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NMR imaging of the chromatographic process. Deposition and removal of gadolinium ions on a reversed-phase liquid chromatographic column

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Abstract

NMR imaging permits the visualization of the adsorption processes which take place in chromatographic columns during the adsorption and concentration of strongly retained analytes from their solutions in weak solvents and during the desorption of these compounds as more concentrated bands in a strong mobile phase. The results obtained demonstrate that the surface of stationary phases is not homogeneous and that column beds are not homogeneously packed. These deviations from an idealized behavior must be taken into account in the interpretation of the results.

1. Introduction

The techniques of NMR imaging constitute powerful tools for the investigation of the various processes involved in the migration of bands along chromatographic columns. Previous publications have illustrated this phenomenon and investigated various aspects of the mechanism of band broadening [1,2]. In another implementation of the method, local values of the axial and transverse dispersion coefficients have been measured [3]. For the visualization of the various effects which take place inside the column, however, the use of Gadolinium(III) chelates is the simplest and most fruitful approach. This method is noninvasive and it allows the continuous monitoring of the bands of compounds injected in the column and the real-time analysis of these chromatographic bands directly inside the column. The method takes advantage of the enhancement by the gadolinium(III) atoms or ions of the rate of relaxation of the protons which are in their immediate neighborhood [4–6].

In spite of thirty years of intense investigations, there are still areas in chromatography which remain poorly understood. The most important includes the phenomena involved in band broadening [1–3] whose understanding is critical for improvement of the performance of columns and which are the topic of ongoing investigations. Other phenomena of importance are those related to analytical applications in which a compound contained in a solution is adsorbed at the column inlet where it is concentrated because the solution is a weak mobile

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phase. A change in the mobile phase composition towards a stronger solution allows the migration of the analyte. This procedure is common in trace analysis. Here we present a study of this phenomenon of adsorption from a weak mobile phase followed by the desorption into a stronger eluent.

2. Experimental

The equipment and experimental design used in the present study are the same as described in our previous study on the migration and separation of gadolinium bands [2]. Thus, they are only briefly described.

2.1. Liquid chromatography

A Merck Superformance (Darmstadt, Germany) glass column (12 cm bed length × 2.6 cm I.D.) was packed with Lichrospher RP18 (Merck), 15 μm average particle size, by sedimentation of a concentrated slurry of the adsorbent. The system was completed by a SYKAM S 1100 pump (Gilching, Germany), a Rheodyne sampling valve (Cotati, CA. USA) and a Linear UVIS 204 detector (Reno, NE. USA). These devices were connected to the column by plastic tubings. Care was taken to avoid the presence of even nonmagnetic metals close to the column, because they could perturb the image, and in the same time to limit the extent of the extra-column contribution to band broadening. The mobile phase was a buffer solution (see below). The water and buffer solution were not filtered before use.

2.2. NMR system

The column was placed inside a solenoid coil (transmitter/receiver coil length, 10 cm), which was positioned horizontally in the middle of the superconducting whole body imager (Magnetom 63, Siemens, Erlangen, Germany) of the University of Tübingen. This imager operates at 1.5 Tesla, corresponding to a frequency of approximately 63.6 MHz for proton imaging. A set of three mutually orthogonal coil groups can produce any desired gradient, in any direction respective to the axis of the main magnetic field, with a maximum gradient strength of 10 mT/m. The column and the solenoid which surrounds it are positioned orthogonal to the tunnel axis, i.e., to the direction of the main magnetic field. The system permits the acquisition of images of the distribution of the gadolinium concentration in any planar direction. The direction is chosen by appropriately directing the magnetic field gradient. The image can be updated every 7 s. The image of the column cross-section (26 × 120 mm) is given in a 166 × 166 mm field of view, as a 256 × 256 matrix, the individual pixel corresponding to a 0.65 × 0.65 mm rectangle of column packing. In the construction of the image, the signal is averaged over a thickness of ca. 1 mm.

2.3. Analytes

The analytes are solutions of Gd(III) and of its complexes with ethylenediaminetetraacetic acid (EDTA), *trans*-1,2-cyclohexanediaminetetraacetic acid (CDTA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), and diethylenetriaminepentaacetic acid (DTPA).

