Review

Chromatographic NMR: a tool for the analysis of mixtures of small molecules

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The addition of a solid to a mixture of small molecules introduces variations on the average translational mobility of these latter, proportional to the affinity of a given moiety for the less-mobile phase. This effect is at the basis of chromatographic separation, but can be used to simplify the NMR analysis of mixtures as well. In fact, as the induced differences in molecular mobility can span orders of magnitude, it becomes a much easier task to split the overall NMR spectrum of the mixture into one of the pure components using pulsed field gradient (PFG) methods. We have demonstrated recently this approach, in the context of HRMAS (high-resolution magic angle spinning) NMR, as required to recover high-resolution spectra in solid/liquid mixtures. In this review, we shall cover some of the principles and the practicality of this HRMAS-PFG approach for the study of mixtures. A comparison of the actual (in LC) and virtual (in NMR) separation capabilities of a few combinations of solid phase material/solvent composition shows similarities but also some striking differences: bare (not functionalized) silica expresses a superior potential for resolving spectral components than expected on the basis of the LC outcome. Copyright © 2007 John Wiley & Sons, Ltd.

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INTRODUCTION

A goal of the technological development in solid-state NMR is to produce spectra that are as close as possible to the ones obtained in liquid-state, thus removing, to some respect, the broadening interactions due to the anisotropy of the environment.

High-resolution magic angle spinning (MAS) deals with a kind of solid materials that, while being impenetrable, may still possess a good deal of microscopic mobility. The dynamics may concern just a part of the sample, as it could be the case of small molecules adsorbed on a rigid framework. In these conditions, most NMR anisotropic Hamiltonians are partially averaged out in the mobile fraction, and thus a large part of the residual line broadening in the NMR spectrum arises from susceptibility variations associated to the structural heterogeneity in the sample. Most of this effect can be span away by magic angle spinning already at moderate rates, and thus high-resolution magic angle spinning (HRMAS) can produce $^1$H spectra that rival, in favorable cases, with liquid-state (ls) NMR ones. This inclined to the introduction of standard ls-NMR procedures, such as extreme $B_0$ field homogeneity enhancement (high-order ‘shimming’) and built-in pulsed field gradient capabilities for coherent pathway selection and, accessorily, for molecular mobility studies.

We review in the following an NMR method that, relying on all of these characteristics of HRMAS probeheads, allows a simplification of the analysis of mixtures of small molecules.

NMR of mixtures of small molecules

NMR, along with mass spectrometry, is the most commonly used detector for unknown molecules of small weight due to the extremely high-resolution of the spectrum and the consequent prompt discrimination of most molecules. However, the case of mixtures remains a challenge as the overlap of the constituent peaks complicates the analysis.

Although $n$D NMR methods (including diffusometry) can provide improved resolution by spreading the signal over several dimensions, their outcome is hardly ever entirely satisfactory, and it often demands lengthy interpretation of the spectra. Therefore, hyphenated techniques have been developed to intimately marry the separative capacity of chromatography and the detection capacity of NMR (or of MS).

Although there is still some debate on the details of its mechanism, LC works by selectively delaying the constituents of a solution due to specific interactions with a solid. Inspired by this, we proposed recently an alternative approach for the characterization of the molecular constituents of mixtures. The technique that we dubbed...
Chromatographic NMR, relies on the reduction of the apparent mobility (as seen by NMR methods) of a given chemical entity, induced by the presence of a typical solid that can be found in LC columns. Then, pure NMR spectra of the single components can be extracted thanks to mobility labeling using standard pulsed field gradient (PFG) methods, according to long established procedures, but in a simplified fashion because of the enhanced spread in their values.

The successful application of this method has been possible thanks to the introduction of the above-mentioned HRMAS probes in which the pivotal solid line-narrowing method (MAS) is coupled to technology designed to deliver high-resolution NMR spectra, including pulse magnetic field gradients.

Alternatively, the required gradient pulse could be created using a microimaging unit located outside a regular MAS probehead. This latter solution allows the creation of stronger PFG to investigate slow-moving molecules such as phospholipids in membranes, but in this option the alignment of the MAS and PFG axes has to be carefully tuned.

In the following, we shall first introduce briefly the NMR ingredients (PFG and HRMAS) of chromatographic NMR as well as their limitations; then we shall cover examples of the NMR separation method and a short analysis of the conditions in which it can be used, namely in terms of solid phase and solvent composition.

