Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1101 (2006) 136–152
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Thermodynamic analysis of the heterogenous binding sites of molecularly imprinted polymers

Hyunjung Kim [a,b], Krzysztof Kaczmarski [a,b,c], Georges Guiochon [a,b,*]

[a] *Department of Chemistry, University of Tennessee, Knoxville, TN 37996-1600, USA*
[b] *Division of Chemical Sciences, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6120, USA*
[c] *Faculty of Chemistry, Rzeszów University of Technology, 35-959 Rzeszów, Poland*

Received 1 August 2005; received in revised form 23 September 2005; accepted 27 September 2005
Available online 2 November 2005

Abstract

The thermodynamic interactions of two polymers, one Fmoc-L-Trp-imprinted (MIP), the other one an unimprinted reference (NIP), with the two Fmoc-tryptophan enantiomers were studied by frontal analysis, which allows accurate measurements of the adsorption isotherms. These isotherms were acquired at temperatures of 40, 50, 60, and 70 °C, for sample concentrations ranging between 0.005 and 40 mM. The mobile phase used was acetonitrile with one percent acetic acid as an organic modifier. Within the measured concentration ranges, the tri-Langmuir isotherm model accounts best for the isotherm data of both enantiomers on the MIP, the bi-Langmuir model for the isotherm data of Fmoc-L-Trp on the NIP. These isotherm models were selected using three independent processes: statistical tests on the results from regression of the isotherm data to different isotherm models; calculation of the affinity energy distribution from the raw isotherm data; comparison of the experimental and the calculated band profiles. The isotherm parameters obtained from these best selected isotherm models showed that the enantiomeric selectivity does not change significantly with temperature, while the affinity of the substrates for both the MIP and the NIP decrease considerably with increasing temperatures. These temperature effects on the binding performance of the MIP were clarified by considering the thermodynamic functions (i.e., the standard molar Gibbs free energy, the standard molar entropy of adsorption, and the standard molar enthalpy of adsorption) for each identified type of adsorption sites, derived from the Van't Hoff equation. This showed that the entropy of transfer of Fmoc-L-Trp from the mobile to the MIP stationary phase is the dominant driving force for the selective adsorption of Fmoc-L-Trp onto the enantioselective binding sites. This entropy does not change significantly with increasing temperatures from 40 to 70 °C.

Keywords: Fmoc-L-tryptophan imprinted polymers; Frontal analysis; Isotherm parameters; Affinity distribution; Heterogeneous binding sites; Temperatures; Overloaded band profiles; Enantiomer selectivity; Van't Hoff plot; Entropy; Enthalpy

1. Introduction

MIPs are chemically and physically very stable polymers that adsorb selectively the molecules of the species present in solution during their polymerization (i.e., the template). The most commonly used strategy to prepare MIPs involves the use of non-covalent interactions between a target molecule (the template) and some suitable functional groups of the monomers. These interactions allow the formation of template-functional monomer complexes in solution before and during their polycondensation. These complexes are then immobilized into the polymer matrix by copolymerization with a high concentration of cross-linking monomers. Complementary size, shape, and functionalities toward the template in the MIPs can be obtained by extracting the template from the polymer matrix after the end of the polymerization process. The MIPs are thermally stable stationary phases that are used for the separation of enantiomers in high-performance liquid chromatography (HPLC). Recent studies on the thermal stability of methacrylic acid–ethylene glycol dimethacrylate imprinted copolymers (the most typical formula for MIPs) showed no loss of affinity for the template after treatment at 150 °C for 24 h [1]. Therefore, it may be most useful to optimize the temperature of the column. Improved column efficiency, selectivity, and separation speed have been reported upon increasing the temperature of other thermally stable stationary phases [2]. To explore the optimization of the temperature of MIPs in HPLC, we need to understand how better the effects of temperature on the binding characteristics of MIPs. Basically,

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

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.09.092

there are two major effects of temperature that can affect the performance of stationary phases in chromatography [3–6]. First, the viscosity of the mobile phase decreases and the diffusion coefficients of the analytes in both the mobile and the stationary phases increase with increasing temperature. Thus, the mass transfer kinetics becomes faster and the separation times can be reduced. The effects of the temperature on the mass transfer kinetics can especially benefit the chromatographic performance of MIPs which are known to exhibit slow mass transfer kinetics and to produce serious peak tailing (particularly for the template). Second, and for completely different reasons, temperature affects the retention and the separation factors. Although the first effect is general (retention factors almost always decrease with increasing temperature), the effect of temperature on the separation factor depends much on the nature of the system studied and there are no general rules [7–10]. This is partly due to a lack of understanding of how temperatures affect the changes of thermodynamic functions, such as entropy, enthalpy, and free energy, that are associated with the transfer of the solutes from the mobile to the stationary phases in different systems. The phenomenon is particularly complex in the case of mixed mechanisms.

