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Retention properties of hydrophobically end-capped poly(ethylene glycol)s on a β-cyclodextrin support

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Abstract

High-performance liquid chromatography (HPLC) was used to examine the retention behavior of monomethoxypoly(ethylene glycol)s bearing one hydrophobic naphthyl end group (Nap-MPEG) on β-cyclodextrin polymer (poly-β-CD) immobilized on a silica support, under isocratic elution conditions and using water as mobile phase. Studies of retentions and theoretical plate heights *H* were conducted at infinite dilution by comparing the behavior of Nap-MPEGs having different molecular weight (750, 1000 and 5000 g/mol). The larger is its molecular size, the lower is the retention of the polymer. The linear increase of *H* with mobile phase velocity reveals slow mass-transfer kinetics arising from the restricted diffusion into the pores of the support. The complexation constants between the Nap-MPEGs and β-CD in solution (around 500 M^{-1}) were determined from the decrease of retention observed by adding increasing concentrations of hydroxypropyl β-CD into the eluent. The peak profiles in mass-overload conditions were studied by fitting a model based upon bi-Langmuir kinetics which assumes a non-uniform support having two types of binding sites and apparent adsorption rate constants are used to describe mass-transfer kinetics. A three-parameter adsorption equilibrium isotherm was sufficient to account for the modifications of peak shapes observed when increasing amounts of polymer were injected. This result indicates an interaction with a heterogeneous poly-β-CD support mainly composed of low affinity groups, non-saturable in the range of polymer concentration studied. An upper limit was estimated for the equilibrium constant (<1000 M^{-1}) characterizing the affinity of Nap-MPEG for the non-saturable sites of the poly-β-CD support. Large affinity constants (8–9 × 10^4 M^{-1}) were found for the interaction of Nap-MPEGs with a small percentage of active sites.

Keywords: β-Cyclodextrin; Poly(ethylene glycol)s; Liquid chromatography; Binding isotherm; Adsorption kinetics

1. Introduction

Recently, it was shown that supramolecular systems involving host–guest interactions allowed the immobilization of hydrophobically modified polymers or biomolecules onto β-cyclodextrin-coated materials [1–3]. β-Cyclodextrin (β-CD) is a cyclic oligosaccharide having an hydrophobic cavity. For this reason, this cone-shaped molecule is able to form inclusion complexes with hydrophobic compounds and with hydrophobically modified macromolecules [4,5]. When both the size and hydrophobicity of the hydrophobic moiety are suitable, the interaction with β-CD may be quite strong. For example, it was shown by surface plasmon resonance that adamantyl-end-capped monomethoxypoly(ethylene glycol)s (Ad-MPEG) were irreversibly adsorbed on β-cyclodextrin-polymer (poly-β-CD) modified gold surfaces when the solvent was pure water. Interestingly, the immobilized polymer layer was desorbed from the surface by using an organic solvent, demonstrating the hydrophobic character of the interaction between the hydrophobic substituent and immobilized β-CD cavities. By contrast, the formation of inclusion complexes between β-CD and naphthyl-substituted monomethoxypoly(ethylene glycol)s (Nap-MPEG) was reversible in water since the polymer layer was removed by rinsing the surface with water, demonstrating that the interaction between naphthyl substituents and β-CD cavities was much weaker than with adamantyl groups. Similar results were obtained by high-performance liquid chromatography (HPLC) when small amounts of hydrophobically modified monomethoxypoly(ethylene glycol)s bearing one naphthyl or phenyladamantyl end group were analyzed on poly-β-CD columns in the presence of pure water [6]. Ad-MPEG was irreversibly adsorbed on the support while Nap-MPEG was

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eluted from the column with peaks of almost symmetrical shape.

The aim of this work is to study the chromatographic behavior of naphthyl-substituted MPEGs, when eluted on poly-β-CD columns in isocratic elution conditions. These studies will be conducted both at infinite dilution and in mass-overload conditions. With experiments performed in the initial range of the equilibrium isotherm, the plate height theory [7,8] will be applied to investigate peak spreading as a function of flow rate. In this work, the analysis in mass-overload conditions will be conducted on the basis of a bi-Langmuir kinetic model by fitting the simulated profiles to the experimental ones for increasing sample size. These different approaches are useful for a better understanding of the retention behavior of macromolecules.

2. Theory

A number of models were developed to describe the propagation of solutes at finite concentration through a chromatographic column [9]. In this work, we shall use a model based upon a bi-Langmuir type kinetics, which assumes an adsorption on a non-uniform adsorbent exhibiting two types of binding sites. In this simplified approach, a global adsorption process is considered which includes both the effective binding process and the transport to the binding sites. The model is thus characterized by apparent adsorption and desorption rate constants. It has been used for studying the breakthrough curves of proteins on various chromatographic supports when adsorption occurs with extremely low desorption rates [10,11]. In this work, the model will be applied in the zonal elution mode, to describe the reversible adsorption–desorption exchanges between the mobile and stationary phases. In the linear range of the adsorption isotherm (small amounts injected), the macromolecules elute isocratically from the column during the time scale of a chromatographic experiment.