**PFG and mobility measurements**

The use of pulsed linear magnetic field gradients to follow the displacement of molecules has grown, from the first experiment, to an autonomous specialty of NMR. While elaborated pulse schemes and new experimental designs are presented regularly, the basic experiment relies on NMR position/frequency labeling of the target, which is then presented regularly, the basic experiment relies on NMR position/frequency labeling of the target, which is then allowed to diffuse during a given time \( \Delta \).

For a spherical molecule moving in an unconstrained environment, the Stokes–Einstein law predicts a correlation between the hydrodynamic radius \( r \) and the self-diffusion coefficient \( D \):

\[
D = \frac{kT}{6\pi\eta r} \quad (1)
\]

where \( \eta \) is the medium viscosity.

The target for assessing the average motion characteristics of a species is thus the diffusion coefficient \( D \), which can be measured in a liquid by monitoring the intensity decay in a PFG experiment as a law of the type:

\[
I = I_0 \exp\left(-D(\nabla g)^2\right) \left(\Delta - \frac{\delta}{3}\right) \quad (2)
\]

where \( \nabla g \) is the gradient strength, \( \delta \) is its duration, and \( g \) is the gyromagnetic ratio of the observed nucleus.

Shape-associated deviations to the Stokes–Einstein law can be predicted qualitatively or quantitatively on the basis of the inertia tensor evaluation. More severe and difficult to model are the effects of an environment not allowing free evolution either because of the presence of physical barriers or of preferential diffusion directions due to a generic anisotropic environment. To summarize briefly, the mean path covered by a molecule in an environment with physical barriers is the composition of a series of steps in which the mobility rate per unit time is not constant. Thus the measured diffusion coefficient becomes a function of the time allowed for a molecule to diffuse:

\[
D = D(\Delta) \quad (3)
\]

The most typical PFG experiment is performed to minimize the role of relaxation phenomena by varying the gradient strength \( \nabla g \) rather than the time \( \Delta \). The impact on the molecular path due to physical constraints on the available space is thus not revealed at first glance, and only several such experiments at different choices of \( \Delta \) can unveil their presence.

Finally, diffusion rates in a heterogeneous medium could be varying in different compartments. If the target molecule equilibrates quickly all of its states, an average diffusion coefficient is measured.

\[
< D > = \sum_i D_i c_i \quad (4)
\]

where \( c_i \) is the fraction of time spent in the \( i \)-th phase, otherwise a multieponential character can be cast upon the decay described by Eqn (2).

**Limitation of the processing of PFG-based mobility experiments**

A specific processing of PFG-associated decay curves that has become synonymous with NMR diffusion experiment is DOSY, and so we shall just refer to this approach, which shares most of the shortcomings of other processing methods. In DOSY, on the verge of nD NMR techniques, the mobility is displayed in an independent dimension, using an appropriate algorithm to best-guess/fit the diffusion curve parameters and then tracing a corresponding spot in this specific dimension with a width linked to the error of the guess. The major difference between other 2D NMR experiments and this approach is that the inversion of an exponential decay is an ill-posed problem, and all algorithms used fail to some degree to provide an accurate response. Thus, the uncertainty on the value of \( D \) may be high and the associated resolution in the diffusion dimension limited. This limitation is exacerbated when the decay curve has a multiexponential character. Unfortunately, this is a very common case, and it may be due to: overlap of peaks of different species, sample polydispersity, co-presence of areas in the sample with different mobility, to name the most common cases.

Alternative processing schemes have been introduced to deal essentially with the enhancement of digital resolution in the diffusion dimension, but only special case solutions have been proposed for the analysis of multieponential decays, which remains an open challenge mathematically. The sum of all these shortcomings is a broadening of the diffusion dimension that has limited the applications of PFG (DOSY) experiments for the investigation of mixtures of small molecules, as only moieties with appreciable mobility differences can be properly differentiated. The method we
propose here introduces a new kink as, instead of improving the resolution in the diffusion dimension, it enhances the differences in mobility of the observed species.

**HRMAS: susceptibility broadening and achievable resolution**

A short summarizing sentence describing the resolution achievable by HRMAS in heterogeneous systems would be that the proton lineshape quality is always inferior to that of the corresponding liquid-state. This is mainly due to susceptibility broadening and, to a minor extent, to relaxation.4 To start with, a heterogeneous sample is characterized by a broad NMR spectrum, due mainly to physical discontinuities, which in turn produce local variations of the effective magnetic field and a consequent scatter in the chemical shifts.