3. Results and discussion

As explained previously [2], the relationship between the intensity of the signal acquired during the imaging experiments and the local concentration of gadolinium is not linear. The signal increases with increasing concentration of gadolinium at low concentrations because the relaxation of the protons is enhanced by gadolinium (T_1 effect). The regions of the column where the concentration of gadolinium is low or moderate appear in shades of gray to white. However, at high concentrations, the relaxation is so fast that there is no signal [4–6]. Thus, the regions where the concentration of gadolinium is high appear black. The nonlinear behavior of the calibration curve explains the

images obtained and the change in appearance of the bands when their concentration varies.

In solution in pure water, Gd^{3+} associates with water molecules and forms a weakly stable complex, $[Gd(H_2O)_9]^{3+}$ or aquo complex. This complex is retained on the C_{18} bonded silica adsorbents used in reversed-phase chromatography, via silanophilic interactions with the residual silanols. Fig. 1 shows a 2D-FLASH image [7] of a longitudinal slice of the column in a horizontal plane shortly after an injection has been made. Note that in all figures, the direction of the mobile phase stream is from left to right. The sample injected is a solution of Gd^{3+}, Gd(III)EGTA, Gd(III)CDTA, and Gd(III)-DTPA. Under the conditions of the experiment (15% acetonitrile in the eluent), the concentration of Gd^{3+} is high enough to saturate the residual silanols and its retention time on chemically bonded C_{18} silica is negligible [8]. Thus, the Gd^{3+} band elutes as an unretained band. This band is seen in Fig. 1 as a thin dark black band. No signal is observed for the water protons in the vicinity of the Gd^{3+} band whose concentration is high. This is because the gadolinium ion has a very strong and efficient "inner sphere relaxation" effect [9–12] on up to the nine water molecules in the aquo complex, $[Gd(H_2O)_9]^{3+}$. Note that in Gd(III)DTPA there is one coordination site left for one water molecule. The rapid exchange with the surrounding water molecules explains the very low signal intensity in the region occupied by the band [13]. The signal is now determined by relaxation time T_2 rather than by T_1. The anomalies in the band profile have been previously explained [2].

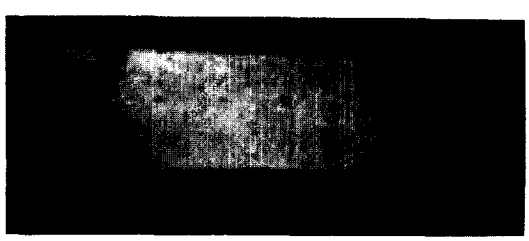

Fig. 1. 2D-FLASH image of a longitudinal, central slice of the column bed (26 mm wide, 95 mm long). The dark band in front is $[Gd(H_2O)_9]^{3+}$. Mobile phase, water–acetonitrile (85:15), 5 m*M* KH_2PO_4 and 4 m*M* octylamine, pH 5.0; flow-rate 3 ml/min (left to right). Acquisition parameters: flip angle $\theta = 90°$; repetition time, $T_R = 0.03$ s; echo time, $T_E = 10$ ms; slice thickness, 4 mm; image matrix, 256 × 256.

If the aquo complex is unretained on C_{18} silica, it is rather strongly retained on the residual silanols [14]. Thus, part of the gadolinium ions remain adsorbed on the surface for a certain time-period after passage of the band. When the concentration of gadolinium is moderate, the enhancement it causes to the relaxation of the water protons is significant but no longer as dramatic observed with the band of concentrated aquo complex. The T_1 effect dominates, leading to a higher signal [13]. As a consequence of the local presence of gadolinium, the contrast between the band of organic complexes and the column background is markedly reduced, as exemplified in Fig. 2.

