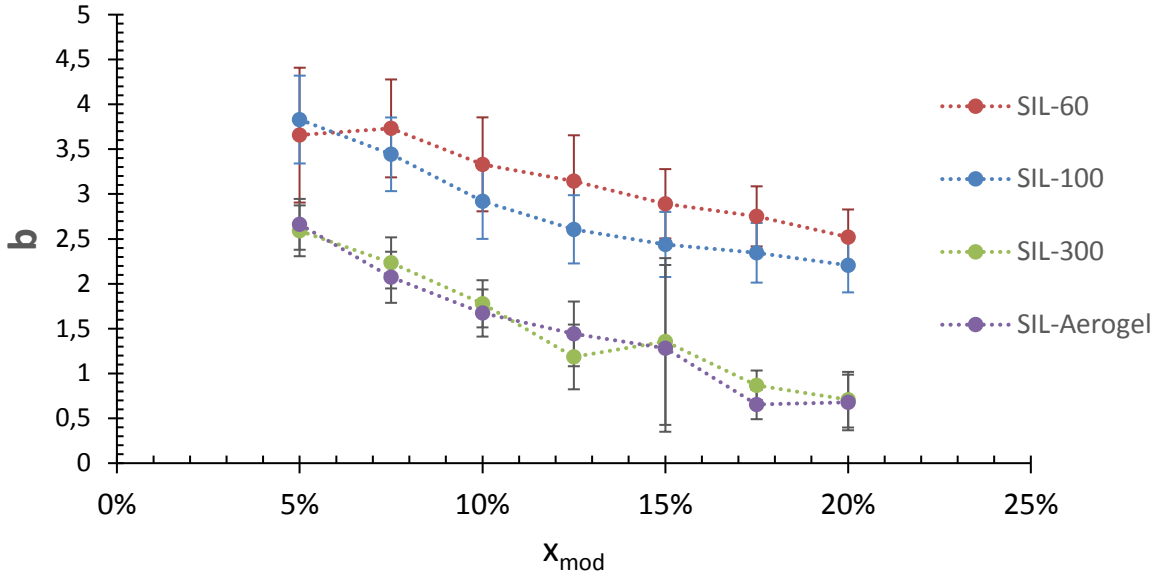


Figure 4.24 Comparison of the b system coefficient among columns at different concentrations of modifier in the stationary phase

The b coefficient is large and positive but it decreases when the percentage of modifier is increased in all the stationary phases, as it is seen in Figure 4.24. This decrease is due to the dynamic coating of the free silanols on the surface of the silica gel packing material (Pyo et al., 1996).

c term

The c term is related to the phase ratio contribution retention (V_s/V_m), and it increases between 5% and 20% modifier percentage. It means that the volume of the stationary phase increases more than the volume of the mobile phase, probably due to the adsorption of the mobile phase. The adsorption of CO_2 with methanol on a stationary phase is cooperative not competitive, so the total amount of adsorbed mobile phase is increased by the addition of modifier (Pyo et al., 1996).

e term

The excess molar refraction term is related to charge transfer, reflecting the interactions between the electronic excess of the solute (π and n electrons) and the surface of silica gel or the mobile phase. $e = e_{stationary} - e_{mobile}$

The *e* coefficient does not vary significantly. This means that, the more percentage of modifier in the mobile phase, the variations of charge-transfer interactions between the solutes and the mobile phase and the solute and the stationary phase compensate. However, lowest values of *e* can be noticed at low modifier percentages in Figure 4.21, which *e* can only be due to low charge-transfer interactions between the solute and the stationary phase when silica gel is covered with methanol (West & Lesellier, 2005).

v term

The v term represents the difference in dispersion interactions between the solute and the stationary phase and also the interactions between the solute and the mobile phase.
$\mathrm{v} = v_{stationary} - v_{mobile}$

The fact that is negative means that the mobile phase is dominant over the stationary phase with respect to this property. It increases slightly when the percentage of modifier is increased (see Figure 4.21). The polar modifier addition mainly increases the mobile phase polarity and decreases the dispersion interaction between the solute and the mobile phase, so $\mathrm{v}_{\mathrm{mobile}}$ decreases (West & Lesellier, 2005).

s term

It can be defined as: $\mathrm{s} = s_{stationary} - s_{mobile}$. $\mathrm{s}_{\mathrm{stationary}}$ represents a measure of the strength of dipolarity-polarizability interactions between the solute and the stationary phase and $\mathrm{s}_{\mathrm{mobile}}$ represents the same interactions between the solute and the mobile phase.

As the concentration of modifier increases, the dipole-dipole interaction between the solute and mobile phase should increase, leading to an increase in the $\mathrm{s}_{\mathrm{mobile}}$ coefficient. A decrease in the s coefficient could also be explained by the dissolution of methanol and carbon dioxide in the stationary phase, which results in dilution of the stationary phase (Pyo et al., 1996).

4.2.2.2 Temperature

LSER regression coefficients were evaluated at different temperatures, from 25 ºC to 60 ºC in Figure 4.25 using SIL-300 as stationary phase. Results show that a and b represent the strongest interaction that affect most the retention time in all cases; s and e have the lowest influence, whereas v and c are negative.

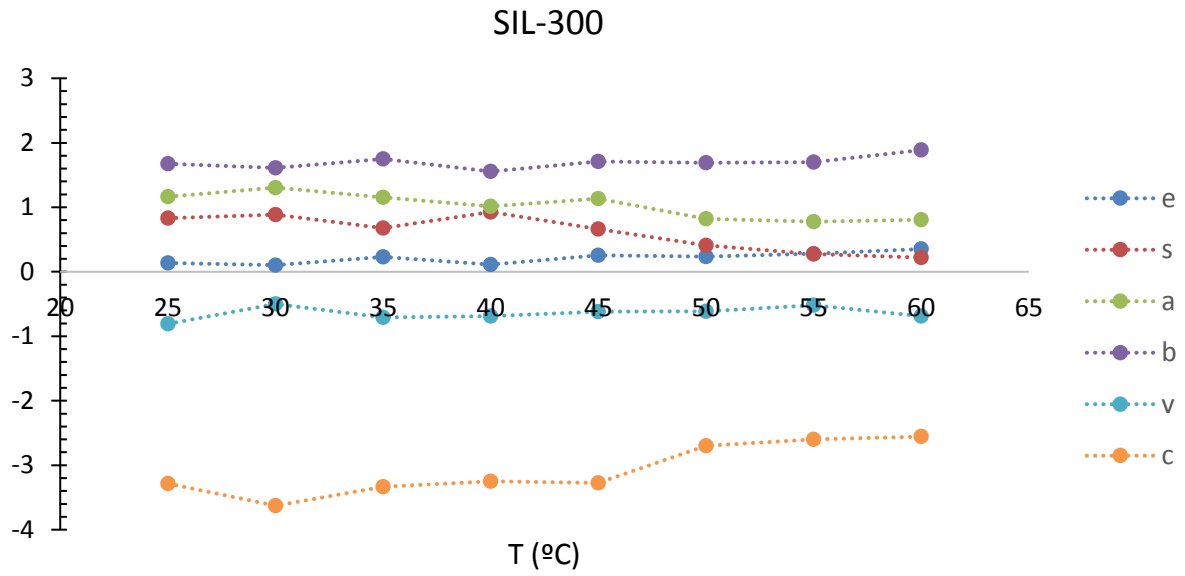


Figure 4.25 LSER regression coefficients evaluated at temperatures from 25 to 60 ºC using SIL-300. Conditions: 10% xmod, 200 bar, 2mL min^{-1}.

It is observed that the temperature does not have a significant effect in the values of the system coefficients, what is somewhat unexpected. As it was seen in the previous study, depending of the considered solute, temperature has or positive or negative or not influence in the retention time. The fact that different natures of solutes are evaluated, the overall study of the different responses of solutes can lead to a counteraction of the effect of temperature.

In Figure 4.26, the medium value of the regression coefficients from 25 ºC to 60 ºC was taken and they were evaluated across columns. The error bars represent the difference in the values of the regression coefficients from 25 ºC to 60 ºC. It is shown that a and b are the strongest interactions that affect most the retention time in all cases.

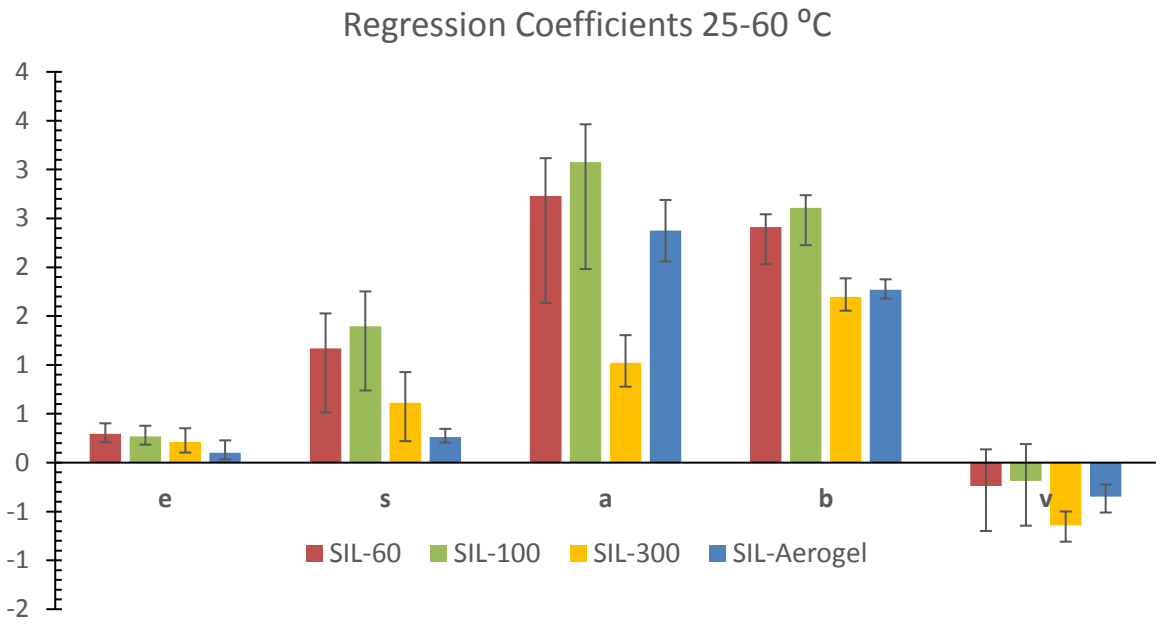


Figure 4.26 Medium value of the regression coefficients from 25 to 60 °C evaluated in SIL-60, SIL-100, SIL-300 and SIL-Aerogel. Conditions: 10% xmod, 200 bar, 2mL min^{-1}.

4.2.2.3 Pressure

LSER regression coefficients were evaluated at different pressures, from 150 to 300 bar in Figure 4.27 using SIL-Aerogel as stationary phase. Results show that a and b represent the strongest interaction that affect most the retention time in all cases; s and e have the lowest influence, whereas v and c are negative.

