



# Investigation of plateau methods for adsorption isotherm determination in supercritical fluid chromatography<sup>☆</sup>



Martin Enmark, Jörgen Samuelsson, Erik Forss, Patrik Forssén, Torgny Fornstedt\*

Department of Engineering and Chemical Sciences, Karlstad University, SE-651 88 Karlstad, Sweden

## ARTICLE INFO

### Article history:

Received 21 January 2014  
Received in revised form 30 April 2014  
Accepted 26 May 2014  
Available online 2 June 2014

### Keywords:

Adsorption isotherms  
Supercritical fluid chromatography  
Perturbation peak method  
Elution by characteristic points  
Antipyrine

## ABSTRACT

The Perturbation Peak (PP) method and Frontal analysis (FA) are considered as the most accurate methods for adsorption isotherms determination in liquid chromatography. In this study we investigate and explain why this is not the case in Supercritical Fluid Chromatography (SFC), where the PP method does not work at all, using a modern analytical system. The main reason was found to be that the solute to be studied must be dissolved in the MeOH reservoir before it is mixed with CO<sub>2</sub>. Since the solute occupies a certain partial volume in the reservoir, the larger the solute content the larger this fractional volume will be, and the final MeOH fraction in the mobile phase will then be smaller compared to the bulk mobile phase without solute in the modifier. If the retention of small injections on the concentration plateaus, i.e., “analytical-size” perturbation peaks, is sensitive to small variations of MeOH in the eluent, this will seriously decrease the accuracy of the PP method. This effect was verified and compensated for and we also demonstrated that the same problem will occur in frontal analysis, another concentration plateau method.

© 2014 Elsevier B.V. All rights reserved.

## 1. Introduction

There is currently a strong trend towards the use of preparative Supercritical Fluid Chromatography (Prep-SFC) for purification and many leading pharmaceutical industries have replaced their preparative Liquid Chromatography (Prep-LC) instruments with Prep-SFC ones, especially for purification of gram amounts in the discovery stage of drug development. The main reason for this is that the production rate can be several-fold higher in prep-SFC compared to Prep-LC [1]. In addition, the main solvent in Prep-SFC (CO<sub>2</sub>) is already in the carbon cycle and therefore much less environmentally harmful than the organic solvents frequently used in Prep-LC. This trend is now also visible in the analytical chromatography area; one sign is that leading instrument manufacturers have recently launched new generations of analytical SFC instruments [2,3].

One inherent disadvantage of SFC is that the method is more complex than LC, mainly due to the compressibility of the supercritical mobile phase fluid that causes local variations in density,

temperature and viscosity in the column. Currently the lack of fundamental knowledge hampers both understanding of the underlying mechanisms as well as reliable computer-assisted optimizations of large scale SFC processes [4]. Computer-assisted optimization can ultimately be used to design more robust separation processes with higher production rates that are cheaper, safer and more environmentally friendly. Reliable computer-assisted optimization of Prep-SFC requires both proper modeling of the separation process and accurate determination of the adsorption isotherms of the component(s) in the used phase system. With accurate adsorption isotherms, over broad solute concentration ranges, it is also possible to obtain deeper mechanistic understanding of the adsorption process; i.e., to determine the degree of heterogeneity and energy of interaction as well as the monolayer capacity of the adsorption sites. For high quality adsorption data it is possible to calculate the adsorption energy distribution (AED) and thereby, prior to the model fitting procedure, determine the particular types of interactions, e.g. dipole–dipole, van der Waals, etc., that are present in the phase system. This was recently demonstrated for liquid-based biosensor systems [5].

In a recent study we investigated the usefulness of a modern analytical SFC instrument for rapid and reliable determination of adsorption data [6]. This is important since process units in the pharmaceutical industry uses new commercial, analytical-scale SFC instruments to scout separation systems prior to scale-up. More specifically, we investigated the possibility to transfer the

<sup>☆</sup> Presented at the 40th International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2013 Hobart), Hobart, Tasmania, Australia, 18–21 November 2013.

\* Corresponding author.

E-mail address: [Torgny.Fornstedt@kau.se](mailto:Torgny.Fornstedt@kau.se) (T. Fornstedt).

following adsorption isotherm determination methods from LC to SFC: Elution by Characteristic points (ECP), the Retention Time Method (RTM), the Inverse Method (IM) and the Perturbation Peak (PP) method. RTM, ECP and IM are based on elution profiles, whereas the PP method and Frontal Analysis (FA) are so called concentration plateau methods based on experiments where the chromatographic column is equilibrated with a constant stream of the component to be studied. For LC it has been verified and validated that concentration plateau methods are more accurate and reliable than methods based on elution profiles [7–10]. In our SFC study, the adsorption isotherm data generated by the different methods were analyzed and validated by comparing computer simulated elution profiles, using the determined isotherm data, with experimental ones [6]. Here we found that the methods based on elution profiles, i.e., ECP, IM and RTM, were able to accurately predict overloaded experimental elution profiles while the PP method, based on generating data from concentration plateaus, was not able to do so in these SFC experiments [6]. The adsorption isotherm obtained from the PP method did only coincide with the ones obtained by ECP, RTM or IM for the initial linear part of the adsorption isotherm, while at increasing component concentrations, the PP method successively deviated more and more from the other methods.

