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Review

# Nuclear magnetic resonance approaches to the rationalization of chromatographic enantiorecognition processes

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Dedicated to Prof. Volker Schurig, Tübingen, on the occasion of his 70th birthday.

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ABSTRACT

NMR spectroscopy represents a valuable tool for obtaining information about structure and dynamics at a molecular level on the diastereoisomeric complexes formed by enantiomeric substrates and chromatographic chiral selectors or modifiers. Some examples collected from the literature show the potentialities of solution NMR spectroscopy in the rationalization of chromatographic enantiorecognition processes and the different NMR approaches needed according to the chiral selector features.



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## 1. Introduction

The considerable importance of chiral separation of drugs, pharmaceuticals and agrochemicals implies the need for the development of rapid, reproducible and efficient chromatographic methods. Knowledge of chiral recognition mechanisms opens new perspectives on the rational design of chiral selectors endowed with widespread applicability. Understanding of the interaction mechanisms, which are often the basis of enantiorecognition processes, relies on the exploitation of the considerable potentialities of spectroscopic methods, above all, nuclear magnetic resonance (NMR). Spontaneous assembly of molecules into non-covalently bound structured aggregates represents the basis of molecular recognition on which separation sciences rely and an understanding of molecular recognition phenomena involves analysis of different aspects of architecture and organization assembly processes.

Various chiral selectors have been used in enantioselective chromatography, such as polysaccharides, cyclodextrins, proteins, Pirkle's types selectors, alkaloids, macrocyclic antibiotics and crown ethers. Polymers imprinted with chiral templates represent tailor-made stationary phases with predictable selectivity.

Chiral selectors provide a diastereoisomeric environment for the enantiomers with formation of transient complexes, which are stabilized by a number of interactions, such as hydrogen bonds, π–π, dipole–dipole, ionic and steric interactions. Diastereoiso-

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meric derivatives of the enantiomeric substrates having different physicochemical properties are formed in the stationary phases or in the mobile phase, and are therefore separated.

NMR spectroscopy is applicable to all the most important aspects of molecular recognition phenomena. Resonances of the single different nuclei which are present in the molecular systems under investigation can be detected and several NMR parameters are correlated to structural features by means of a large number of NMR pulse sequences, which are in continuous evolution, also by virtue of the widespread availability of highly sophisticated high-field NMR spectrometers. As a matter of fact, since Pirkle's pioneering work in 1986 [1], the last 20 years have witnessed an intense synergy between NMR and chromatographic methods.

Here a general survey of NMR investigations of chromatographic enantioselective molecular recognition processes will be presented, without claiming to give an exhaustive and complete analysis of the data in the literature. Even though there are numerous valuable papers on this topic, only those examples will be selected that are relevant from a historical point of view or that highlight the ability of NMR spectroscopic techniques to satisfy the investigation needs of chiral selectors with very different structural features.

## 2. NMR methods

Primarily, NMR value relies on the opportunities it offers for the investigation of recognition phenomena at a molecular level by determination of: (a) stereochemistry and dynamics of supramolecular complexes, also including macromolecular systems; (b) evaluation of their thermodynamic parameters. The stereochemistry and dynamics of assembled species can be investigated using NOE or ROE methods [2]. The dependence of NMR parameters on concentration or temperature gradients, assisted by suitable data analysis methods, can be exploited for the analysis of thermodynamic parameters [3,4]. DOSY diffusion NMR methods [5–7] of measurement of translational diffusion coefficients D can be applied to the monitoring of assembly at the oligomer level, complementary to light-scattering techniques for larger size particles in solution or transmission electron microscopy in the solid state, by virtue of D dependence on hydrodynamic radius ($R_H$). Diffusion methods are very effective in the analysis of complexation phenomena that lead to a remarkable increase of apparent sizes of complexing species.

Anisochrony of corresponding nuclei of enantiomeric mixtures is the manifestation of chiral recognition phenomena in NMR spectra, which is due to the ability of selected chiral auxiliaries to generate their detectable diastereoisomeric derivatives.