To demonstrate the actual influence of the gadolinium adsorbed on the stationary phase on the separation between bands of organic complexes, the column was opened and the first centimeter of packing material was removed from the bed. Then the column was filled with an equivalent amount of virgin material from the same batch, flushed with pure acetonitrile, then with pure water. An injection of a solution of $[Gd(H_2O)_9]^{3+}$, Gd(III)CDTA, Gd(III)DOTA, and Gd(III)DTPA [15] leads to the band profiles illustrated in Fig. 3. When the mixture of chelates reaches the beginning of the column (frames 2–4), they begin to separate on the new packing material. The bands are rather concentrated at this stage and appear in black (see above, T_2 effect). They become progressively bright bands as they dilute (dominating T_1 effect). When they reach the unchanged part of the column, which is loaded with gadolinium, the bands merge into a black zone (frames 4–9). Most of the amount of analytes contained in this band seems to remain immobile, as if sticking to the old, unreplaced, packing material, while only part of it migrates slowly along the column (frames 11–16). The passage of this band darkens the column bed, meaning probably that part of the migrating chelates are strongly adsorbed on the Gd^{3+}-treated C_{18} packing materi-

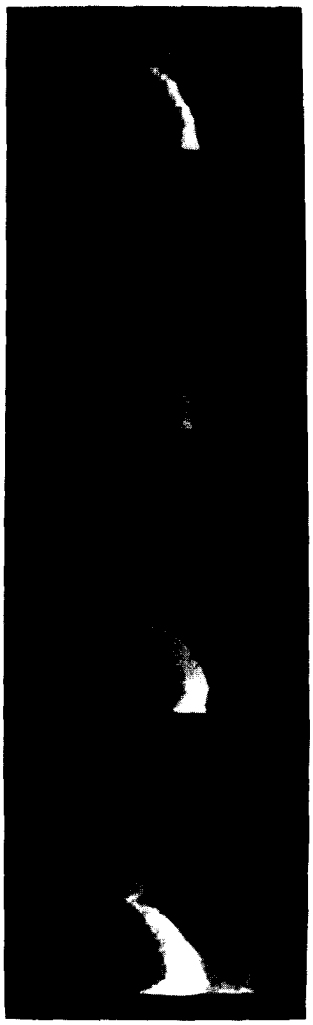

Fig. 2. 2D-FLASH images of the initial part (26 × 52 mm) of longitudinal, central slices comparing Gd(III)DTPA bands obtained at the same migration distance, for increasing amounts of gadolinium deposited on the column. Same acquisition parameters as for Fig. 1.

al. This strong retention is a combined result of the presence of residual silanols and adsorbed gadolinium ions. When the elution is over, the region at the beginning of the old material (i.e., the part of the bed not removed) is dark while the new material added has become bright (compare frames 1 and 16). The interface between new and old material is underlined by a nearly straight black line, suggesting that a certain amount of chelates has remained strongly adsorbed there (frame 10, arrow).

The simplest explanation for this behavior is a secondary retention mechamism due to electrostatic interactions with the Gd^{3+} ions adsorbed on the stationary phase of the negatively charged chelates (with formulas $[Gd(III)CDTA]^-$, $[Gd(III)DOTA]^-$, and $[Gd(III)DTPA]^{2-}$). This would explain why they exhibit a stronger retention on the stationary phase modified by the adsorption of the Gd^{3+} ions on the residual silanols than on the untreated adsorbent. Note that there is no *n*-octylamine present in the mobile phase used for these experiments (pure water), in contrast to the experimental conditions used for the data in Figs. 1 and 2 and in our previous study [2]. When present, this compound could mask the residual silanols (since it is basic), or the adsorbed gadolinium ions (because of its nucleophilic character [16]). As a matter of fact, metallic adsorption sites, apart from the residual silanol groups have been shown to be responsible also for the strong adsorption of nucleophilic organic compounds [14,17].