It is observed that the pressure does not have a significant influence in the values of the system coefficients. According to the previous study, only retention of polar solutes is affected by the effect of pressure, but in less proportion than by the effect of temperature. In this case, the no effect of pressure on the values of the regression coefficients could be explained due to the fact that there are evaluated more nonpolar solutes than polar, so the contribution of these is not enough to have significant effects in the regression coefficients.

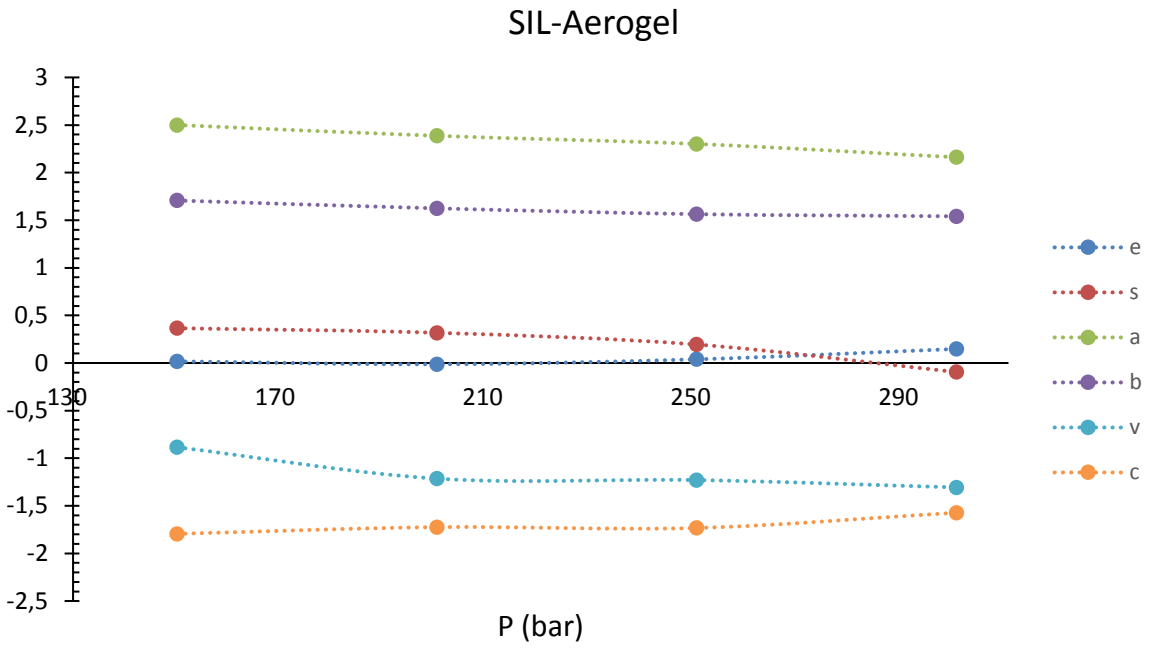


Figure 4.27 LSER regression coefficients evaluated at pressures from 150 to 300 bar using SIL-Aerogel. Conditions: 10% xmod, 35 °C, 2mL min^{-1}.

In Figure 4.28, the medium value of the regression coefficients from 120 to 300 bar was taken and they were evaluated across columns. The error bars represent the difference in the values of the regression coefficients from 150 to 300 bar. It is shown that a and b are the strongest interaction that affect most the retention time in all cases.

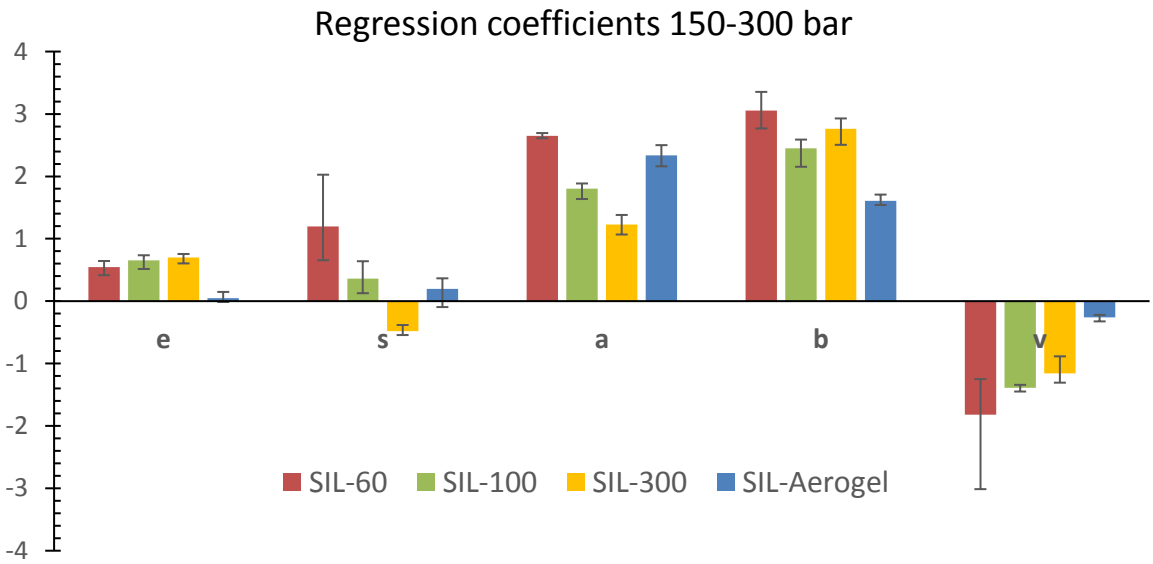


Figure 4.28 Medium value of the regression coefficients from 150 to 300 bar evaluated in SIL-60, SIL-100, SIL-300 and SIL-Aerogel. Conditions: 10% xmod, 35 °C, 2mL min^{-1}.

4.3 Mixed Retention Model

4.3.1 Adaptation of the Mixed Retention Model

In Chapter 2.2.3.1 “Effect of modifiers in pSFC”, it was described that molecules of modifier can partition into the stationary phase and can be adsorbed on the active sites of the packing, which leads to changes in the properties of the stationary phase.

In this chapter, it is suggested that the adsorbed modifier molecules still contribute to the retention of a solute, which leads to the assumption that there are two kinds of active sites. In such a way, the retention of a solute a is due to a mixed retention mechanism between the adsorption of the solute on the free silanol groups that are accessible for solute molecules and the adsorption on the molecules of modifier that are adsorbed on the silanol groups. In Figure 4.29, the two types of interactions between the solute, modifier of the mobile phase and the stationary phase are represented.

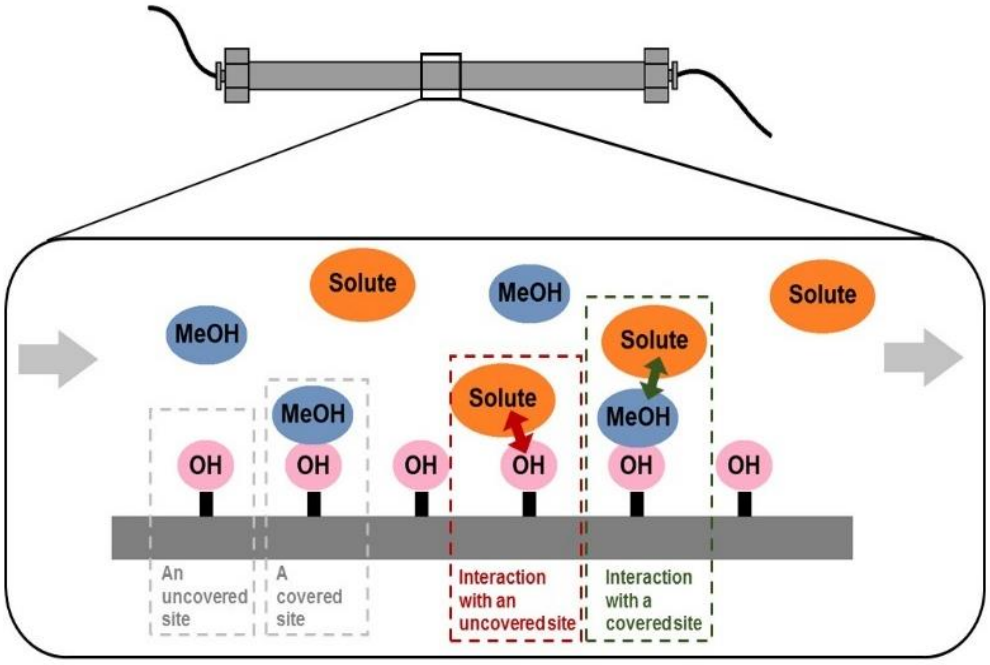


Figure 4.29 Two types of interactions between the solute, modifier and stationary phase.

The observed capacity factor now can be written as:

$$k_{obs} = k_{sil} + k_{mod} \tag{4.8}$$

where k_{obs} is the experimentally observed capacity factor, k_{sil} is the capacity factor due to the interaction of the solute with the silanol groups and k_{mod} is the capacity factor due to the interaction of the solute with the modifier adsorbed on the stationary phase. The fractional occupancy of the modifier on adsorption sites is defined by θ as:

$$\theta = \frac{N_s}{N_{max}} \tag{4.9}$$

where N_s is the number of modifier molecules adsorbed on the mobile phase and N_{max} represents the maximum number of modifier molecules that can be adsorbed on the surface. In such a way, the Equation 4.8 can now be written as:

$$k_{obs} = k_0(1 - \theta) + k_c\theta \tag{4.10}$$

where k_0 is the contribution to the capacity factor from uncovered active sites, in other words, the contribution of the silanols to retention at zero modifier concentration, k_c represents the contribution to the capacity factor from active sites covered with the modifier. It is not possible to obtain the value of k_0 experimentally, due to a minimal concertation of modifier in the mobile phase is needed to elute most of the solutes.

The retention is considered as a partitioning mechanism, which can be described using an adsorption isotherm model, this is, a plot of the concentration of a solute on a surface as a function of its concentration in the mobile phase (Enmark et al., 2013). In Figure 4.30, the meaning of the parameters k_0 and k_c is represented in a curve in which the observed capacity factor, k_{obs}, is plotted against the concentration of modifier at a certain temperature and pressure.