This effect can be sketched according to the formula,

\[ B(r) = \mu_0 (1 + \chi(r)) H_0 \]

(5)

that describes the approximate induction field B experienced by a spin in a sample as a function of the applied magnetic field \( H_0 \) and of the surrounding magnetic susceptibility \( \chi \), which may vary in space. This latter is a tensorial quantity, so that a spin embedded in the medium experiences a variable field as a function of its position and orientation.15

The symmetry of the problem is such that the field induced on a spy spin by a sample volume \( \Delta V \) of susceptibility \( \chi^{AV} \) is:16

\[ B^{AV} = \left( \frac{9}{\pi} W_{10} - \frac{5}{\pi} W_{20} \right) B_0 \Delta V \]

(6)

with

\[ W_{10} = \sum_w \alpha (22l, m - m) D_{2m} X_{2m}^{AV} \]

(7)

where \( D_{2m} \) is describing a dipolar-like coupling between the volume \( \Delta V \) and the observed spin. The second term in Eqn (6) has the characteristic behavior of a second order rank tensor component, and so it can be averaged out by adequate rotation at the magic angle, similar to chemical shift anisotropy (CSA) or dipolar coupling.15 On the other hand, the first term in Eqn (2) is isotropic and thus cannot be removed by any sample reorientation, and it can be one of the causes of the residual broadening even in heterogeneous samples with high local molecular mobility. Interestingly, no higher-order rank tensor components are predicted by this formula, so HR-DOR is not expected to produce higher resolution than HRMAS. If the magnetic susceptibility is isotropic, instead, its effect amounts to just an extra effective (demagnetizing) field, which can be easily removed by moderate MAS.17,18 The presence of anisotropic susceptibility has been clearly shown in cases in which aromatic moieties are abundant in the medium, as in some resins used for solid-phase organing synthesis (SPOS).4,19

The magnitude of the susceptibility broadening naturally increases linearly with the magnetic field size, a point that should not be forgotten when designing HRMAS experiments at high field, and variable field experiment actually demonstrated that this mechanism may be the dominant one behind residual line broadening in HRMAS.19

Further enhancement of the resolution can be achieved though partial compensation for line distortions, which in MAS can be achieved by magnetic field compensation coils (shims), as in regular nonspinning or parallel spinning NMR. However, the field corrections should be now performed considering the spinning axis as the principal (quantization) direction. In ssNMR terminology, shimming in (HR)MAS corresponds to homogenizing the magnetic field in the rotor(MAS) frame, instead of the more common, field parallel, laboratory (LAB) frame. Although the tensorial shim set on the spectrometer is usually expressed in Cartesian coordinates, we shall use spherical coordinates to exemplify the question more concisely, and the two representations can be quickly interconverted using tables.20

The transformation between the LAB and MAS reference frames is achieved according to the standard use of Wigner rotation matrices.

In the hypothesis that the MAS and LAB frames share the y axis, we have:

\[ T_{nm}^{\text{MAS}} = \sum D_{mn}^{\text{mas}} (0, \theta_{\text{MAS}}, 0) T_{nm'}^{\text{LAB}} = \sum D_{mn}^{\text{mas}} (\theta_{\text{MAS}}) T_{nm'}^{\text{LAB}} \]

(8)

For example, the main correction to the magnetic field along the \( z' \) direction is the \( T_{10} \) component, which is built as:

\[ T_{10}^{\text{MAS}} = T_{10}^{\text{LAB}} = \sqrt{\frac{2}{3}} T_{3}^{\text{LAB}} - \sqrt{\frac{2}{3}} T_{3}^{\text{LAB}} \]

(9)

A similar approach can be applied to higher-order terms, resulting in the fact that the \( n \)th-order \( \theta' \) shim in the MAS frame is made up of \( 2n + 1 \) spectrometer shims. Thus, already up to seven shim coil adjustments must be engaged to achieve a basic third order correction to the field homogeneity. The shim set necessary to achieve the same level of finesse in the homogenization procedure in MAS as in solution become quickly so large that it is seldomly performed. We shall omit here a discussion on the MAS shim set perpendicular to the spinning axis, as they play a secondary role on resolution already for moderate spinning rates, and interested readers are referred to Ref. 21. Finally, note that shimming and susceptibility effects are deeply linked, as both deal with local variations of the magnetic field induction.