There are a small number of articles describing the determination of adsorption isotherms for SFC [11–16]. Lübbert et al. [16] used the PP method, however, the authors did not verify the determined adsorption model's ability to predict overloaded elution which makes it difficult to judge the reliability of the method for SFC. Nevertheless, the authors presented a sound approach to control pressure and temperature and in our SFC study [6] we confirmed another recent publication [17] regarding the importance of using external sensors for temperature, mass flow and pressure. In our study these sensors were used to ensure near isopycnic and isothermal conditions, which are needed to transfer adsorption isotherm determination methods from LC to SFC. Under these conditions we also showed that the ideal model, that for example are used to derive the ECP method [18,19] and RTM, can accurately describe the system [6] because modern analytical SFC systems usually have very high column efficiencies.

The aim of this study is to investigate and explain why the PP method results in an adsorption isotherm that differs from the one determined using ECP. We will also investigate if the same problem occurs in SFC for the FA method that is considered to be the most accurate method in LC [7,20–22]. Finally, AED-calculations is conducted, this has only been done once as far as we know [23].

## 2. Theory

As in our previous study [6], it is assumed that the column is operated under close to isopycnic and isothermal conditions and this assumption is verified experimentally by external measurements of mass flow, pressure and temperature. A short description of how to calculate density from an Equation of State is presented in Section 2.1 and the bi-Langmuir adsorption isotherm model is presented in Section 2.2. The PP, ECP and FA adsorption isotherm determination methods are presented in Sections 2.3–2.5. The theory for gradient elution is presented in Section 2.6 and Section 2.7 describes the processing of the adsorption isotherm data.

### 2.1. Mobile phase density and effective volumetric flow

To calculate the density of the mobile phase fluid at a certain point in the chromatographic system the Kunz and Wagner [24] Equation of State, as implemented by the National Institute of Standards and Technologies in REFPROP v 9.1 [25] was used. The inputs are the mass fractions of CO<sub>2</sub> and MeOH ( $w_{\text{CO}_2}$  and  $w_{\text{MeOH}}$ ) and the

temperature and pressure at a specific point on the column. For practical purposes, the pressure and temperature was measured at the inlet and outlet of the column ( $P_{\text{inlet}}$ ,  $P_{\text{outlet}}$ ,  $T_{\text{inlet}}$ ,  $T_{\text{outlet}}$ ). The mass fractions of CO<sub>2</sub> and MeOH were calculated from the total mass flow,  $\dot{m}_{\text{total}}$ , measured by a mass flow sensor, and the MeOH mass flow,  $\dot{m}_{\text{MeOH}}$ , according to,

$$w_{\text{CO}_2} = \frac{\dot{m}_{\text{total}} - \dot{m}_{\text{MeOH}}}{\dot{m}_{\text{total}}}, \quad w_{\text{MeOH}} = 1 - w_{\text{CO}_2} \quad (1)$$

The explicit statement of near isopycnic conditions is that the density in the axial direction is nearly constant. For practical purposes, this is verified by comparing the density at the column inlet and outlet,

$$\rho(P_{\text{inlet}}, T_{\text{inlet}}) \approx \rho(P_{\text{outlet}}, T_{\text{outlet}}) \approx \rho(P_{\text{average}}, T_{\text{average}}). \quad (2)$$

The effective volumetric flow rate  $F_{V,\text{average}}$  is calculated from the total mass flow and the average density,  $\rho_{\text{average}}$ ,

$$F_{V,\text{average}} = \frac{\dot{m}_{\text{total}}}{\rho_{\text{average}}}. \quad (3)$$

In some experiments antipyrine will be introduced in the MeOH fractions. We will ignore the effect of antipyrine in the estimated mass fraction, Eq. (1), and average density, Eq. (3), since the maximum possible mass fraction of antipyrine is about 1% in the experiments. We therefore only expect small errors in these calculations. In addition, to account properly for this a suitable Equation of State for the mixture CO<sub>2</sub>/MeOH/antipyrine will be needed and it is not available today.

### 2.2. The adsorption isotherm model

The adsorption isotherm relates the concentration in the mobile phase,  $C$ , and in the stationary phase,  $q$ , and in this study the bi-Langmuir model, the sum of two single-site Langmuir terms, are used,

$$q = q_{s,1} \frac{K_1 C}{1 + K_1 C} + q_{s,2} \frac{K_2 C}{1 + K_2 C}, \quad (4)$$

where  $q_{s,1}$ ,  $q_{s,2}$  is the monolayer saturation capacity and  $K_1$ ,  $K_2$  the association equilibrium constant for site 1 and 2. Here we define the first adsorption site as the low energy site and the second one as the high energy site, i.e.,  $K_1 < K_2$ .

### 2.3. The Perturbation Peak (PP) method

In the PP method the adsorption isotherm is determined by injecting a small excess or deficiency of the studied component into a column that is already equilibrated with an eluent containing a constant stream of identical molecules, i.e. a concentration plateau. The perturbation peak will be a single peak in the chromatogram and its retention time is related to the slope of the adsorption isotherm according to,

$$\frac{dq}{dC_0} = \frac{V_R(C_0) - V_M}{V_a}, \quad (5)$$

where  $V_R(C_0)$  is the retention volume of the perturbation peak at an established concentration plateau with concentration  $C_0$ ,  $V_a$  is the stationary phase volume,  $V_M$  is the column hold up volume and  $dq/dC_0$  is the slope of the adsorption isotherm. By repeating this experiment at several different concentration plateau levels, the whole adsorption isotherm can be determined. Eq. (5) is derived from the ideal model of chromatography and assumes constant volumetric flow and constant amount of modifier in the eluent. However, because only the first moment of the perturbation peak is used, low column efficiency will only reduce the height of the perturbation peak.

Download English Version:

<https://daneshyari.com/en/article/1199970>

Download Persian Version:

<https://daneshyari.com/article/1199970>

[Daneshyari.com](https://daneshyari.com)