The theoretical profiles were generated by simulating numerically the chromatographic process. The method consists in solving the differential equation describing the solute migration through the column [12,13]:

$$\frac{\partial c}{\partial t} + u\frac{\partial c}{\partial z} + \frac{1}{\varepsilon}\frac{\partial (q' + q)}{\partial t} = D'\frac{\partial^2 c}{\partial z^2} \tag{1}$$

where z the abscissa along the column and c is the solute concentration in the bulk liquid phase. The linear flow velocity u is measured from the hold-up time of a non-sorbed compound totally excluded from the pores of the packing. The concentrations of solute in the pore solution and in the adsorbed state calculated by reference to the volume of the column empty tube are q' and q, respectively. The column void fraction is ε defined as the ratio of the flowing mobile phase volume V_e (elution volume of an excluded non-retained solute) to that of the column empty tube. D' is a global dispersion coefficient [12] accounting for both the molecular diffusion in the bulk liquid and the eddy-diffusion effect. In this work, we assumed a negligible contribution of the global dispersive effect ($D' = 0$).

With the assumption of an instantaneous equilibrium of the solute between the pores and the bulk liquid phase, we can write $q' = \varepsilon k_0 c$, in which k_0 is the solute permeation coefficient. The equation describing the solute migration through the chromatographic column is then:

$$\frac{\partial c}{\partial t} + \frac{u}{(1+k_0)}\frac{\partial c}{\partial z} + \frac{1}{\varepsilon(1+k_0)}\frac{\partial q}{\partial t} = 0 \tag{2}$$

A model with a bi-Langmuir-kinetics [10,11] was used to describe a global adsorption process by assuming the binding of the macromolecules M with two types of binding sites (X_1 and X_2). The following kinetic laws are considered for the adsorption process:

$$\frac{\mathrm{d}q_1}{\mathrm{d}t} = k_1^a[\mathrm{M}](q_1^\infty - q_1) - k_1^d q_1 \tag{3a}$$

$$\frac{\mathrm{d}q_2}{\mathrm{d}t} = k_2^a[\mathrm{M}](q_2^\infty - q_2) - k_2^d q_2 \tag{3b}$$

where [M] is the concentration of the free macromolecules in solution. The apparent rate constants for adsorption are k_1^a and k_2^a and for desorption k_1^d and k_2^d. The apparent rate constants describe a lumped adsorption process that includes the various causes of band broadening: mass transfers (transport to the sites, effective binding) and global dispersion. The concentrations of solute (quantities per unit volume of empty column) adsorbed on sites 1 and 2 are q_1 and q_2, with $q = q_1 + q_2$. The corresponding concentrations at saturation capacity are q_1^∞ and q_2^∞. The equilibrium constants characterizing the affinities of the macromolecules to both types of binding sites are $K_1 = k_1^a/k_1^d$ and $K_2 = k_2^a/k_2^d$, respectively.

In the presence of a ligand L in the eluent, which competes with the immobilized sites for the binding to the macromolecules M, the equilibrium occurring in solution is:

$$\mathrm{M} + \mathrm{L} \rightleftarrows \mathrm{ML}, \quad \text{with} \quad K_\mathrm{L} = \frac{[\mathrm{ML}]}{[\mathrm{M}][\mathrm{L}]} \tag{4}$$

where [L] is the ligand concentration and K_L is the equilibrium constant characterizing the binding of the macromolecules to the ligand in solution. We assumed there is neither adsorption of the ligand nor of the ligand–macromolecule complex on the support and an instantaneous equilibrium takes places for the interaction between the species in solution. With these hypotheses the total concentration of the macromolecules [$\bar{\mathrm{M}}$] is identical to c in Eq. (1). It is related to the concentration of the free species [M] in solution by:

$$[\bar{\mathrm{M}}] = [\mathrm{M}](1 + K_\mathrm{L}[\mathrm{L}]) \tag{5}$$

At equilibrium, $\mathrm{d}q_1/\mathrm{d}t$ and $\mathrm{d}q_2/\mathrm{d}t$ are equal to zero and the adsorption isotherm is given by the bi-Langmuir expression:

$$q = q_1^\infty \frac{K_1[\bar{\mathrm{M}}]}{1 + K_\mathrm{L}[\mathrm{L}] + K_1[\bar{\mathrm{M}}]} + q_2^\infty \frac{K_2[\bar{\mathrm{M}}]}{1 + K_\mathrm{L}[\mathrm{L}] + K_2[\bar{\mathrm{M}}]} \tag{6}$$

At infinite dilution, the retention factor of the solute is given by:

$$k = k_1 + k_2 = \frac{1}{\varepsilon(1+k_0)}\frac{q_1^\infty K_1 + q_2^\infty K_2}{1 + K_\mathrm{L}[\mathrm{L}]} \tag{7}$$