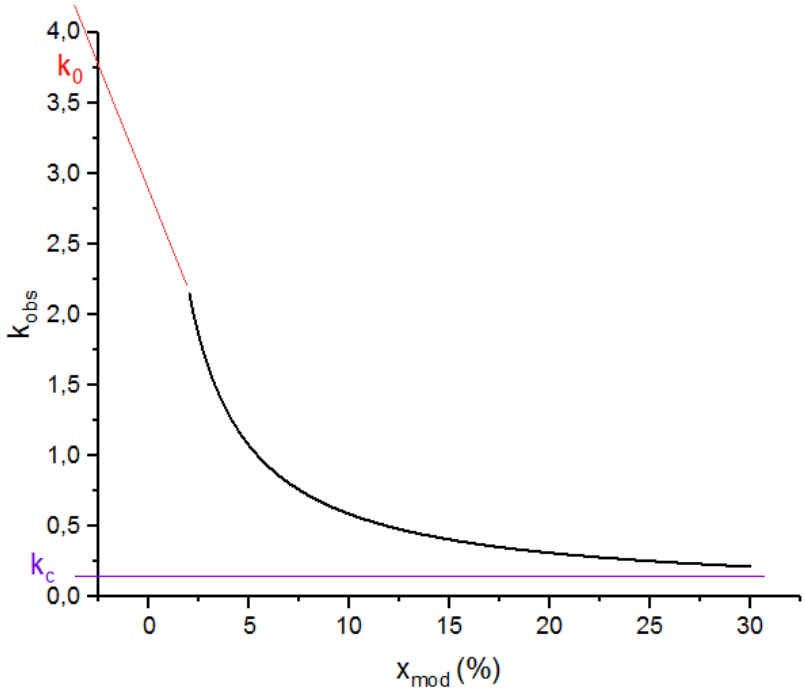


Figure 4.30 k_{obs} is plotted against the concentration of modifier, and the parameters k_0 and k_c are represented

The parameter k_0 is estimated at the intersection of the curve with the y axis, whereas the parameter k_c is obtained at a value in which the observed capacity factor is not affected anymore by the concentration of the modifier. This is, the surface of the stationary phase is totally covered by the modifier. Different adsorption models are used in order to determine the values of the k_0 and k_c. Furthermore, a modification of the Van't Hoff plot described in Chapter 2.3.2.1 "Van't Hoff Plot" will be applied in order to determine the enthalpy and the entropy of the adsorption of the target solute on the material of the stationary phase at every temperature and pressure, as it follows:

$$(\ln k_0)_p = -\frac{\Delta H_T^0}{RT} + \frac{\Delta S_T^0}{R} - ln\beta \qquad (4.11)$$

Equation 4.11 represents a linear correlation of the capacity factor at zero modifier concentration to 1/T at a certain pressure and temperature.

To this aim, the retention time of phenol was measured at concentration of modifiers from 2% to 30% in increments of 2% at temperatures from 25 °C to 60 °C in increments

of 5 ºC. Temperatures no higher than 60 ºC were decided not to be used due to the possible modification of the surface of the stationary phase, as described in Chapter 4.1.2 "Stability of the columns". Phenol was chosen because it was decided to be the representative compound due to its good peak shape, fast elution and it is properly influenced by the modifier concentration. Toluene is almost not affected by the concentration of modifier due to the lack of interactions with the mobile phase, whereas the used models were not possible to be applied to the caffeine curves due to its strong interaction with the stationary phase.

4.3.2 Langmuir model

The Langmuir adsorption model was used to describe the adsorption of modifier in the first place. The adsorption is explained by assuming that the modifier molecule behaves as an ideal gas at isothermal conditions. The adsorbent is assumed to be an ideal solid surface composed of series of active sites. Then, a molecule of the modifier, Mod, reacts with an empty site, S, and the reaction yields an adsorbed complex Mod_{ad} with an associated equilibrium constant K_{eq}.

$$\mathrm{Mod} + \mathrm{S} \leftrightarrow Mod_{ad} \tag{4.12}$$

The Langmuir equation is described by:

$$N_s = \frac{N_{s,max} \cdot K_{eq} \cdot x_{mod}}{1 + K_{eq} \cdot x_{mod}} \tag{4.13}$$

where N_s is the number of modifier molecules adsorbed on the stationary phase, $N_{s,max}$ is the maximum molecules that can be adsorbed, x_{mod} is the concentration of modifier and K_{eq} is the constant equilibrium of the reaction. Thus, the fractional occupancy of the adsorption sites, θ, can be calculated by (Janssen et al., 1991):

$$\theta = \frac{N_s}{N_{max}} = \frac{K_{eq} x_{mod}}{1 + K_{eq} x_{mod}} \tag{4.14}$$

If Equation 4.14 is introduced in the Equation 4.10, the observed capacity factor can be described as:

$$k_{obs} = \frac{k_0 + k_c \cdot K_{eq} \cdot x_{mod}}{1 + K_{eq} x_{mod}} \tag{4.15}$$

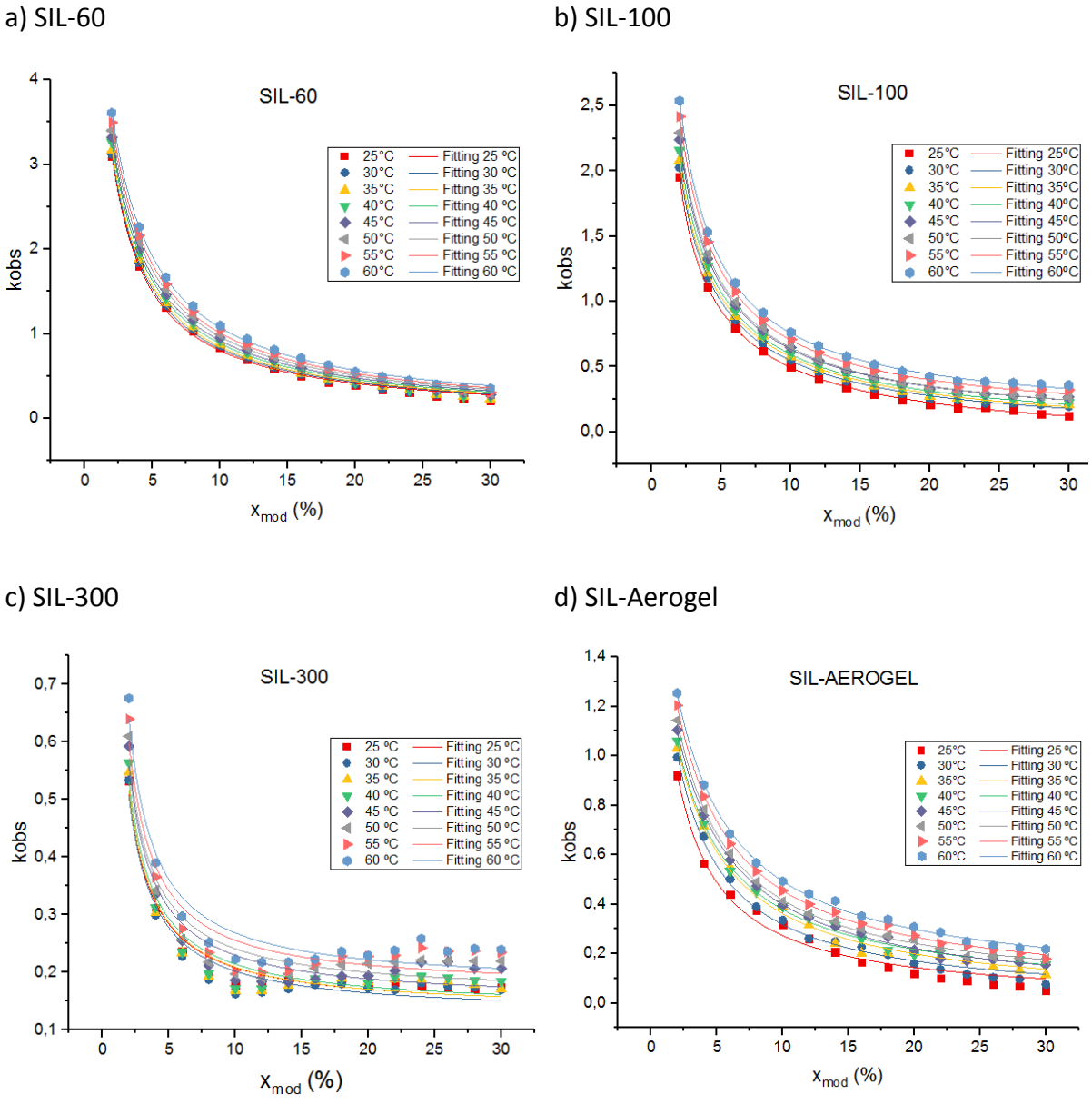


Figure 4.31 Observed capacity factor of phenol at concentrations of modifier from 2% to 30% in increments of 2% and at temperatures from 25 ºC to 60 ºC in increments of 5 ºC was fitted by the Langmuir model in the different stationary phases: a) SIL-60; b) SIL-100; c) SIL-300; d) SIL-Aerogel

In Figure 4.31, the observed capacity factor of phenol at different concentrations of modifier and at different temperatures was fitted by the Equation 4.15 in the different stationary phases: a) SIL-60; b) SIL-100; c) SIL-300; d) SIL-Aerogel. The values of k_0, k_c and Keq as well as the statistics of the regressions are shown in Table 4.6 for SIL-60 and in Appendix for SIL-100, SIL-300 and SIL-Aerogel.

Table 4.6 Values of k_0, k_c and Keq as well as the statistics of the Langmuir regressions of SIL-60

T (^{0}C)	k_0	k_c	Keq	Reduced Chi-Sqr	Adj R^2
25	11.02 *(± 0.96)*	$1.51 \cdot 10^{-17}$ *(± 0)*	1.27 *(± 0.14)*	$1.76 \cdot 10^{-3}$	0.997
30	10.28 *(± 0.62)*	$1.97 \cdot 10^{-17}$ *(± 0)*	1.14 *(± 0.09)*	$1.03 \cdot 10^{-3}$	0.998
35	10.15 *(± 0.69)*	$1.67 \cdot 10^{-17}$ *(± 0)*	1.10 *(± 0.10)*	$1.46 \cdot 10^{-3}$	0.997
40	9.97 *(± 0.65)*	$1.64 \cdot 10^{-17}$ *(± 0)*	1.02 *(± 0.08)*	$1.65 \cdot 10^{-3}$	0.998
45	9.52 *(± 0.46)*	$1.97 \cdot 10^{-17}$ *(± 0)*	0.93 *(± 0.06)*	$1.08 \cdot 10^{-3}$	0.998
50	9.17 *(± 0.34)*	$2.12 \cdot 10^{-17}$ *(± 0)*	0.85 *(± 0.04)*	$8.08 \cdot 10^{-4}$	0.999
55	9.12 *(± 0.37)*	$1.92 \cdot 10^{-17}$ *(± 0)*	0.80 *(± 0.04)*	$1.12 \cdot 10^{-4}$	0.999
60	8.85 *(± 0.27)*	$2.17 \cdot 10^{-17}$ *(± 0)*	0.72 *(± 0.03)*	$8.10 \cdot 10^{-4}$	0.999

The values of k_0 are reasonable and they decrease when the temperature increases, which is in concordance with the adsorption exothermal process. In the Figure 4.31, it is seen that the capacity factor is higher at high temperatures, so the fitting of the experimental data is supposed to crossed at some point.

Values of k_c are close to zero, which is also logic because no retention of the solute is expected at the total surface coverage of the stationary phase. Keq values decrease

when temperature increases, which indicates that higher temperatures have negative effects in the adsorption of modifier, according also to the exothermic process.

In Figure 4.32, Van't Hoff plot was represented, in which the ln k_0 is plotted against the inverse of Temperature in kelvin multiplied by a factor of 10^3. The scatter plot was adjusted by a linear regression, where R^2 coefficient was 0.97.