In summary, the composition of possible effects of anisotropic magnetic susceptibility and the difficulty of proper shimming result thus in a decreased resolution in HRMAS spectra compared to their liquid-state counterpart. However, the spectral quality may attain a level sufficient to distinguish a multiplet structure, a prerequisite for proper NMR analysis. In favorable cases, even linewidths comparable to liquid-state can be achieved, but otherwise the spectral resolution achieved HRMAS is extremely variable, depending essentially on the local mobility of the observed species and on the physical constitution of the medium.19 Interestingly, variations in mobility can be expected to induce a plethora of effects, from different relaxation times, possibly residual homonuclear dipolar broadening, to interference with the averaging MAS process. Limitations of the sample
holder volume as well as a careful design of its geometry may counter, to some extent, the contribution to the resolution loss due to variable magnetic susceptibility.

**Chromatographic NMR**

Variations in the apparent mobility of small molecules caused by interactions with larger species have long been used as one out of many NMR ways of revealing labile or stable complex formation in solution. The displacement rate of the target, registered over several milliseconds, results from the composition of the fast motion of the free molecule, characterized by a diffusion coefficient \( D_{\text{free}} \), and the slower wandering of the complexed species, associated to a smaller coefficient, \( D_{\text{bound}} \), to produce an average apparent diffusion coefficient:

\[
\langle D \rangle = D_{\text{bound}} f_{\text{bound}} + D_{\text{free}} f_{\text{free}}
\]

where \( f_{\text{bound}} \) and \( f_{\text{free}} \) are the residence fractions spent in either state.

If the slower partner is a solid, for example a silica gel, the effect is expected to be all the most dramatic. Differential affinities toward immobilized phases play a pivotal role in all mechanisms involved in separation science. It is thus natural to put to test the capacity of a chromatographic solid to improve pure NMR separation of mixtures.

**Demonstration of Chr-NMR**

A demonstration of the principle of the pseudo-chromatographic separation induced in the NMR spectra is shown in Fig. 1.7

Figure 1(A–B) concerns a set of molecules (ethanol, dec-1-ene, and napthalene) with increasing hydrophobic character and of comparable hydrodynamic ratio (as seen in Fig. 1(A), the regular DOSY spectrum). Upon addition of an octadecylsil (ODS)-functionalized silica gel, the molecules slow down proportionally to their affinity for the solid, and thus to their hydrophobic character (Fig. 1(B)).

A second test, rather based on hydrophobicity variation, is depicted in Fig. 1(C). Again, a set of molecules (heptane, ethanol, dichlorophenol) of roughly the same mobility in solution (regular DOSY shown in Fig. 1(C)) modifies its behavior drastically when contacted with bare silica (Fig. 1(D)).

**Limitations to the resolution in chromatographic NMR**

The introduction of a solid in the mixture and the use of PFG labeling for separation are both factors of limitation of the attainable resolution. Note that the actual resolution degrades in both the NMR and diffusion dimensions, due to the effects explained above, but the enhancement in difference of mobility outperforms largely the degradation in the diffusion dimension, while the NMR spectrum remains of a more than sufficient quality to be exploited. With respect to the discussion outlined above on HRMAS attainable resolution, Chr-NMR may suffer further broadening if the key exchange phenomena is not very fast on the NMR timescale or from a heterogeneous distribution of the analyte inside the sample. This latter aspect may have an impact on the susceptibility broadening, and it is currently under investigation. As explained in the previous sections, the decaying exponential behavior of PFG-based diffusometry is prone, as all curves of this type, to imprecision in the determination of the exponent. Although chromatographic NMR has been devised to bypass this aspect, it remains affected in principle by the same vice, further fostered by the possible multieponential decay induced by the anisotropic environment. For example, inspection of Fig. 1(B) and (D)

![Figure 1](image-url)

**Figure 1.** Demonstration of chromatographic NMR. The regular DOSY spectra of a mixture of molecules with increasing hydrophobicity (ethanol, dec-1-ene, napthalene dissolved in deuterated ethanol) are poorly separated (A), while the addition of a functionalized silica achieves excellent spectral separation (B). Similarly, the NMR spectra of a mixture of increasingly hydrophilic character (heptane, ethanol, dichlorophenol dissolved in cyclohexane-\( d_8 \)) are only separated upon addition of a silica gel (C), (D).

