Journal of Chromatography A, 1216 (2009) 8496–8504

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Comparison of the adsorption mechanisms of pyridine in hydrophilic interaction chromatography and in reversed-phase aqueous liquid chromatography

Fabrice Gritti[a], Alberto dos Santos Pereira[b], Pat Sandra[a], Georges Guiochon[b,*]

[a] Department of Chemistry, University of Tennessee, Knoxville, TN 37996-1600, USA
[b] Research Institute for Chromatography, Kennedypark 26, 8500 Kortrijk, Belgium

ARTICLE INFO

Article history:
Received 22 May 2009
Received in revised form 8 September 2009
Accepted 2 October 2009
Available online 9 October 2009

Keywords:
Hydrophilic interaction
HILIC
Per aqueous liquid chromatography (PALC)
Hydrophobic interactions
Adsorption mechanism
Frontal analysis
Minor disturbance method
Excess adsorption isotherm
Total adsorption isotherm
Adsorption energy distribution
Pyridine
Refractive index detection

ABSTRACT

The adsorption isotherms of pyridine were measured by frontal analysis (FA) on a column packed with shell particles of neat porous silica (Halo), using water–acetonitrile mixtures as the mobile phase at 295 K. The isotherm data were measured for pyridine concentrations covering a dynamic range of four millions. The degree of heterogeneity of the surface was characterized by the adsorption energy distribution (AED) function calculated from the raw adsorption data, using the expectation-maximization (EM) procedure. The results showed that two different retention mechanisms dominate in *Per* aqueous liquid chromatography (PALC) at low acetonitrile concentrations and in hydrophilic interaction chromatography (HILIC) at high acetonitrile concentrations. In the PALC mode, the adsorption mechanism of pyridine on the silica surface is controlled by hydrophobic interactions that take place on very few and ultra-active adsorption sites, which might be pores on the irregular and rugose surface of the porous silica particles. The surface is seriously heterogeneous, with up to five distinct adsorption sites and five different energy peaks on the AED of the packing material. In contrast, in the HILIC mode, the adsorption behavior is quasi-homogeneous and pyridine retention is governed by its adsorption onto free silanol groups. For intermediate mobile phase compositions, the siloxane and the silanol groups are both significantly saturated with acetonitrile and water, respectively, causing a minimum of the retention factor of pyridine on the Halo column.

1. Introduction

Reversed-phase liquid chromatography (RPLC) is the most commonly applied chromatographic mode in analytical laboratories, particularly in the chemical and pharmaceutical industries, and in biochemical, clinical, and forensic laboratories. However, the analysis of samples containing very polar compounds and the resolution of their main components is difficult because many of these components are so weakly retained that they are eluted close to the hold-up elution time. Normal phase liquid chromatography (NPLC) would appear to be an efficient alternative to RPLC but its use is hampered by two important drawbacks: (1) NPLC solvents are mostly hazardous and environmentally unfriendly; and (2) NPLC mobile phases are not compatible with most RPLC eluents, making arduous the combination of both modes for 2D separation purposes.

Highly polar compounds are also retained on bare silica when eluted with concentrated aqueous solutions of organic solvents [1]. This mode is called hydrophobic interaction chromatography or HILIC. Acetonitrile is the most popular solvent used in HILIC. In this mode, the separation mechanism is based on the differential distribution of the sample components between a water-rich layer adsorbed onto the silica surface and the acetonitrile-rich bulk phase [2,3].

Recently, the production of acetonitrile has been so strongly reduced that its availability is limited, even at a price that is now nearly an order of magnitude larger than it was a year ago. Academic and industrial laboratories involved in separation and purification processes are facing the challenge of finding alternatives to the HILIC and RPLC modes, which use acetonitrile-rich mobile phases. Switching from acetonitrile to solvents of comparable elution strength could be a solution. Ethanol, which is produced in large amounts and is biodegradable, appears an attractive candidate. Another solution would consist in using water-rich mobile phases to elute columns packed of neat silica particles, in order to benefit from the hydrophobic character conferred to the silica surface by the siloxane groups [4]. This mechanism was called *per* aqueous liquid chromatography (PALC) by Sandra et al. [5]. These authors measured the retention factors of seven amino-acids eluted on a column packed with Zorbax Rx-SIL silica, with water containing between 0 and 60% of acetonitrile. They observed U-shaped

* Corresponding author. Tel.: +1 865 974 0733; fax: +1 865 974 2667.
E-mail address: guiochon@utk.edu (G. Guiochon).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.10.009

graphs when plotting the retention factor of these compounds versus the acetonitrile concentration, with sharp increases of k' below 5% and beyond 50% of acetonitrile for the most hydrophobic amino-acids (isoleucine, leucine, methionine, valine, and proline). The other two amino-acids (glutamic acid and lysine) showed no significant increase of their retention factors in water-rich mobile phases because they are very polar compounds. In both cases, hydrophobic interactions are too weak to promote retention in the PALC mode. Several scientists found U-shaped retention patterns, e.g., with polyphenols onto cross-linked agarose gel media following the same retention modes [6], with epirubicin and analogs [7] and in the investigation of the mobile phase layer rich in water on the silica surface, using toluene as the analyte [3].