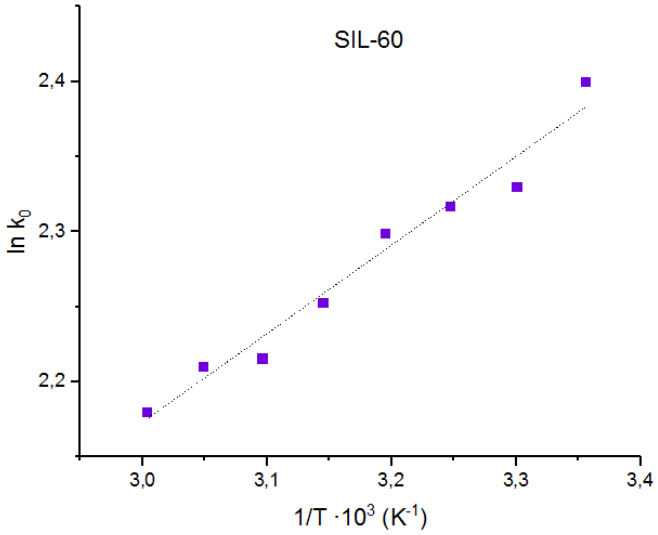


Figure 4.32 Van't Hoff plot for the Langmuir regressions in SIL-60.

It is seen that the values have a linear and positive slope, in which the higher the temperature is, the lower the k_0 is, which is consistent with the previous result and with the application of the Van't Hoff plot.

The adjusted equation of the fitting combined with the Van't Hoff equation represented would give the values of the enthalpy entropy of the adsorption. The phase ratio can be obtained from the void volume, v_0, the inlet flow and the density of the mobile phase at the pumps and at the column.

Although the results of this model for the SIL-60 stationary phase are in concordance with expected, the adjustment is unsatisfactory for the rest of columns. In the case of SIL-100 and SIL-Aerogel, the regressions adjust the experimental data but it is not possible to obtain coherent values from the Van't Hoff plot. In case of SIL-300, the

model does not fit the experimental data so reasonable values of k_0 and k_c are not possible to be obtained.

Values of the surface coverage fraction are represented against the concentration of modifier at 40 ºC in SIL-60, SIL-100 and SIL-Aerogel in Figure 4.33. It is shown that, at a certain amount of modifier, the less temperature causes a more coverage of the surface of the stationary phase, which is expected.

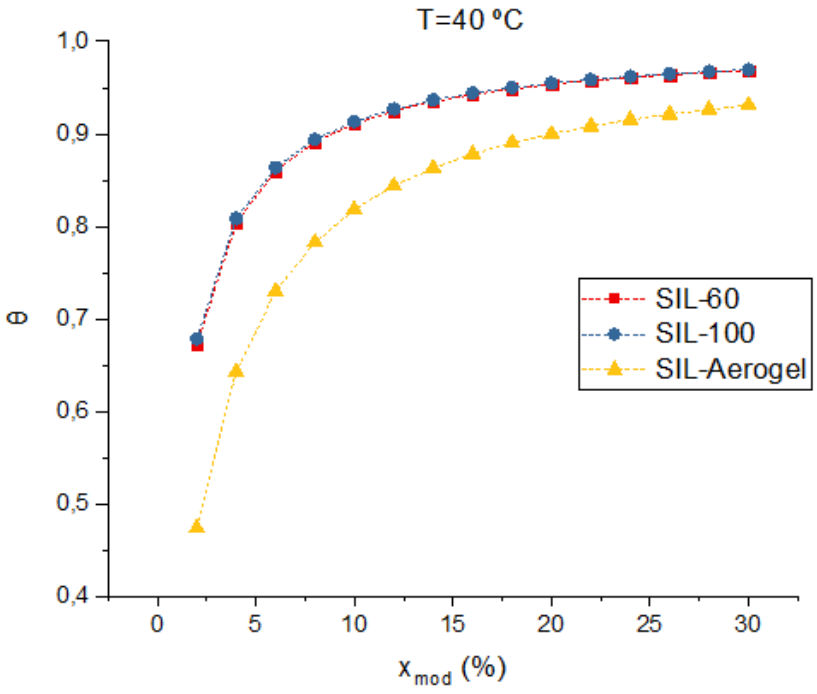


Figure 4.33 Surface coverage fraction is represented against the concentration of modifier at different temperatures. Conditions: 40ºC, 200 bar, 2mL min^{-1}

The fact that the Langmuir model fits the experimental data of SIL-60, SIL-100 and SIL-Aerogel stationary phases but physically sound information can only be taken from SIL-60 can be due to two reasons. On the one hand, the Langmuir model could not be able to describe all the interactions between the mobile and the stationary phase, and more complex models are needed to be used in order to get proper information. On the other hand, the proposed adaptation of the mixed retention model can have some limitations and it is needed to be improved. In the following section, some other models are used to fit the experimental data to try to clarify the present dilemma.

4.3.3 Other adsorption models

Four more adsorption models were used to fit the experimental data of the k_{obs} plotted against the concentration of modifier in different stationary phases. The Tóth adsorption isotherm has a unimodal heterogeneous adsorption energy distribution that tails forward a lower energy. The Jovanovic adsorption isotherm is similar to Langmuir but has taken into account that the adsorption and desorption of the solute is not instant. The Moreau model is an expansion of the Langmuir model, with the addition of solute-solute interactions. The BET model consider multilayer adsorption (Samuelsson, Zang, Murunga, Fornstedt, & Sajonz, 2008).

The equation of the surface coverage fraction as well as the adsorption isotherm of the mentioned models are shown in Table 4.7. . In Figure 4.34, the fitting of the Jovanovic model is shown for the experimental data of SIL-60, SIL-100, SIL-300 and SIL-Aerogel.

Table 4.7 θ and the adsorption isotherm of Tóth, Jovanovic, Moreau and BET models. Extracted from (Samuelsson et al., 2008).

ADSORPTION ISOTHERM MODELS	
Tóth Model	$\theta = \frac{a \cdot x_{mod}}{(1 + (b \cdot x_{mod})^{v})^{\frac{1}{v}}} \quad ; k_{obs} = k_0 + \frac{(k_c - k_0) \cdot a \cdot x_{mod}}{(1 + (b \cdot x_{mod})^{v})^{\frac{1}{v}}}$
Jovanovic Model	$\theta = \left(1 - e^{kx_{mod}}\right) \quad ; \quad k_{obs} = k_0 + (k_c - k_0) \cdot \left(1 - e^{kx_{mod}}\right)$
Moreau Model	$\theta = \frac{k \cdot x_{mod} + I \cdot k^2 \cdot x_{mod}^2}{1 + k \cdot x_{mod} + I \cdot k^2 \cdot x_{mod}^2} \quad ;$ $k_{obs} = k_0 + \frac{(k_c - k_0) \cdot (k \cdot x_{mod} + I \cdot k^2 \cdot x_{mod}^2)}{1 + k \cdot x_{mod} + I \cdot k^2 \cdot x_{mod}^2}$
BET Model	$\theta = \frac{c \cdot x_{mod}}{(1 - x_{mod})(1 - x_{mod} + c \cdot x_{mod})} \quad ;$ $k_{obs} = k_0 + \frac{(k_c - k_0) \cdot c \cdot x_{mod}}{(1 - x_{mod})(1 - x_{mod} + c \cdot x_{mod})}$

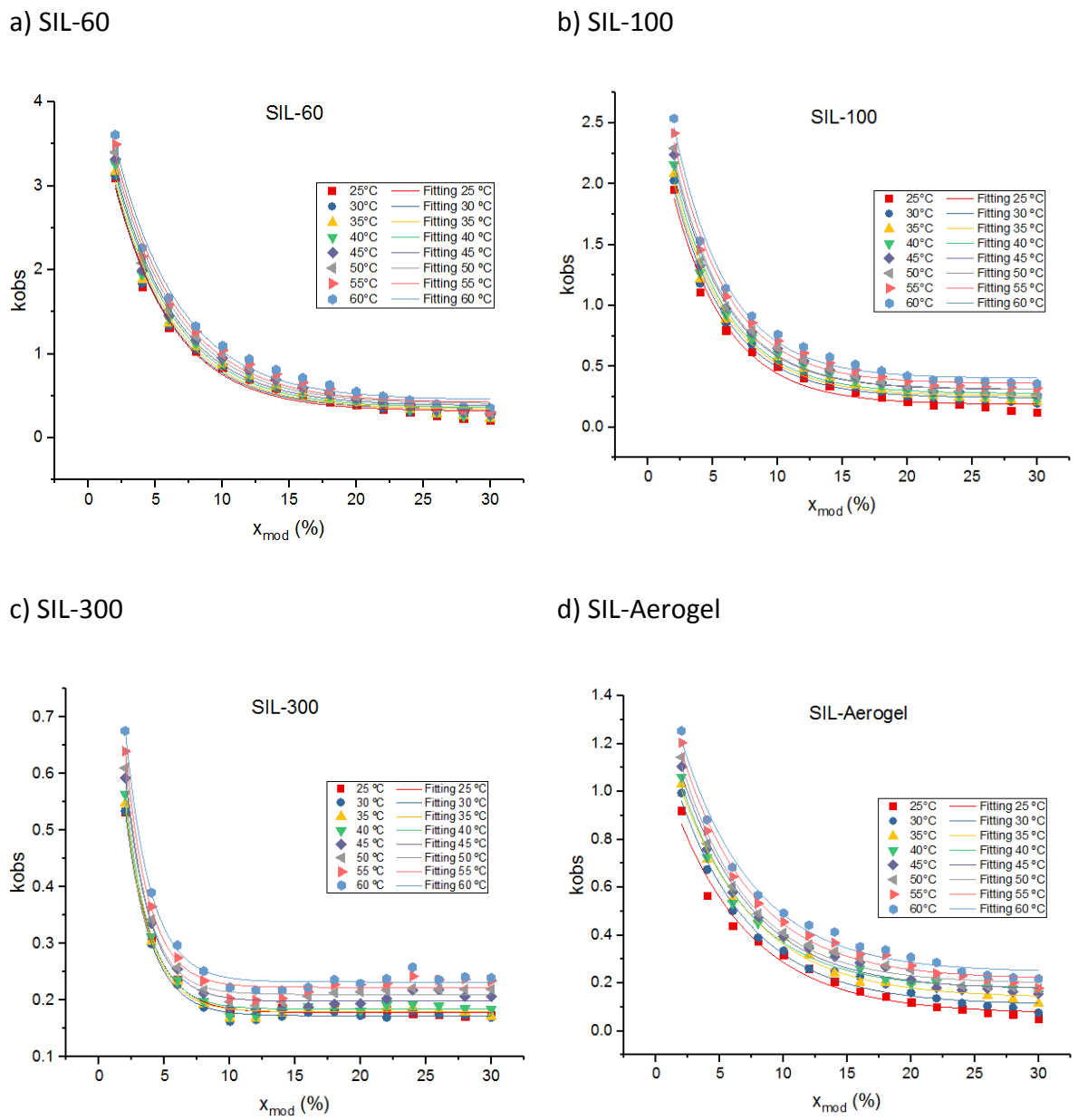


Figure 4.34 Observed capacity factor of phenol at concentrations of modifier from 2% to 30% in increments of 2% and at temperatures from 25 ºC to 60 ºC in increments of 5 ºC was fitted by the Langmuir model in the different stationary phases: a) SIL-60; b) SIL-100; c) SIL-300; d) SIL-Aerogel

The Jovanovic model is the only one that fits the experimental data of the k_{obs} plotted against the concentration of modifier in all the stationary phases and with reasonable values of k_0 and k_c, whereas the BET model was not able to fit any of them.

