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Nonlinear adsorption isotherm as a tool for understanding and characterizing molecularly imprinted polymers

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Abstract

Molecularly imprinted polymers (MIPs) have frequently been characterized by quantities which are easily determined from experiments but have no theoretical foundation. This makes it difficult to compare different MIP preparations or to transfer MIP based methods to different experimental conditions. Since the adsorption isotherms of MIPs are markedly nonlinear, one can build a better characterization strategy on isotherms as shown by examples in this paper.

Keywords: Retention factor; Selectivity; Imprinting factor

1. Introduction

Molecularly imprinted polymers (MIPs) [1–20] are selective sorbents. Their unique selectivity is due to the procedure for making MIPs. This procedure consists essentially of polymerizing some monomers in the presence of "template" molecules identical with or similar to the future analyte. If monomers with appropriate functional groups are chosen, they may form before or during polymerization complex or loose covalent bonds with the template molecules. These structures are then retained even after polymerization of the monomers into a rigid, crosslinked network. The template can be leached out at this point, leaving its empty binding sites behind.

Real life applications of MIPs have been rare until now. Despite of this, the interest in MIPs has not abated. This is perhaps because MIPs provide an extremely elegant way of synthesizing selective, solid phase complexing agents by self-organization.

If we look for the causes of the slower-than-expected progress with MIPs, the lack of detailed understanding of the templated polymerization process comes first to mind. A second important factor is the difficulty of investigating the chemical structure of the binding sites, this difficulty lying mainly in the low concentration and the apparent heterogeneity of such sites and their being confined to a solid, macroporous, insoluble polymer.

In this paper, we want to discuss a further, perhaps even more important but less obvious cause for the slow progress with MIPs. This is the problem of using inadequate, incomparable measures to characterize MIPs. Due to using inadequate measures, it has become increasingly difficult to gauge the progress in the field or to transfer results obtained in one application into another kind of application.

In most experimental reports (including some of our own previous work), MIPs have been characterized by quantities which had been traditionally used in the particular field of application where the MIP was to be introduced. Such quantities are, for example k and α values in chromatography or IC_{50} values in binding assays. The limitations of these parameters for characterizing MIPs should have become apparent long time ago. It has been well known in the MIP field that the adsorption isotherms of MIPs are nonlinear. It has also been well documented in the field of nonlinear chromatography that quantities like k have little meaning if the isotherm is nonlinear. The communication gap between the two communities may have been so large that the few attempts from both sides to bridge this gap have still not been sufficient. Our goal here is to make the MIP community more aware of these problems.

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2. Nonlinear isotherm and the shape of the chromatographic peaks

MIPs have been frequently applied as HPLC or SPE stationary phases. Adsorption isotherms of different templates on their respective MIPs have also been frequently measured and found to be nonlinear even at fairly low template concentrations, certainly well below 10^{-4} M. It is, therefore, quite natural to try to apply the principles of nonlinear chromatography [21–23] to HPLC with MIP stationary phases. This has indeed been done by Guiochon and coworkers [24–26]. These authors have shown that chromatographic peak shapes or breakthrough curves obtained with MIPs can be reasonably well calculated from the corresponding nonlinear isotherm data. As the fits based on equilibrium (i.e. isotherm) data were not perfect, these were improved by using additional kinetic terms. Nevertheless, the overall shape of the chromatogram was mainly determined by the nonlinear isotherm.

Despite the success of the Guiochon group, there has been little done by others to test quantitatively the validity of nonlinear chromatographic theory with MIPs other than those studied by the Guiochon group. Recently, we have reported such work [27] with a MIP of very different nature from those studied by these previous workers.

It seems now that one may generalize the observations of the Guiochon group and say that most of the HPLC peak shapes presented with MIP columns in the literature have been strongly if not mainly determined by the nonlinear adsorption isotherms of the respective analytes. Thus, in agreement with the theory of nonlinear chromatography, the peaks had a relatively steep ascending part and a rather extended tailing part. The ideal nonlinear model of chromatography describes the ascending part as a vertical line, whereas the descending part obeys the following function:

$$t = t_0 \left(1 + F\frac{\mathrm{d}q}{\mathrm{d}c}\right) \quad (1)$$

with t_0 the holdup time, F the phase ratio and q the concentration of the analyte in the stationary phase when equilibrated with mobile phase of concentration c.

Eq. (1) is an implicit expression for the shape of the tail, which would normally be expressed as a concentration (c) against time (t) function (Fig. 1). Eq. (1) shows instead t as a function of the derivative $\mathrm{d}q/\mathrm{d}c$ which in turn is a function of c, since q itself is a function of c. This latter function, i.e. $q = q(c)$ is the adsorption isotherm.

3. Peak tailing cannot be reduced by smaller and more uniform particles or by dilution

The first conclusion that arises from the nonlinear chromatographic theory is that peak tailing cannot be substantially improved on by preparing smaller, spherical, uniformly sized MIP particles. This is simply so because such changes do not influence the isotherm.

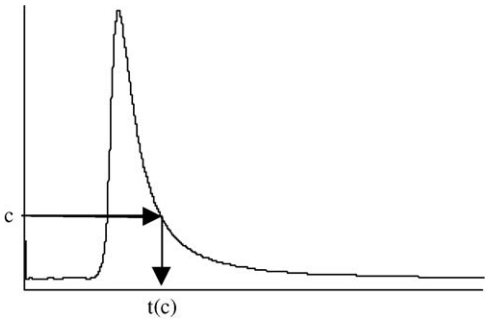

Fig. 1. A typical chromatographic peak measured with a MIP column. The arrows show a corresponding pair of c and $t(c)$ values, respectively, on the rear part of the chromatogram.

The conclusion may appear trivial, but much experimental effort for improving MIPs had gone just into better particle geometry in the hope of reducing peak tailing. The results had to be meager unless the particle size to start with was quite large, like well over 15 μm.

On the other hand, even the Guiochon group had hinted that at high dilution, the isotherms are linear. While this statement may have been valid for the particular MIP used by them, it is certainly not general. We could not reach, for example, the linear range down to about 10^{-7} M peak maximum concentrations with a phenytoin MIP. Even more importantly, there have been many reports of successfully using MIPs in homologous binding assays down to 10^{-8} M or less. Such binding assays work, however, only if the isotherm has marked nonlinearity in the measuring range [28]. Umpleby et al. [29] have also found that many of the published MIP isotherms follow the nonlinear Freundlich isotherm in the whole concentration range investigated. Thus, one can conclude that with many types of MIPs, the adsorption isotherm will not be linear even at very low concentrations. The consequence is then, that even excessive dilution of the sample and the concomitant use of more sensitive detectors, e.g. fluorescence or MS detectors, will not help to obtain less tailing of the HPLC peaks.

It is important to call here the attention to an easily misunderstood statement by experts of nonlinear chromatography. They refer to the low enough concentration range where the isotherm becomes (in their experience) linear, as the, "analytical range", to differentiate this range from the "preparative range" where the isotherm is nonlinear. As mentioned before, with many MIPs such "analytical range" may not exist practically. On the other hand, the typical concentration range where MIPs have been used lies between 10^{-10} and 10^{-1} M. For any analytical chemist, this whole range would qualify as "analytical range". This difference of vocabulary may have contributed to the misunderstandings because a typical statement from a nonlinear chromatography specialist about MIPs would be like this: "In the analytical range, the peak shape on MIP columns should be symmetrical", which is just the opposite of what an analytical chemist – with his interpretation of "analytical range" – observes in his experiments.