The washing of undesired impurities from stationary-phase surfaces has been investigated [18], with special attention to metallic ions [18–20]. It has been claimed that EDTA removes all metallic impurities from surfaces [19]. Others have found acid washing to be more effective [20]. We attempted to extract the gadolinium ions adsorbed on the C_{18} bonded silica with injections of a dilute solution of EDTA. Fig. 4 illustrates typical results obtained after the injection of a 40-μl solution. This compound is not visible. Only its effect on the local concentration of gadolinium can be recognized on the figures. When it comes into contact with the gadolinium ions adsorbed on the old stationary phase, EDTA reacts to form the complex $[Gd(III)EDTA]^-$ which is much less retained and migrates along the column. As a result, gadolinium is entirely and easily leached from the beginning of the column (new material) which darkens again (compare frames 1 and 16). It is also removed partly from the beginning of the old material section which turns from black (T_2 effect) to intense white (T_1 effect). The end

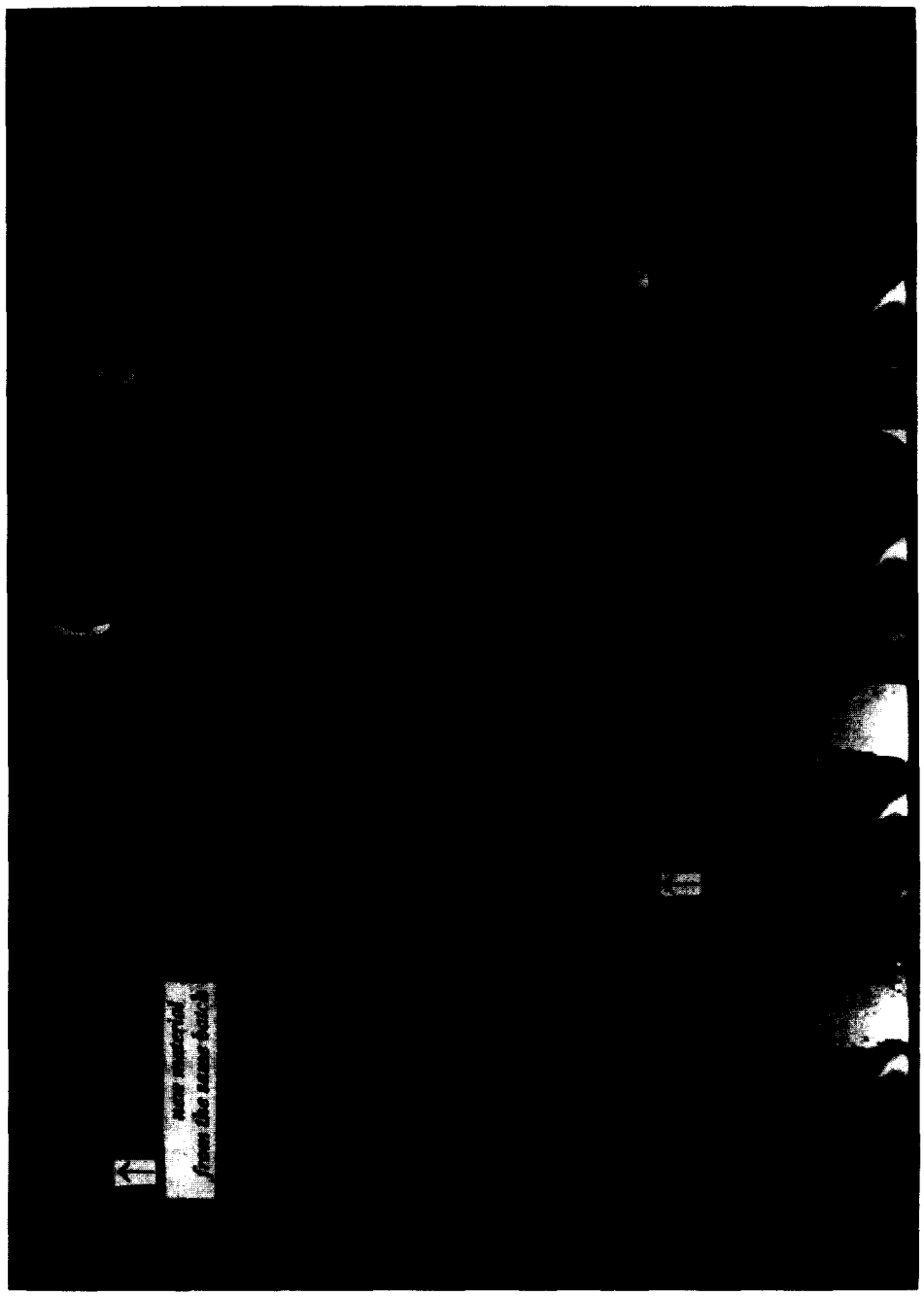