The values of k_0, k_c and *k* as well as the statistics of the regressions are shown in Table 4.8 for SIL-60 and in Appendix for SIL-100, SIL-300 and SIL-Aerogel. The parameter k is a numerical coefficient.

Table 4.8 Values of k_0, k_c and Keq as well as the statistics of the Jovanovic regressions of SIL-60

T (^{0}C)	k_0	k_c	k	Reduced Chi-Sqr	Adj R^2
25	4.51 *(± 0.25)*	0.32 *(± 0.04)*	0.23 *(± 0.02)*	$1.02 \cdot 10^{-2}$	0.983
30	4.55 *(± 0.23)*	0.34 *(± 0.04)*	0.23 *(± 0.02)*	$8.63 \cdot 10^{-3}$	0.986
35	4.57 *(± 0.25)*	0.35 *(± 0.04)*	0.23 *(± 0.02)*	$1.04 \cdot 10^{-2}$	0.983
40	4.66 *(± 0.25)*	0.36 *(± 0.04)*	0.22 *(± 0.02)*	$1.12 \cdot 10^{-2}$	0.983
45	4.73 *(± 0.24)*	0.39 *(± 0.04)*	0.22 *(± 0.02)*	$1.07 \cdot 10^{-2}$	0.984
50	4.83 *(± 0.24)*	0.41 *(± 0.04)*	0.22 *(± 0.02)*	$1.04 \cdot 10^{-2}$	0.985
55	4.91 *(± 0.24)*	0.42 *(± 0.04)*	0.21 *(± 0.02)*	$1.11 \cdot 10^{-2}$	0.985
60	5.04 *(± 0.23)*	0.45 *(± 0.04)*	0.21 *(± 0.02)*	$1.11 \cdot 10^{-2}$	0.986

However, the Van't Hoff plot was not possible to be applied in any case. Due to this, it is concluded that the proposed modification from the Mixed Retention Model from Janssen et al. is a good starting point in order to consider the adsorption of the modifier on the surface of the stationary phase in the retention time. However, apart from k_0 and k_c, some other parameters are needed to be considered in order to take into account all the interactions. The Mixed Retention Model is limited to the phenomena of the surface of the stationary phase, and it does not consider the effect of modifier in the mobile phase.

Furthermore, in the experimental data from SIL-300 it is seen that, after a certain amount of modifier, the experimental points go up in the curve, what means that high concentrations of modifier lead to larger retention times. The fact that this effect only occurs in the SIL-300 stationary phase can be explained from the active sites point of view: SIL-300 is the stationary phase that presents the lowest value of active sites according to Chapter 4.2.1.4 "Classification of stationary phases", which means that the surface can be saturated at lower concentrations of modifier. Once the surface is saturated, the adsorbed molecules of methanol can form a liquid phase (a film) on the stationary phase, what leads to an increase in the retention time.

Furthermore, the fractional surface coverage is obtained from the values of k_0 and k_c and it is calculated from the Equation 4.10. It would be recommended to obtained the value of the surface coverage fraction directly by dynamic analysis of modifier adsorption (Frontal Analysis) to compare with the data extracted from modeling.

5 Conclusions and Future work

In this work, SFC analyses were performed in order to study the interactions between the solid carriers, CO_2, methanol as modifier and different solutes at different temperature, pressure and concentration of modifier. Silica aerogel and kromasil particles were used as solid carriers (stationary phases).

The hold-up time of the columns was obtained by the injection of an unretained peak. N_2O was shown to be a good marker for hold-up time measurements for all the stationary phases because it was not affected by the concentration and the nature of the mobile phase. The stability of the columns at different temperatures and modifier concentrations was tested. While Kromasil particles showed some discrepancies in the retention time at high temperatures and high concentration of modifier, aerogel particles-packed column showed a good stability in an operation time of 48 hours in all the study cases. Thus, we identified a temperature range where the analysis of the retention can be performed without affecting the chemical nature of the stationary phase due to the reaction with modifier.

The applicability of LSER methodology in studying the retention behaviors in SFC was demonstrated for Kromasil particles and for aerogel particles-packed columns. The results showed that LSER is capable of describing reliably the retention factors using hydrogen bond acidity (a), hydrogen bond basicity (b), McGowan's characteristic volume (v), polarizability (s), and excess molar refraction (e) as parameters. We clearly

show that for the stationary phases studied, the coefficients a and b have more influence on the retention time across all the studied conditions.

Solutes were classified in families according to the results of the LSER regressions. Each family of compounds produced a unique response to changes in concentration of modifier, temperature and pressure, so that higher values increases, decreases, or has no effect on the capacity factor. The retention time of the different solutes was also compared across the different stationary phases. Aerogel particles showed reliable results in terms of physical-chemical analysis with SFC, wider peaks were observed, most likely due to the irregular shape of the aerogel powder.

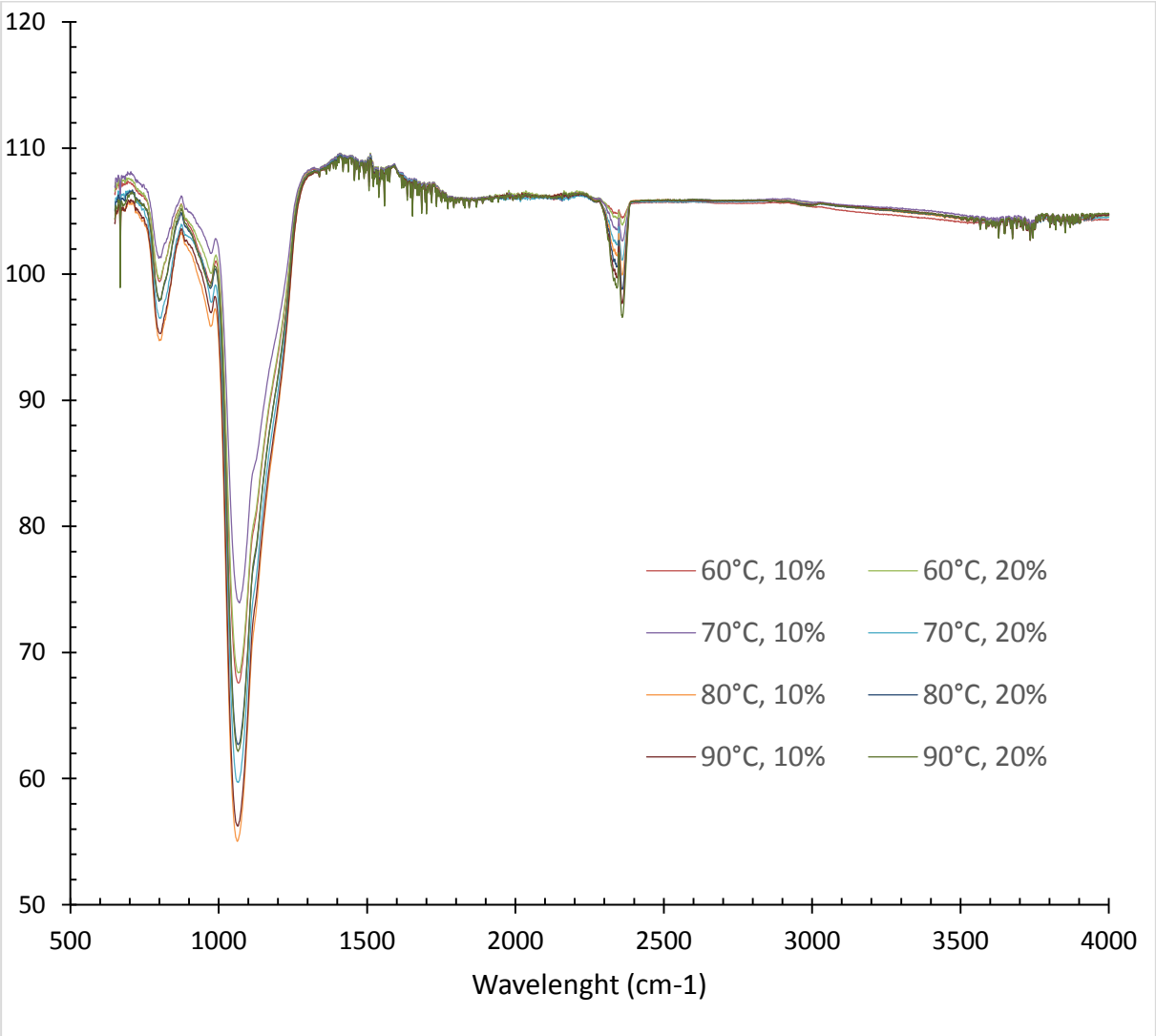
A Mixed Retention Model was employed, considering that, apart from the contribution of the silanol groups, represented by k_0, the adsorption of modifier on the surface of the stationary phase, represented by k_c, can also have an influence in the retention time. The influence of both contributions is described by the surface coverage fraction, which is approximated by the Langmuir adsorption model.

The results from the mixed retention model combined with Langmuir adsorption isotherm showed good agreement with the experimental data, but further validation is needed to extract more thermodynamic information from the fitting. Other adsorption isotherms were used in combination with the mixed retention model, however with a limited improvement compared to the Langmuir model.

Due to this findings, it was concluded that the employed mixed retention model has some limitations and needs improvements. One possibility would be to consider effects of the modifier in the mobile phase, *e.g.*, clustering around the solutes in the mobile phase resulting in faster elution of polar solutes. It is also suggested that, at high concentration of modifier, a liquid layer of modifier may form on the surface of the stationary phase, so that the dissolves in the liquid layer and retains stronger on the stationary phase. It is clear that these questions should be attacked by direct measuring the surface coverage fraction. This is a part of our ongoing work.