Adapted from Ref. 7, copyright (2003) National Academy of Sciences (USA).
shows that, in the apparent diffusion dimension span several orders of magnitude, and that the lines remain broad. A curious visual analogy with LC can be found in the broadest peaks in the mobility direction corresponding to the slowest compound, as it is the case for most retained compounds in LC.

**Parametrization of the separation**

Once the principle has been put forward, it becomes interesting to try and understand the rationale for the increased separation obtained in DOSY-like experiments. This is tantamount to revealing and describing all sources of modification of the apparent displacement rate observed by NMR. We are currently exploring factors based on the thermodynamics of the solid/liquid/vapor interaction as well as the effect of pure spin dynamics, and shall report in the following sections on the first results obtained in this direction.

**The role of the solid retardant**

Most LC is nowadays performed in reverse phase (RP). Chromatography on silica has quickly faded due to difficulty in reproducibility and, most importantly, to its lack of impact on the separation of hydrophobic molecules, the most important case in pharmaceutically relevant compounds. Reverse-phase chromatography typically uses solids functionalized by grafting of alkyl chains that cast a hydrophobic (and thus reversed) attitude on silica. This configuration is also suitable for testing and predicting permeability of species through cellular membranes. Consequently, hundreds of solids for RPLC are commercially available.

The mechanism of action of grafted solids in RP is highly debated as it involves the onset of several solvent/organic moieties equilibria prior to utilization, and then partition of the analyte among all these phases, composed with the driving forces behind the molecule displacement along the column (the pressure applied at the inlet, the permeability of each phase, the diffusion on the solid surface, etc.)

**Figure 2.** Surprising separation capacity of chromatographic NMR. Left panels: A mixture of aromatic homologues is easily separated by LC (top) in reversed phase conditions (ODS column and column material and eluent/solvent: ACN/water, 90/10, v/v) and by chromatographic NMR (bottom). Right panels: Coelution (top) is produced by HILIC (silica gel stationary phase and column material and eluent/solvent: ACN/water, 90/10, v/v), while a spectral separation completely analogous to the previous case is observed via NMR. Adapted with permission from Guilhem Pages, Corinne Delaurent, and Stefano Caldarelli. 'Simplified Analysis of Mixtures of Small Molecules by Chromatographic NMR Spectroscopy'. *Angew. Chem*. 2006; 45: 5950-5953. Copyright (2006) Wiley-VCH Verlag GmbH & Co. KGaA.
and the average path accessible to them. Most of these parameters can be probed only indirectly, but Albert and coworkers showed that the grafted chains on silica do play a selective role through preferential interactions with analytes. This was revealed by uncovering molecular contacts on the time scale of NOE and saturation transfer buildup.\textsuperscript{23,24} Impressively, the source of chromatographic chiral separation at least in one case could be linked to such a pairing mechanism.\textsuperscript{25} Functionalized silica may maintain an activity as an adsorbant, essentially due to residual silanols. In fact, bare silica possess at least two classes of silanols, accessible or not to water and other chemicals. Only the accessible ones are available for grafting, but once the first organic molecules start dwelling the surface covalently, a portion of the originally accessible silanols become hindered by the long organic arm and thus, it will not further react while it can still play a role in retention.

Recently, a renaissance of bare silica as a working substrate has been witnessed in LC,\textsuperscript{26} while using water/organic solvent pair as a mobile phase. This condition, [hydrophilic interaction chromatography (HILIC)] relies on a different retention mechanism. For example, the retention behavior is commonly inverted with respect to RPLC. On the other hand, chromatographic NMR, surprisingly, managed to produce a perfectly equivalent separation for a homologous series of polyaromatic hydrocarbons using either bare or ODS-grafted silica, a case for which LC produces coelution (Fig. 2).\textsuperscript{27} Although the reason behind this puzzling result has not been unveiled yet, the possibility of using bare silica to produce separated NMR spectra largely simplify the applicability of chromatographic NMR in routine analysis. Along these lines, it could be conceived that any solid or immobilized phase could be used to induce the desired separation effect in the NMR spectrum once a suspect exists of a selective interaction between this immobile object and the target molecule.