Fig. 3. 2D-FLASH images of the initial part (26 × 52 mm) of a longitudinal, central slice obtained after the injection of 50 μl of a mixture of Gd^{3+} and of its complexes with CDTA, DOTA, and DTPA. Mobile phase: pure water, flow-rate 3 ml/min. Time elapsed, 15 min between frames 2 and 9, 30 min between frames 9 and 16. Same acquisition parameters as for Fig. 1.

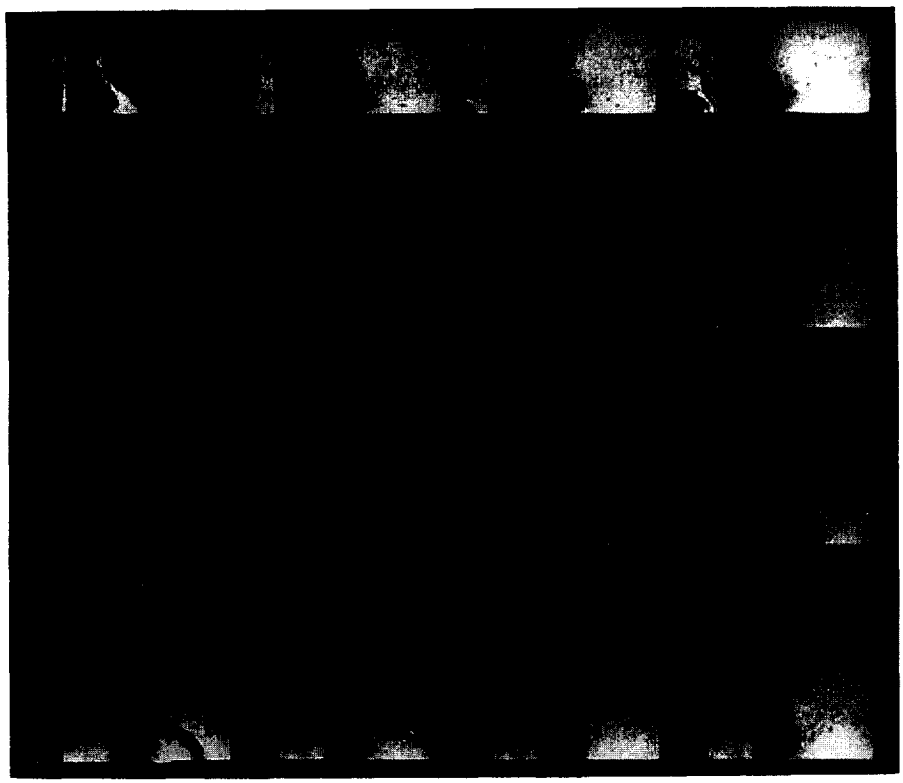

Fig. 4. 2D-FLASH images of the initial part (26 × 52 mm) of a longitudinal, central slice obtained after the injection of a dilute solution of EDTA. Flow-rate, 3 ml/min. Same acquisition parameters as for Fig. 1.

of the dark region in the old packing material remains unmodified because there was not enough EDTA in the sample. The band of newly formed complex migrates slowly across the black zone of modified material on which it is strongly retained (see above, Fig. 3). It emerges from that zone at frames 8–9 (see arrow) as a narrow concentrated band. It is much less retained on the unmodified adsorbent, migrates rapidly and disperses. Thus, it fades to gray, then disappears (frames 13–16).

Comparison of frames 2 and 16 shows the substantial removal of gadolinium by EDTA. The unexpected shape of the bands has been explained previously [2]. The center part of the inlet frit is obstructed by an accidental deposit.