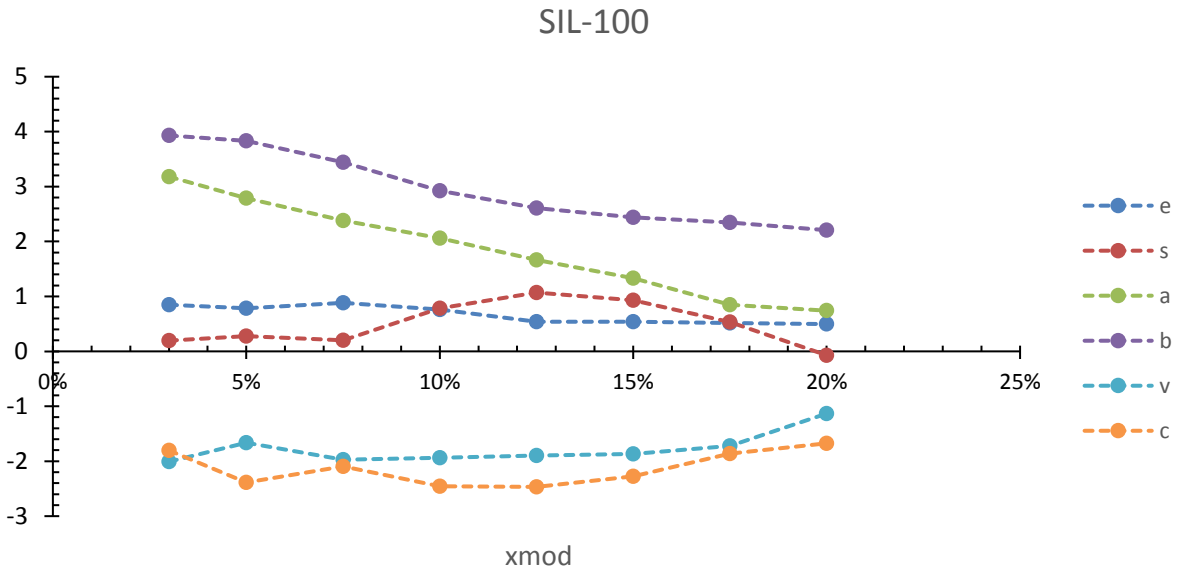
Appendix

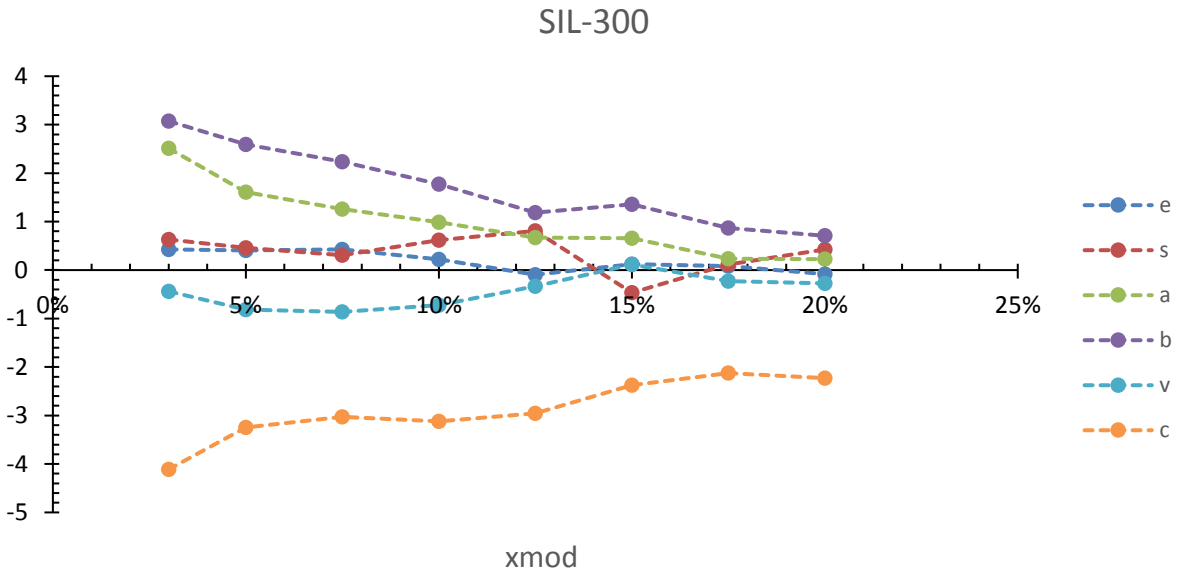
Appendix I: FTIR measurements

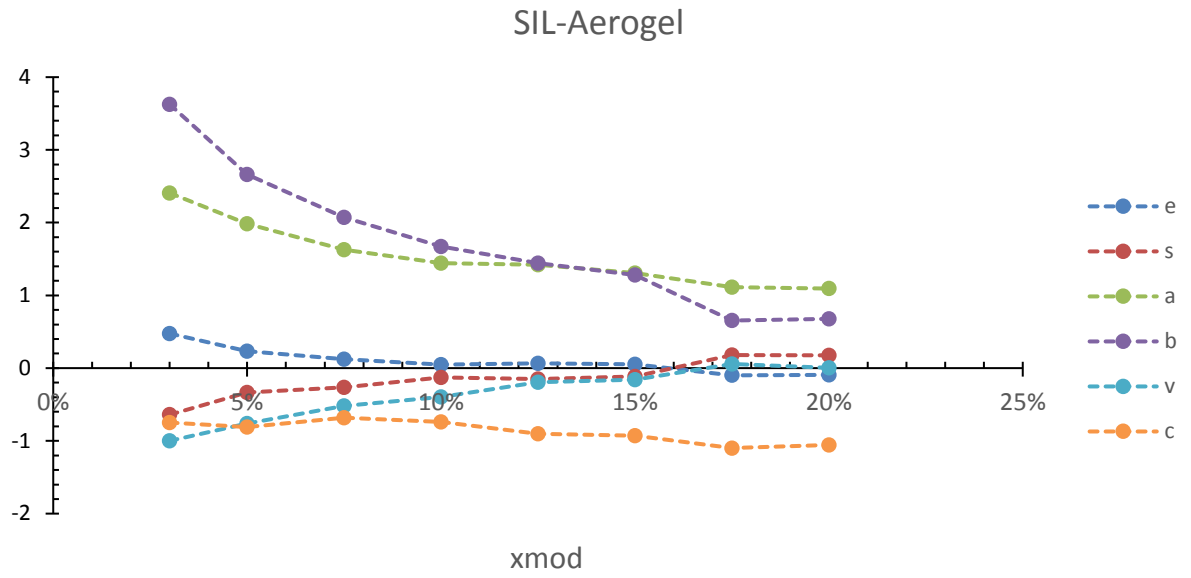


Appendix II: LSER Regressions

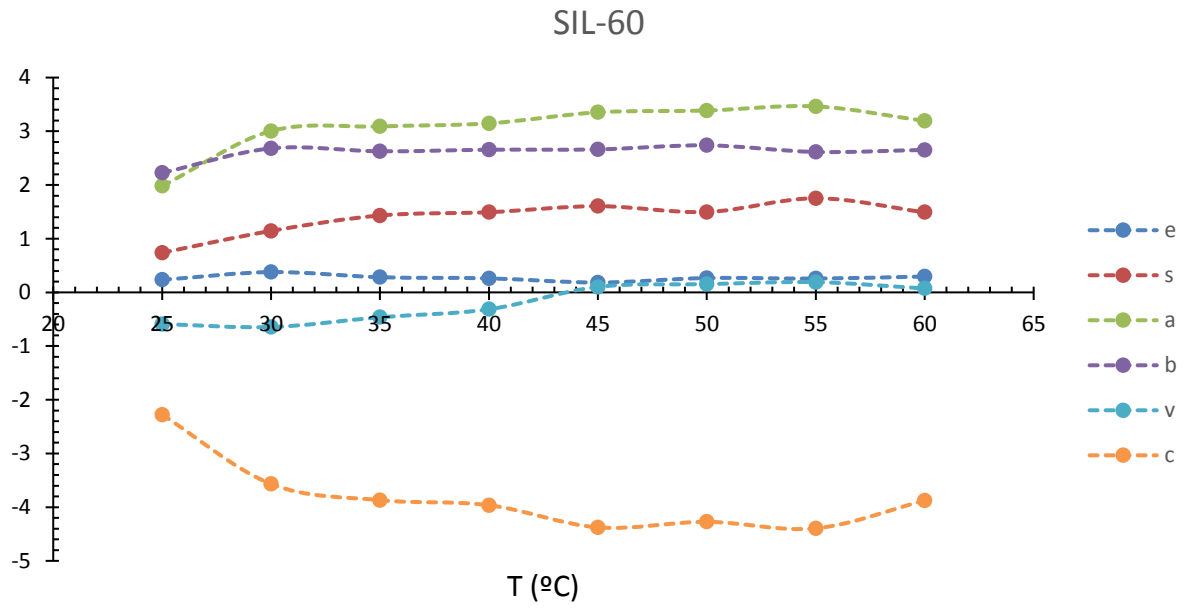
Concentration of modifier regressions

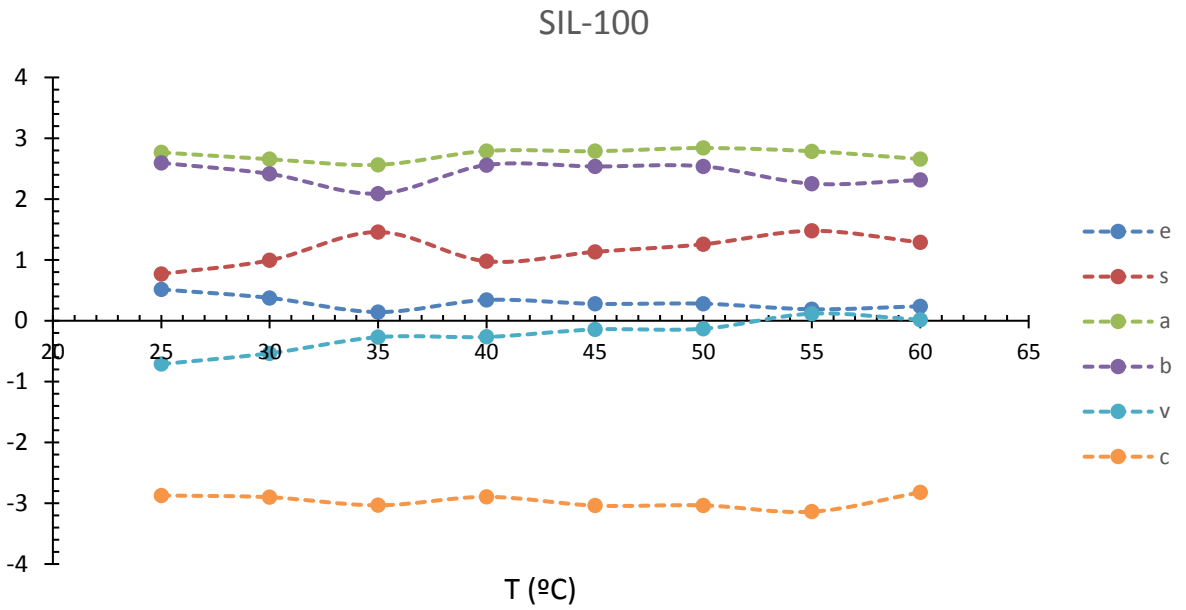


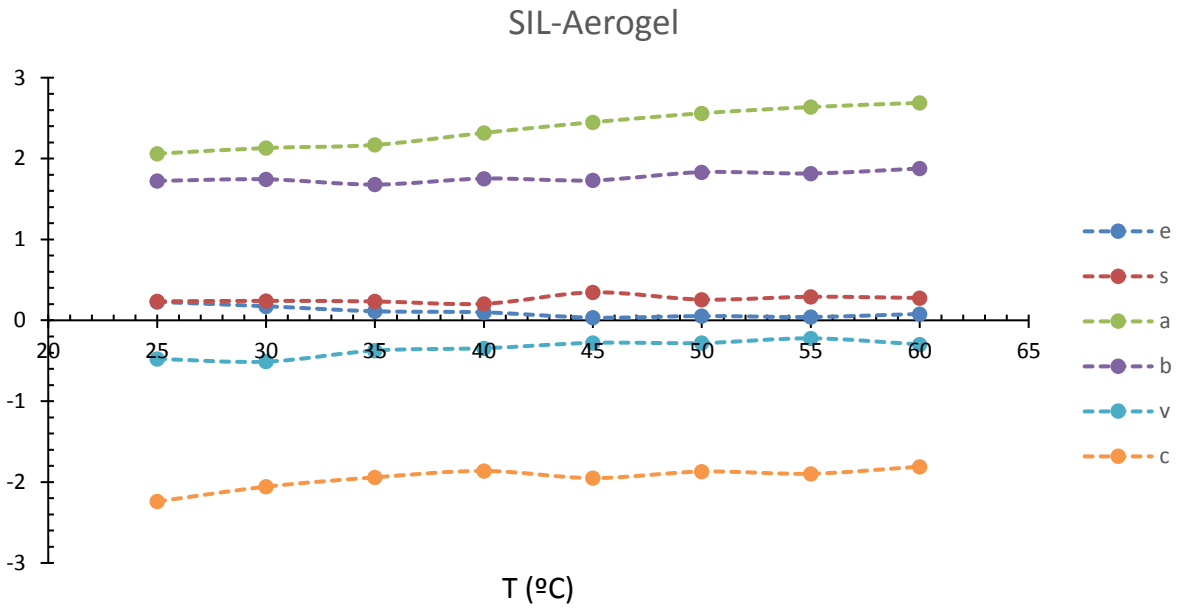




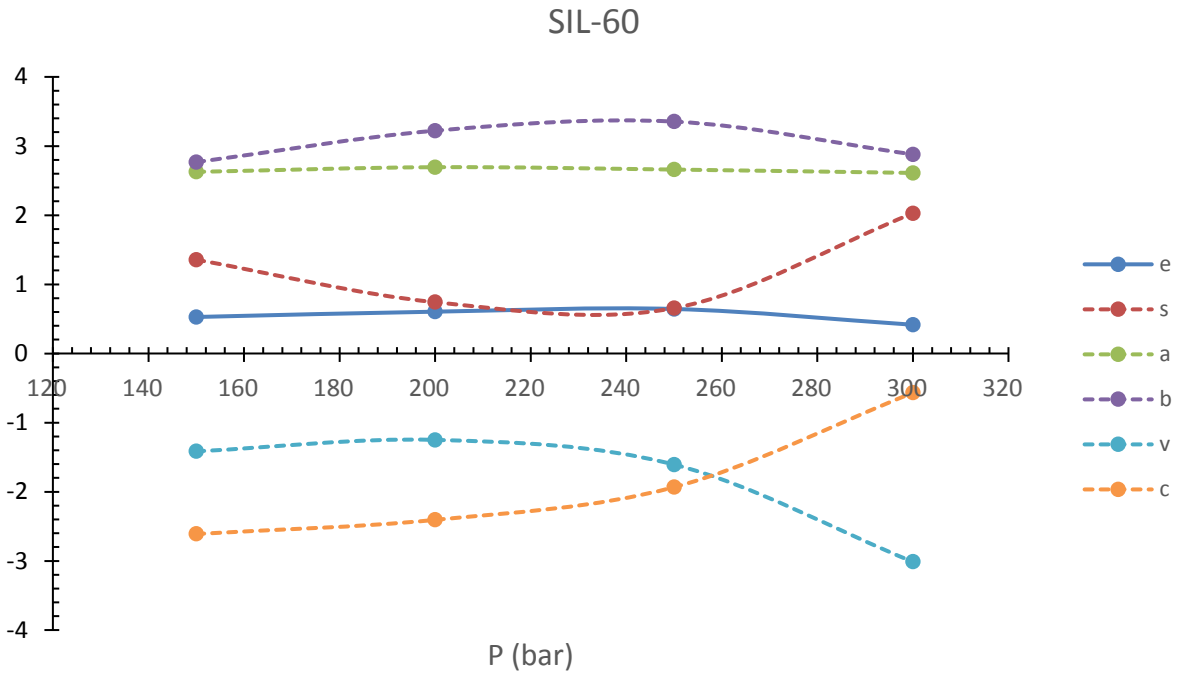
