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Comparison of pyrimethanil-imprinted beads and bulk polymer as stationary phase by non-linear chromatography

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Abstract

A pyrimethanil-imprinted polymer (P1) was prepared by iniferter-mediated photografting a mixture of methacrylic acid and ethylene dimethacrylate onto homemade near-monodispersed chloromethylated polydivinylbenzene beads. The chromatographic behaviour of a column packed with these imprinted beads was compared with another column packed with irregular particles obtained by grinding a bulk pyrimethanil-imprinted polymer (P2). The comparison was made using the kinetic model of non-linear chromatography, studying the elution of the template and of two related substances, cyprodinil and mepanipyrim. Extension of the region of linearity, capacity factors for the template and the related substances, column selectivity, binding site heterogeneity, apparent affinity constant (K) and lumped kinetic association (k_a) and dissociation rate constant (k_d) were studied during a large interval of solute concentration, ranging between 1 and 2000 μg/ml. From the experimental results obtained, in the linearity region of solute concentration column selectivity and binding site heterogeneity remained essentially the same for the two columns, while column capacity (at 20 μg/ml, P1 = 23.1, P2 = 11.5), K (at 20 μg/ml, P1 = 8.3×10^6 M^{-1}, P2 = 2.5×10^6 M^{-1}) and k_a (at 20 μg/ml, P1 = 3.5 μM^{-1} s^{-1}, P2 = 0.47 μM^{-1} s^{-1}) significantly increased and k_d (at 20 μg/ml, P1 = 0.42 s^{-1}, P2 = 0.67 s^{-1}) decreased for the column packed with the imprinted beads. These results are consistent with an influence of the polymerisation method on the morphology of the resulting polymer and not on the molecular recognition properties due to the molecular imprinting process.

Keywords: Molecular imprinting; Pyrimethanil; Grafting; Non-linear chromatography; Affinity constant; Dissociation rate constant; Association rate constant; Selectivity

1. Introduction

Chromatographic stationary phases with predetermined and specific molecular recognition properties can be conveniently obtained using the molecular imprinting technique. This method has attracted large attention because of its versatility and simplicity of execution. Applications of the molecular imprinted polymers (MIPs) in many fields of separation science have been extensively reviewed in the recent years, with particular attention to separation of natural products [1], capillary electrophoresis [2,3], chiral chromatography [4] and solid phase extraction [5,6].

Commonly, the preferred method to produce MIPs consists of the bulk radical polymerisation of a mixture of porogenic solvent, functional monomers, cross-linkers and template molecules in a monolithic polymer. It should be crushed and accurately sieved before any practical use. This method, by far the most popular, presents many attractive properties, especially to newcomers. In fact, it is fast and simple in its practical execution and it does not require particular operator skills or sophisticated instrumentation. Bulk polymerisation method presents many drawbacks anyway. First of all, the particles obtained after the last sieving step have a highly irregular shape and are of variable dimensions. This dramatically reduces the efficiency of an HPLC column packed with such a material. Moreover, the procedure of grinding and sieving is cumbersome, and it causes a substantial loss of useful polymer, that can be estimated between 50 and 75% of the initial amount of bulk material. Last, but not least, due to its exothermical nature, bulk polymerisation cannot be scaled-up without danger of sample overheating.

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In recent years, much effort has been dedicated to developing alternative methods to prepare imprinted stationary phases, which are superior in terms of efficiency and mass transfer properties. Micrometer-sized spherical imprinted polymers with narrow size distribution have been prepared through several techniques. Suspension polymerisation in water with additives acting as microdroplet stabilizers [7–9], in presence of perfluorocarbon liquid phases [10–12], multi-step swelling polymerisation [13–15], bulk polymerisation in bead pores [16–18] have been proposed as potential substitutes of bulk polymerisation. It should be considered that all these procedures show some practical drawbacks: high sensitivity to small changes in polymerisation conditions, polymerisation medium not compatible with weak non-covalent interactions between functional monomers and templates, presence of residual emulsifier or stabilizer and high costs or procedure complexity, make a wide application of these techniques as valid substitutes to bulk polymerisation method difficult.