In this work, we investigated the transition from the HILIC to the PALC adsorption mechanism for pyridine when the concentration of acetonitrile in the aqueous mobile phase is decreased from 99.6 to 10%. The adsorption data were measured by frontal analysis on a column packed with Halo shell particles [8,9]. The particle shell being made of porous silica with no chemically bonded ligands, this adsorbent is suitable for our purpose. The pyridine concentrations was increased from 2×10^{-5} to 80 g/L. The low initial value is necessary to measure accurately the initial slope of the adsorption isotherm, the high value is required to measure adsorption data when the weak adsorption sites are highly populated. The adsorption energy distribution (AED) was derived from the raw adsorption data, using the expectation-maximization (EM) procedure and assuming local Langmuir isotherm behavior [10–12]. The degree of heterogeneity of the adsorbent surface, the saturation capacities, and the equilibrium constants measured under HILIC and PALC mode, and in the transition between these two modes are discussed.

2. Theory

2.1. Frontal analysis

The measurement of adsorption data were conducted by the frontal analysis method. This method is very precise (±1%) and accurate (±2%), provided that the temperature of the column is controlled (±0.5 K), the hold-up time, t_0, of the column is accurate within 0.5%, and the extra-column time, t_{ex} from the mixer to the detector, is known within 1% [12]. A concentration step of height C is injected during a time, t_p, sufficiently long for thermodynamic equilibrium between the stationary and the bulk phase to be achieved all along the column. The breakthrough curve is recorded and the elution time of its front, t_{eq}, is measured. The amount of compound, $q^*(C)$, adsorbed at equilibrium per unit volume of adsorbent is given by the mass conservation law:

$$q^*(C) = F_v C \frac{t_{eq} - t_0 - t_{ex}}{V_c - F_v t_0} \quad (1)$$

where F_v is the flow rate applied during the frontal analysis experiments and V_c is the volume of the empty column tube. This experiment is repeated for a number of values of the concentration step, C.

2.2. Minor disturbance method

When the compound studied is weakly adsorbed or when its concentration is very high, the composition of the adsorbed phase is barely different from that of the bulk phase. Then, the total and the excess number of moles adsorbed are significantly different. Frontal analysis is not an accurate method of measurement of excess adsorption isotherms of weakly adsorbed compounds. In contrast, the minor disturbance method using RI and/or MS detection is very suitable to detect the perturbation of the equilibrium plateau of binary [13,14] or ternary eluents [15]. If we neglect the changes in the partial molar volumes of each solvent component upon adsorption and mixing, the excess number of mole of component i adsorbed from a binary mixture writes:

$$n_i^e = \frac{1}{v_i^*} \int_0^1 [V_R(x_i) - V_M] dx_i \quad (2)$$

where v_i^* is the molar volume of the pure component i, $V_R(x_i)$ is the retention volume (corrected for the extra-column contributions) of the perturbation peak when the column is equilibrated with the eluent containing a volume fraction x_i of component i, and V_M is the thermodynamic void volume of the column determined by

$$V_M = \int_0^1 V_R(x_i) dx_i \quad (3)$$

2.3. Adsorption energy distribution

Almost all adsorption mechanisms are heterogeneous because (1) the surface of actual adsorbents is heterogeneous by nature; and (2) the molecules of the sample components have multiple functional groups which may interact specifically with the stationary phase. As a result, if the local adsorption mechanism follows a Langmuir adsorption model, the total overall adsorption isotherm may be written:

$$q^*(C) = \int_0^\infty F(\epsilon) \frac{b(\epsilon)C}{1 + b(\epsilon)C} d\epsilon \quad (4)$$

In Eq. (4), $F(\epsilon)$ is the AED function expressed as the fraction of the total saturation capacity q_S for which the adsorption energy of the sample remains in between ϵ and $\epsilon + d\epsilon$. By definition:

$$q_S = \int_0^\infty F(\epsilon) d\epsilon \quad (5)$$

Experimental results give only the overall adsorbed amount, $q^*(C)$. The difficulty of the procedure consists in estimating the most likely AED function, $F(\epsilon)$, based on the sole measurement of $q^*(C)$, without introducing arbitrary assumptions such as an analytical expression of the overall isotherm and/or of the AED function. The method used in this work is the EM procedure elaborated by Stanley et al. [10]. All the details of this procedure are given in [12]. In the EM method, Eq. (4) is discretized, and an iteration calculation procedure is used, assuming an initial adsorption energy distribution function that is uniform over all the N adsorption sites. The iteration equation estimates the AED function at step $k+1$ from the one calculated at step k [10]:

$$F^{k+1}(\epsilon_i) = F^k(\epsilon_i) \sum_{C_{min}}^{C_{max}} \frac{b(\epsilon_i)C_j}{1 + b(\epsilon_i)C_j} \Delta\epsilon \frac{q^*(C_j)}{q_{cal}^k(C_j)} \quad (6)$$

The procedure ends either when the difference between F^{k+1} and F^k is less than a preset threshold or when the number of iterations reaches a preset number. In this work, the procedure was always stopped after one million iterations.

Provided that the experimental range of sample concentration, $[C_{min}, C_{max}]$, allows accurate estimates of the Henry constant K (that is obtained from the adsorption data at the smallest concentrations) and corresponds to populations of the weakest adsorption site (that correspond to the highest adsorbate concentrations) exceeding 50%, the initial uniform AED function eventually converges towards a well-resolved energy distribution from which the degree of adsorption heterogeneity can be determined.

Note that the iteration number should match the precision of the adsorption data. It is unnecessary to use too large iteration numbers if the precision of the adsorption isotherm achieved is poor. There