In solutions containing mixtures of the selected enantiomerically pure chiral auxiliary (**A**) and enantiomeric mixtures of the chiral substrate (**B**), two complexation equilibria must be considered in which each enantiomer is present as a free and bound species:

$$\boldsymbol{A} + (S)\text{-}\boldsymbol{B} \rightleftarrows (S)\text{-}\boldsymbol{B}\cdot\boldsymbol{A} \quad \text{and} \quad \boldsymbol{A} + (R)\text{-}\boldsymbol{B} \rightleftarrows (R)\text{-}\boldsymbol{B}\cdot\boldsymbol{A}$$

In the fast-exchange conditions, which are frequently fulfilled, only one signal is observed for the bound and free forms of each enantiomer and the observed NMR parameter ($P^R_{obs}$, $P^S_{obs}$), Eq. (1), is the molar fraction weighted average of the same parameters in the free ($P^R_f = P^S_f$) and bound ($P^R_b$, $P^S_b$) forms

$$P^R_{obs} = X^R_f P^R_f + X^R_b P^R_b \quad \text{and} \quad P^S_{obs} = X^S_f P^S_f + X^S_b P^S_b \tag{1}$$

where $X_f$ and $X_b$ are the molar fractions of the free and bound species, respectively.

Possibility to devise interaction models relies on the availability of several local parameters, such as chemical shifts, coupling constants and relaxation rates that assume different values for the different nuclei inside the molecule and are highly responsive to local effects produced by selector–selectand interactions. Investigations of thermodynamic features of diastereoisomeric solvates not only rely on the dependence of the above-mentioned local parameters on concentration or selector–selectand molar ratios, but also on the opportunity offered by NMR spectroscopy to detect rotational or translational motions by determining diffusion coefficients or reorientational correlation times, which assume only one value (isotropic motions) for the whole molecule and, hence, are particularly responsive to the slowing-down of molecular motions due to complexation phenomena.

Analysis of complexation parameters [3] of the two diastereoisomeric solvates should include analysis of the self-aggregation propensity of both chiral auxiliaries and enantiomeric substrates, which is fundamental in selecting the optimal experimental conditions for the analysis of heterocomplexation phenomena. The presence of self-association phenomena is clearly evaluated by analysing the NMR spectra of the pure component in progressively diluted solutions: when chemical shifts depend on the concentration, the self-association constant should be determined. To this end, in more simple and common self-aggregation, i.e. dimerization, we combine Eq. (2), which defines the measured chemical shift ($\delta_{obs}$) as the weighted average of its value in the monomer ($\delta_m$) and dimer ($\delta_d$), with that of dimerization constant, Eq. (3), in order to obtain the dependence of observed chemical shifts on the initial concentration $C_0$.

$$\delta_{obs} = X_m\delta_m + X_d\delta_d \tag{2}$$

$$K_d = \frac{X_d}{2C_0(1-X_d)^2} \tag{3}$$

The dimerization constant can be determined by non-linear fittings of experimental dilution data on the basis of suitable equations (Eqs. (4)–(6) are examples) describing such a dependence [8–10].

$$\Delta_{obs} = \frac{4K_dC_0 + 1 - \sqrt{1+8K_dC_0}}{4K_dC_0}\Delta_d - \Delta_r \tag{4}$$

where $\Delta_{obs} = \delta_r - \delta_{obs}$, $\Delta_d = \delta_m - \delta_d$, $\Delta_r = \delta_m - \delta_r$ and $\delta_r$ is the chemical shift of a reference compound;

$$\delta_{obs} = \delta_m + (\delta_d - \delta_m)\frac{\sqrt{1+8K_dC_0}-1}{\sqrt{1+8K_dC_0}+1} \tag{5}$$

and

$$C_0 = \frac{(\delta_{obs}-\delta_m)(\delta_d-\delta_m)}{2K_d(\delta_d-\delta_{obs})^2} \tag{6}$$

Alternatively, diffusion coefficients, strongly sensitive to aggregation phenomena, could reveal the real nature of the self-associated forms.

The dependence of diffusion coefficients on molecular sizes is given by the Stokes–Einstein equation, Eq. (7), which strictly holds only for spherical molecules

$$D = \frac{kT}{c\pi\eta R_H} \tag{7}$$

where $R_H$ is the hydrodynamics radius, $k$ the Boltzmann constant, $T$ the absolute temperature, $\eta$ the solution viscosity and $c$ a numerical factor, which is assumed to be equal to 6 when solvent radius is significantly smaller than molecule radius; alternatively it can be suitably corrected by exploiting semi-empirical approaches [11,12]. Solution viscosity is usually considered approximately equal to solvent viscosity, even though use an internal standard is recommended [13] to correct viscosity changes caused by solute presence. Suitable viscosity standards are spherical molecules