The role of the solvent

Analogous to LC, solvent mixtures can be used to enhance/modulate the target separation.\textsuperscript{28} We explored, in this sense, the effect of varying the solvent composition, an acetonitrile/water pair, on the observed mobility in chromatographic NMR of two homologous series of molecules, aromatic molecules, and ketones with a single functional group and increasing number of carbons. Figure 3 shows the variations in apparent mobility measured by PFG for the two test sets as a function of the solvent composition, both in solution and in the presence of a solid ODS chromatographic phase.

Figure 3. Evolution of the apparent diffusion coefficients of two homologous series as a function of the solvent composition in a water/acetonitrile mixture. Top: Pure solution measurements show an increase of the solute mobility as a function of the acetonitrile content for both the aromatic and ketone homologue sets, consistent with a variation in the density of the mix. Bottom: A reverse trend is observed for the same conditions as in the previous case when a chromatographic solid is added. In this case, a competition of water toward adsorption on the solid may be invoked. Adapted with permissions from Ref. 28. Copyright (2006) American Chemical Society.
The evolution of the mobility in solution is likely dominated by the density of the mix that also varies with the composition, as all molecules go faster at higher concentrations of acetonitrile. However, in the presence of a solid the trend is reversed, and all molecules tested have faster attitudes with increasing water concentration. This is opposite to what is observed in LC, where the molecules slow down with increasing water content, as this latter is meant to induce a more effective organization in the organic arm. The NMR result is, again, pointing toward a lack of dependency in this experiment on the presence of a grafted chain.

On the other hand, if silanols were the binding sites, water may be considered as an antagonist for the interaction with the solid and thus, the higher its content, the faster the test molecules will move. The reasons why this effect is not predominant in LC are still under scrutiny.

It is particularly complicated to describe with a few simple parameters the role of the mobile phase composition in LC, particularly in reversed phase conditions, and many established rules remain empirical ones, although well parametrized.29,30 Here, we would like stress that a much larger flexibility in the choice of the solvent is likely to be available to chromatographic NMR than in LC. In the latter, picking the solvent is dominated by the imperative that molecules do exit the column after a tolerable amount of time. In the case of chromatographic NMR, the major role that the solvent has is just to assure a proper solubility of the analytes, to get acceptable signal-to-noise ratios, and to assure that the analyte remains in solution even in the presence of a solid.

Other parameters at work
The details of the technicality required for a proper setup of the experiment will be the object of a specific publication. We should notice here only that sensitivity and line narrowing by HRMAS are prerequisites to obtain the spectral separation and molecule identification as wished. Moreover, several mechanisms contribute to the build-up of the average mobility when a porous solid is involved, either speeding up or slowing down the analytes. All of the above depend on the liquid-to-solid ratio, which becomes important to optimize.

Perspectives
Chromatographic NMR as an analytical tool has just begun to show its potential, particularly in the use of bare silica and of a large selection of solvents. All molecules that maintain a certain degree of mobility in the presence of a solid can be submitted to this analysis.

The investigation of part of the chromatographic process in this way has the particular advantage of bearing a visual resemblance to LC, which facilitates a comparative analysis.

Some effects deserving future exploration are those that could stem purely from spin dynamics. For instance, Chen and Shapiro demonstrated that NOE could be effective in transferring mobility encoding intermolecularly, thus producing changes in the apparent diffusion rate of the partners involved in the cross-relaxation.31

In analyzing the potential of the technique, it should be considered that the setup of effective spectral separation in Chr-NMR does share some of the characteristics of LC, mainly the need of understanding a specific separation in order to get sensible results that may include acting on the solvent, on the temperature, or on the solid.

Conclusions
This review shortly analyzed some aspects of solid-enhanced PFG/HRMAS-based methods for the study of mixtures of small molecules. Although this approach is just beginning to show its potential (and shortcomings), it appears that it can induce order of magnitude differences in the apparent mobility of molecules with similar hydrodynamic ratios. Therefore, the pure spectra of a mixture of constituents can be more easily reconstructed through mobility labeling. In principle, the technique can be used with a large variety of solvent and solid selections, which leaves an ample margin to the creativity of the analyst to find conditions effective for a desired mixture.

The separation achieved is purely spectral and so, the method is a mere complement to preparative LC. In this respect, it may serve as a prescreening test to select the best solid to provide a given separation which is a costly and time consuming step in the LC setup.

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