Temperature regressions

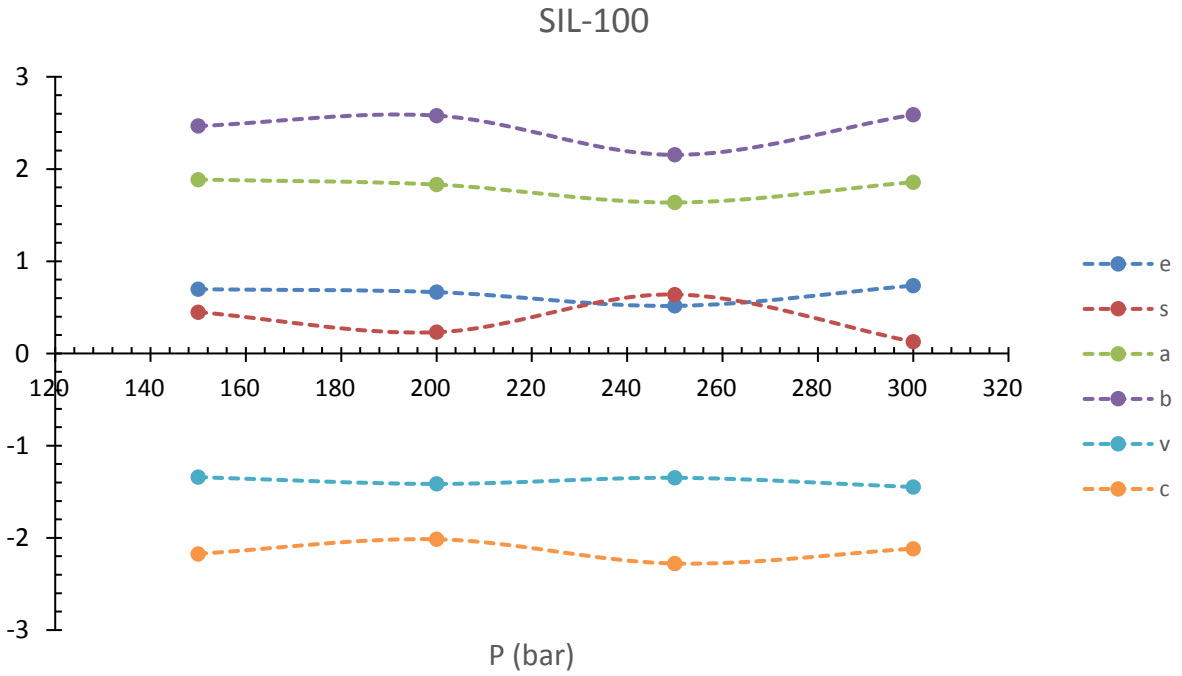


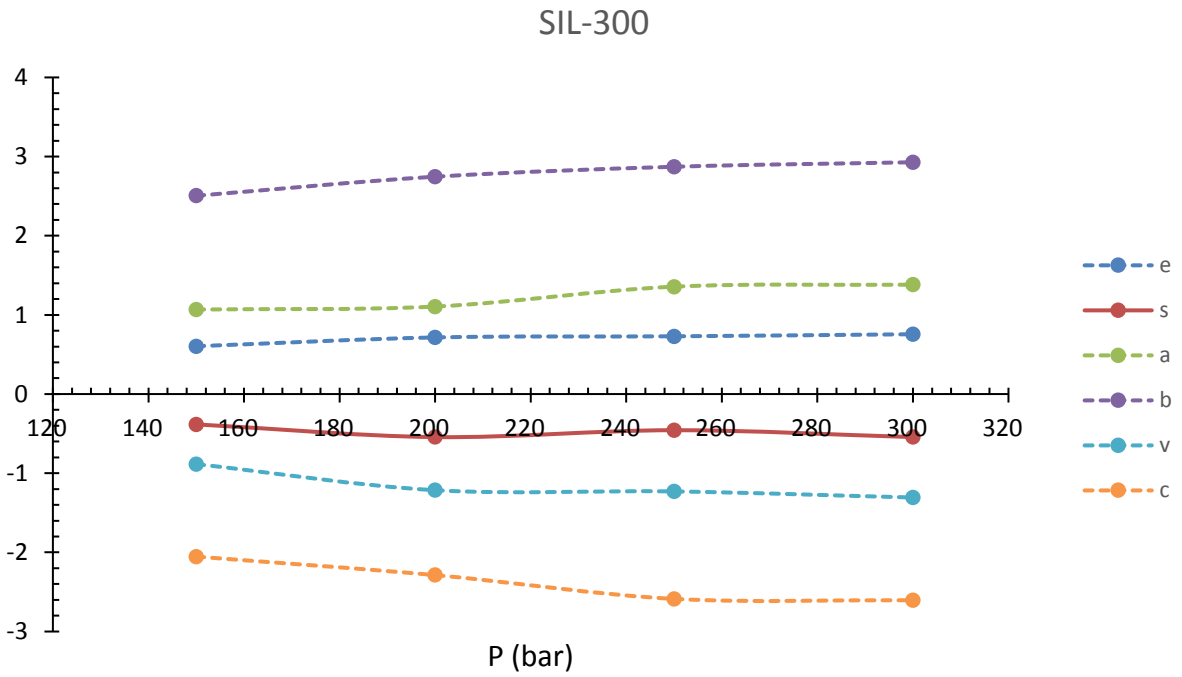

SIL-100
4
3
2
1
0
-1
-2
-3
-4
20
25
30
35
40
45
50
55
60
65
T (ºC)
e
s
a
b
v
c


SIL-Aerogel
3
2
1
0
-1
-2
-3
20
25
30
35
40
45
50
55
60
65
T (ºC)
e
s
a
b
v
c

Pressure regressions



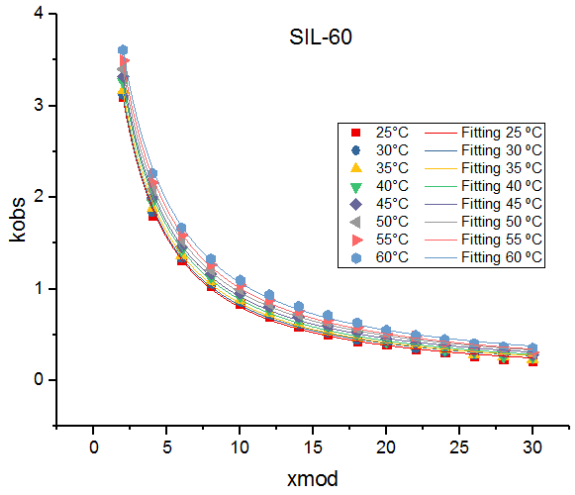



SIL-300
4
3
2
1
0
-1
-2
-3
120
140
160
180
200
220
240
260
280
300
320
P (bar)
e
s
a
b
v
c