This work had several purposes. First, we wanted to determine how should be selected the most appropriate isotherm model that accounts best for the isotherm data obtained for the system studied. Second, we wanted to investigate how temperature affects the isotherm parameters and the enantiomeric selectivity of a MIP. Third, we wanted to determine the thermodynamic functions of each type of adsorption sites that we had identified on the surface of the MIP and use this information better to understand the temperature effects on the thermodynamics of the separation. In a separate publication, we investigate how temperature affects the mass transfer kinetics on a MIP and on its reference (i.e., non-imprinted polymer). To achieve our goals, we measured the adsorption isotherms of the Fmoc-Trp enantiomers on a Fmoc-L-Trp MIP and on the reference polymer (NIP), in an organic-based mobile phase, at different temperatures. The isotherm parameters estimated from the isotherm data for each system were used to understand the temperature effects on the MIP and the NIP.

2. Theoretical background

The principle of our approach consists of measuring, in as wide a concentration range as possible, the adsorption equilibrium isotherm data of the template and of its antipode on the MIP polymer (MIP) and the similar data for one of the enantiomers or for the racemic mixture for the NIP. These data are modeled on the one hand, used to calculate the affinity energy distributions of the two solutes on the two polymers, on the other hand. As explained elsewhere [11], the data for both polymers are consistent with heterogeneous surfaces paved with different homogeneous surfaces having markedly different properties. The combination of the results of these thermodynamic determinations provides useful clues regarding the surface heterogeneity and the enantioselectivity of MIPs. The fundamental basis of the method is discussed elsewhere [12–14].

Obviously, in this work, as in all applications of thermodynamics to chromatography, we assume that the rates of the different contributions to the mass transfer kinetics across the columns are sufficiently fast and that the data derived from frontal analysis measurements are adsorption data. This is justified in the experimental problem studied here [20].

2.1. Isotherm models

The isotherm models used in this work are the bi- and the tri-Langmuir models:[11]

$$q = \frac{q_{s1}b_1C}{1+b_1C} + \frac{q_{s2}b_2C}{1+b_2C} \tag{1}$$

$$q = \frac{q_{s1}b_1C}{1+b_1C} + \frac{q_{s2}b_2C}{1+b_2C} + \frac{q_{s3}b_3C}{1+b_3C} \tag{2}$$

where q_{s1}, q_{s2}, and q_{s3} are the saturation capacities for the first, the second, and the third types of sites on a heterogeneous surface, respectively; and b_1, b_2, and b_3 are the corresponding adsorption constants. Each of these terms has the form of a Langmuir isotherm and corresponds to one type of sites.

2.2. Affinity energy distributions

The affinity energy distribution (AED) of a surface is the distribution of the values of the association constants over the area of this surface. The heterogeneous nature of the surface of the MIPs and NIPs mean that these surfaces are covered with different types of sites, each type being nearly homogeneous, hence having a narrow affinity adsorption energy distribution [11]. Accordingly, the distribution for the whole polymer surface is multimodal. The adsorption energy distributions of the surfaces corresponding to the isotherm models in Eqs. (1) and (2) display two or three isolated peaks which should be infinitely narrow. In practice, these modes are merely narrow, well resolved, and their small width does not cause any significant deviation from the langmuirian behavior of each terms of the isotherm. Detailed investigations of the properties of the binding sites on the surface of MIPs are possible only by determining the AEDs of the template and its enantiomer and comparing them to their AED on the NIP. Among the several possible methods used directly to derive the AED from the set of experimental isotherm data ($q(C)$), the expectation maximization (EM) method is the most successful for the investigation of the binding sites on MIPs [11,15–18]. The EM method allows the direct calculation of the adsorption energy distribution from the raw experimental isotherm data, without making any prior assumption regarding the isotherm model nor the shape of the AED. The EM method is described, justified, and validated elsewhere [19].

2.3. Modeling of peak profiles

The degree of agreement between the experimental band profiles obtained for large samples and those calculated with a suitable model of chromatography and the best isotherm model derived from the set of adsorption data informs on the validity