As alternatives to the above-mentioned approaches, surface grafting of MIP layers onto preformed beads and precipitation polymerisation in surfactant-free medium have been recently proposed as attractive and apparently general techniques to obtain chromatography-grade imprinted materials. In the first method, thin imprinted layers have been successfully used as coatings on chromatography-grade porous silica using several techniques to restrain the radical polymerisation at the surface of the beads (it should be noted that imprinting on Merrified-type lightly cross-linked polystyrene beads failed, presumably due to the low density of chlormethyl groups on the surface of the beads) [19,20]. As regards precipitation polymerisation, this technique involves coagulation of nanogel beads followed by ordered particle growth due to capture of oligomers from surrounding solution [21–23]. In this manner, near-monodispersed spherical beads can be prepared, and size and porosity can be fine-tuned thereby changing the polymerisation conditions. This technique has been reported in MIP-based competition assays [24–26] and capillary electrochromatography [27–29], but only recently works have been published, in which it is clearly shown that precipitation polymerisation can be a potentially fruitful technique for preparing chromatography-grade molecularly imprinted beads [30–32].

In this paper, we present the results obtained by coupling these two techniques to prepare molecularly imprinted pellicular beads with selective molecular recognition properties towards the template molecule pyrimethanil (*N*-(4,6-dimethylpyrimidin-2-yl)-phenylamine, **1**).

The chromatographic behaviour of HPLC columns packed with these pellicular imprinted beads or with a polymer prepared by bulk polymerisation has been investigated according to non-linear chromatography (NLC) theory, by eluting the template and the related molecules cyprodinil (*N*-(4-cyclopropyl-6-methylpyrimidin-2-yl)phenylamine, **2**) and mepanipyrim (*N*-(4-methyl-6-prop-1-ynylpyrimidin-2-yl)phenyl amine, **3**), Fig. 1. The choice of NLC theory to calculate significant parameters such as apparent affinity constant, lumped kinetic association and dissociation rate constants is justified by the nature of the peaks obtained by zonal chromatographic experiments performed on imprinted columns. These peaks usually show a marked asymmetry that can arise from a variety of sources, including column overloading, slow mass transfer processes, non-linear isotherms and the heterogeneity of the binding sites in the stationary phase [33–36]. The NLC theory copes well with this kind of peaks, provided that some experimental conditions will be fulfilled. In this paper, the NLC problem was approached using the kinetic model given by Wade et al. and Lucy et al. [37,38]. This model neglects the solute axial dispersion and it is more computationally demanding than analogous equations such as the equilibrium-dispersive model of Houghton [39], or the equilibrium model of Haarhoff and Van der Linde [40]. Notwithstanding, it presumes that the chromatographic process of adsorption proceeds on a limited number of active binding sites, that these binding sites are non-interacting and with a non-linear (Langmuirian) isotherm and slow kinetic rates of adsorption and desorption. Moreover, it is compatible with the heterogeneous binding site classes of non-covalently imprinted polymers and many protein-based affinity stationary phases (as successfully shown by the description of the binding behaviour of stationary phases based on Concanavalin A [37] and α3β4 nicotinic acetylcholine receptor [41]). On the basis of all these peculiarities, we think that the kinetic model will be suitable to characterise the binding behaviour of molecularly imprinted stationary phases.

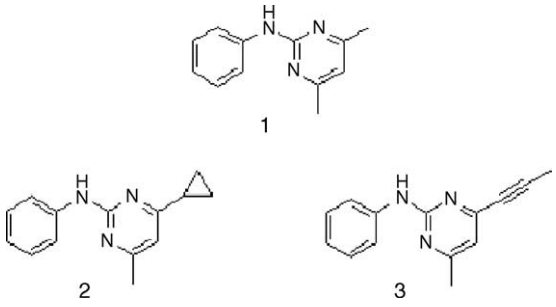

Fig. 1. Template molecule pyrimethanil (**1**) and related solute cyprodinil (**2**) and mepanipyrim (**3**).

2. Experimental

2.1. Materials

4-Chloromethylstyrene (CMS), divinylbenzene (DVB-80, 80% in *meta*/*para* isomers, remaining ethylstyrene), ethylene dimethacrylate (EDMA) and methacrylic acid (MAA) were from Sigma-Aldrich (Milan, Italy). Acetone, acetonitrile (MeCN, HPLC-grade), 2,2′-azobis-(2-methylpropionitrile) (AIBN), chloroform, ethanol (96%, v/v), formic acid, sodium *N*,*N*-diethyldithiocarbamate trihydrate (NaDEDTC)