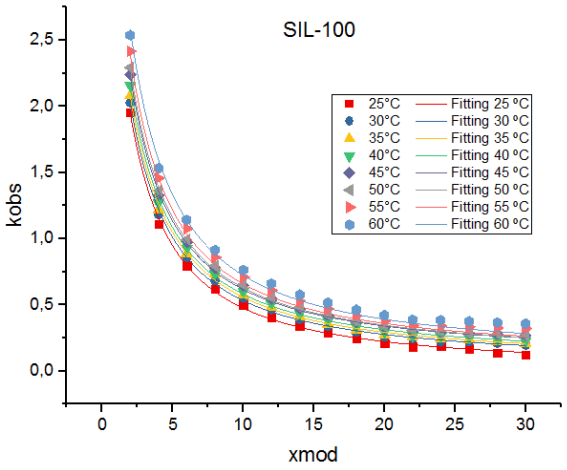
Appendix III: Adsorption isotherm models

Tóth model

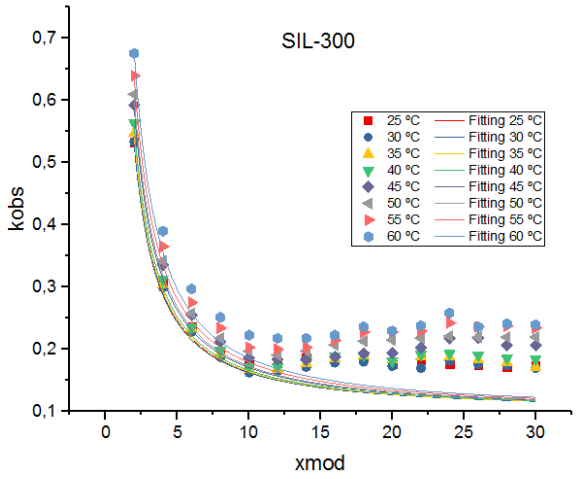
a) SIL-60



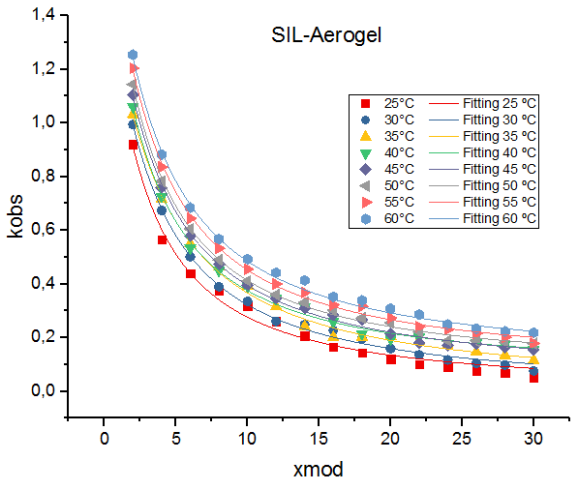
b) SIL-100



c) SIL-300

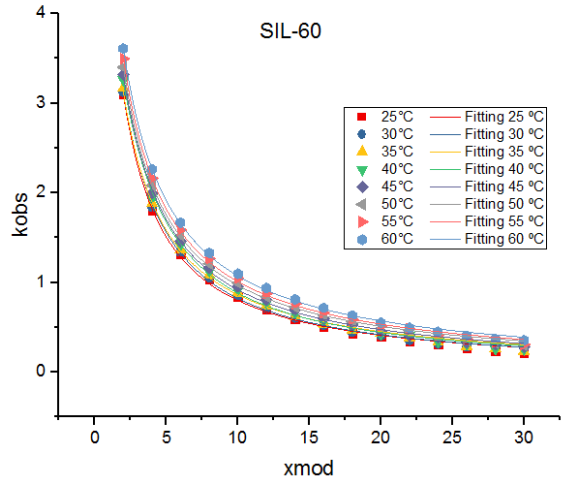


d) SIL-Aerogel

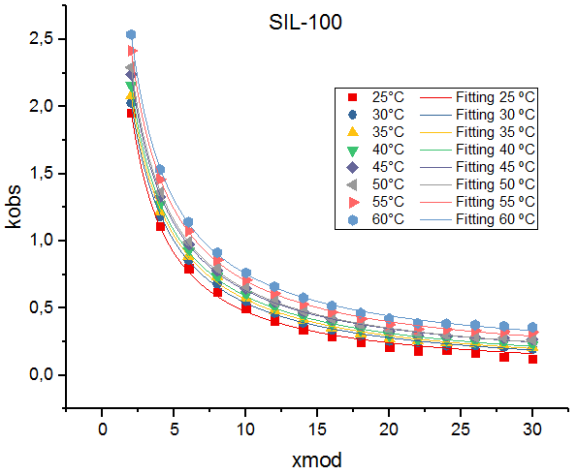


Moreau model

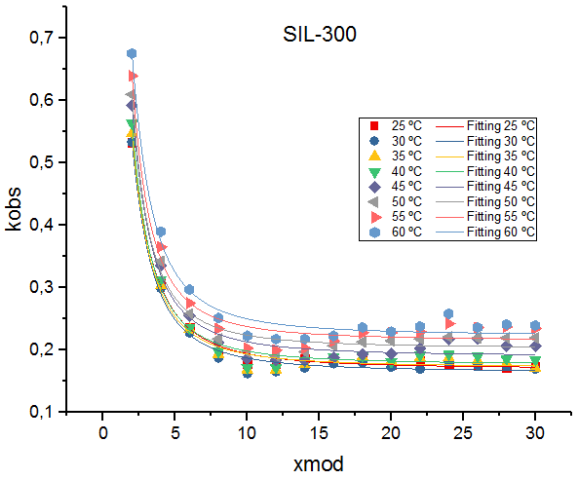
a) SIL-60



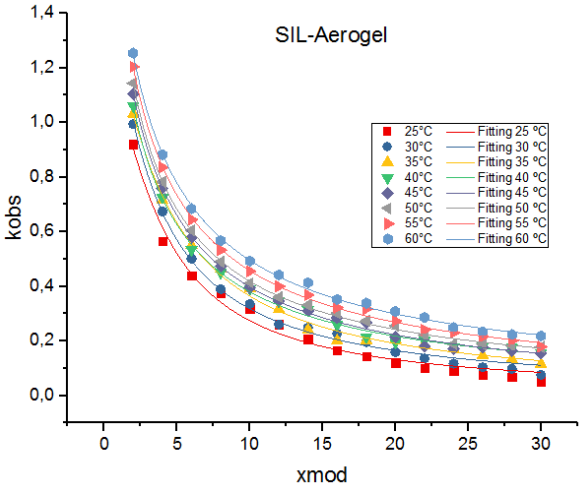
b) SIL-100



c) SIL-300

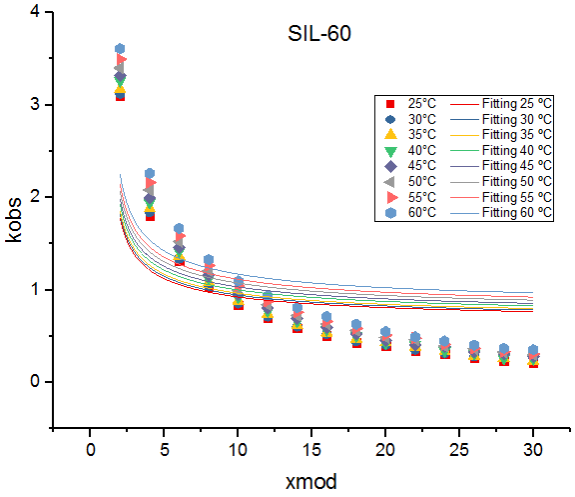


d) SIL-Aerogel

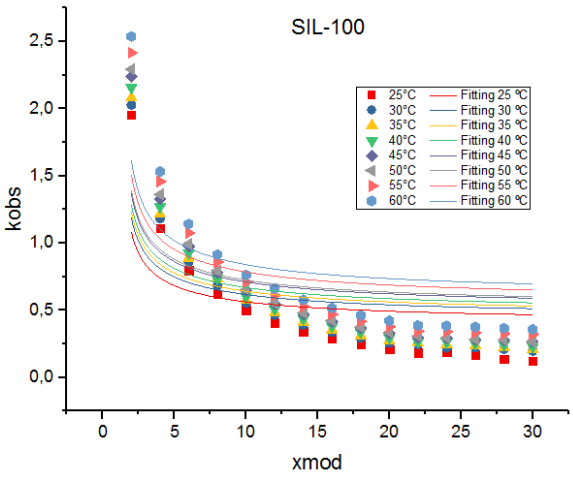


BET model

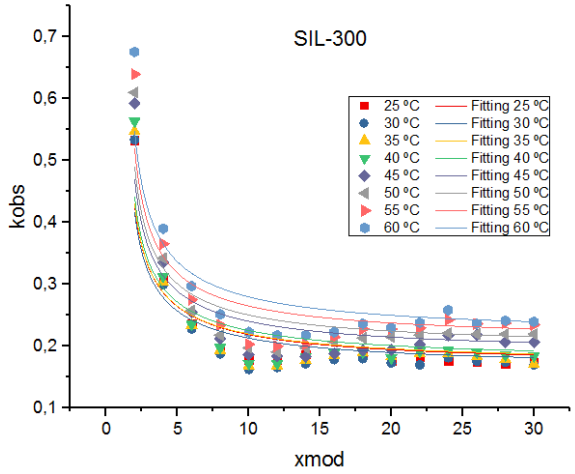
a) SIL-60



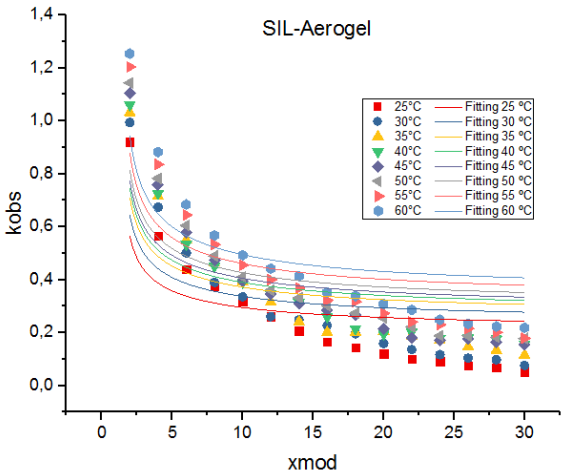
b) SIL-100



c) SIL-300



d) SIL-Aerogel



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