Fig. 5 illustrates the washing of gadolinium from the column by flushing it with a solution of EDTA of constant concentration (0.01 *M*). The EDTA band reacts with the gadolinium ions adsorbed on the modified adsorbent, forming the less adsorbed complex which migrates slowly and accumulates, forming an intense black band which appears in frames 5 and 6 (see arrow). The sequence of images in Fig. 5a shows that there are several stages in the removal. The black part, corresponding to the zone heavily loaded with gadolinium, has disappeared in frame 6, but several fronts are seen in frames 9 and 10 and, although the material appears to be homogeneous in frame 12, the image darkens progressively until frame 16. Even then, some trace amount of gadolinium remains in the first 15 mm of old packing material, as shown by the light gray area.

Fig. 5b illustrates the kinetics of the phenomenon. The signal intensity integrated in the selected spot during the washing of the column

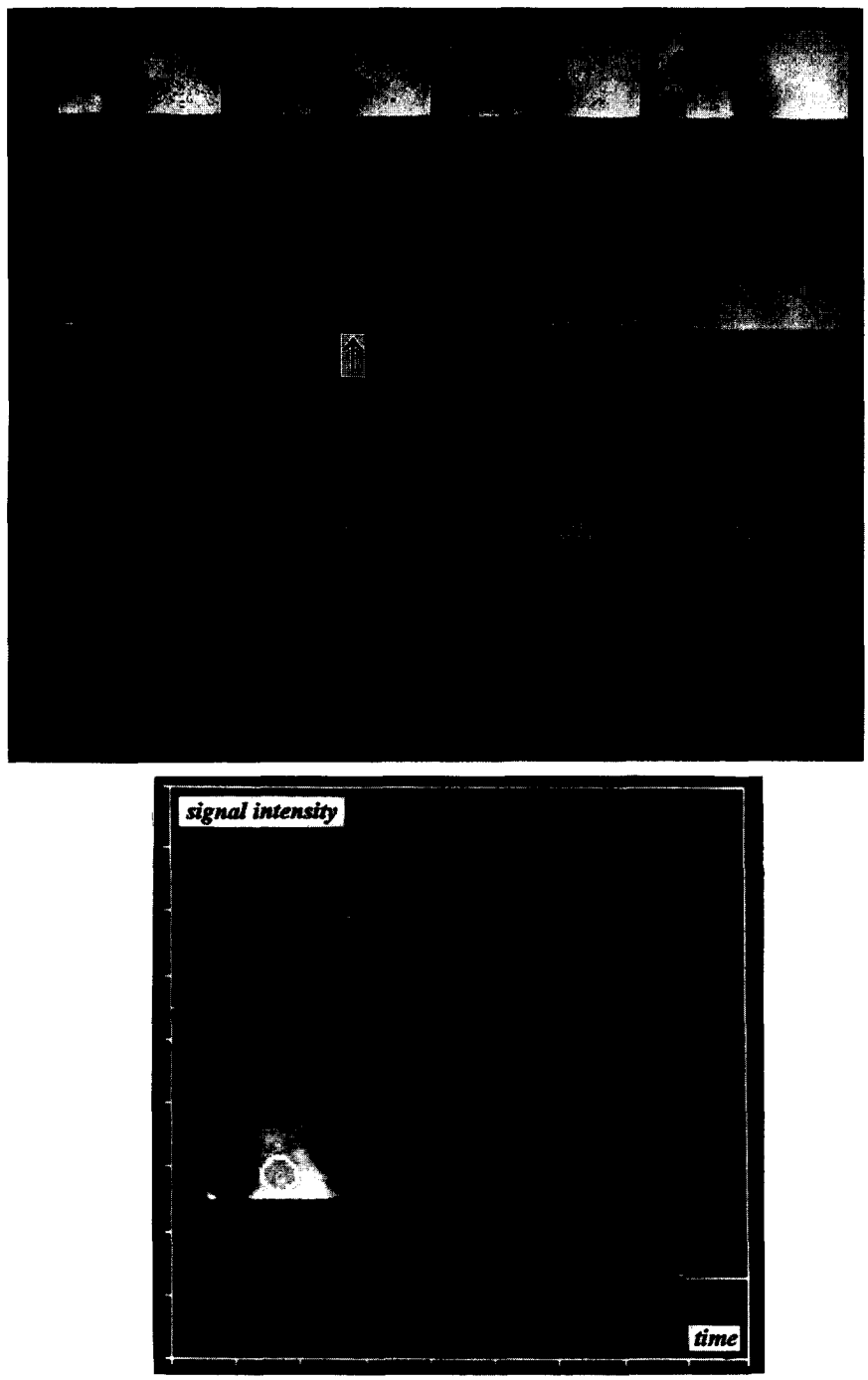

Fig. 5. Concentration distribution of gadolinium during washing of the column by a dilute EDTA solution (flow-rate, 3 ml/min). (a) 2-DFLASH images of the column bed. Time evolved between frames 1 and 13, 40 min. (b) Plot of the total signal intensity of the area marked on the image versus time. For the relationship between signal and gadolinium concentration, see text and Ref. [2]. Same acquisition parameters as for Fig. 1.

with the solution of EDTA is plotted versus time. The curve has a maximum, as does the calibration curve relating the signal intensity and the concentration of gadolinium (see explanation of the T_1 and T_2 effects, at the beginning of this section).

Washing small amounts of a strongly retained compound from a chromatographic column is not the simple process that the simplistic assumptions of linear chromatography would suggest. Stationary phases are not homogeneous. There is a wide distribution of adsorption energy on their surface. Even if we elect to assume that the retention mechanism is unique, compounds adsorbed on sites of different adsorption energies are washed out at different velocities.

References

[1] M. Ilg, J. Maier-Rosenkranz, W. Müller, K. Albert, E. Bayer and D. Höpfel, J. Magn. Reson., 96 (1992) 335.
[2] U. Tallarek, E. Baumeister, K. Albert, E. Bayer and G. Guiochon, J. Chromatogr. A, 696 (1995) 1.
[3] E. Baumeister, U. Klose, K. Albert, E. Bayer and G. Guiochon, J. Chromatogr. A, 694 (1995) 321.
[4] V.M. Runge (Editor), Enhanced Magnetic Resonance Imaging, Mosby, St. Louis, MO, 1989, Ch. 5.
[5] S. Aime, M. Botta and G. Ermondi, J. Magn. Reson., 92 (1991) 572.
[6] D.G. Gadian, J.A. Payne, D.J. Bryant, I.R. Young, D.H. Carr and G.M. Bydder, J. Comput. Assist. Tomogr., 9 (1985) 242.
[7] A. Haase, J. Frahm, D. Matthaei, W. Hänicke and K.-D. Merboldt, J. Magn. Reson., 67 (1986) 258.
[8] M.M. Vora, S. Wukovnig, R.D. Finn, A.M. Emran, T.E. Boothe and P.J. Kotari, J. Chromatogr., 369 (1986) 187.
[9] R.B. Lauffer, Chem. Rev., 87 (1987) 901.
[10] S.H. Koenig, C. Baglin, R.D. Brown and C.F. Brewer, Magn. Reson. Med., 1 (1984) 496.
[11] S.H. Koenig and R.D. Brown III, Progress in NMR Spectroscopy, *22* (1990) 487.
[12] C.A. Chang, H.G. Brittain, J. Telser and M.F. Tweedle, Inorg. Chem., 29 (1990) 4468.
[13] W. Grodd and R.C. Brasch, Fortschr. Röntgenstr., 145 (1986) 130.
[14] J. Nawrocki, Chromatographia, 31 (1991) 177, 193.
[15] K. Kumar, K.V. Sukumaran and M.F. Tweedle, Anal. Chem., 66 (1994) 295.
[16] G. Szepesi, How to Use Reversed-Phase HPLC, VCH Publishers, New York, 1992, Ch. 4.
[17] B. Buszewski, Chromatographia, 34 (1992) 573.
[18] J. Nawrocki, D.L. Moir and W. Szczepaniak, Chromatographia, 28 (1989) 143.
[19] J. Köhler, D.B. Chase, R.D. Farlee, A.J. Vega and J.J. Kirkland, J. Chromatogr., 352 (1986) 275.
[20] M. Verzele, M. de Potter and J. Ghysels, J. High Resolut. Chromatogr., 2 (1979) 151.