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Magnetic resonance imaging in reversed-phase liquid chromatography

MARTIN ILG, JÜRGEN MAIER-ROSENKRANZ, WINFRIED MÜLLER and ERNST BAYER*
Institut für Organische Chemie der Universität, Auf der Morgenstelle 18, D-7400 Tübingen (F.R.G.)

ABSTRACT

The observation of band profiles on preparative columns in liquid chromatography was hitherto not possible in real time. With the help of nuclear magnetic resonance imaging it has been possible to reveal band profiles of a reversed-phase chromatographic system completely non-invasively. The wall effect, which has been theoretically predicted, was confirmed in these proton images for the first time. Additionally, thermal effects could be revealed directly on the column. This technique is expected to have great potential for future investigations in chromatography.

INTRODUCTION

Within the last decade, nuclear magnetic resonance imaging (MRI) has become a powerful tool in medical diagnosis[1]. This imaging technique is based on the same physical properties as nuclear magnetic resonance spectroscopy, and with the help of magnetic field gradients yields an image of any desired volume element in an object[2]. At present, it is mainly limited to the visualization of protons in gel-like states. Whereas the application of MRI in the medical field has experienced rapid development, its application to non-medical problems has been less extensive and has centred largely on materials science investigations[3].

MRI, however, also offers a means of investigating elution profiles and interactions in liquid chromatography, all in real time and completely non-invasively. This is of special interest with preparative columns, where even coloured substances provide information only about the zone boundaries and afford no insight into the profile within the column. In a recent publication, we demonstrated the feasibility of using MRI for the investigation of size-exclusion and ion-exchange chromatography[4]. This paper deals with the observation of elution profiles in reversed-phase liquid chromatography (RPLC) with the help of fast proton MRI. RPLC is based on the specific and non-specific interactions between the sample, the solute and the surface of the stationary phase.

Using two-dimensional imaging, a slice is selected in the object first. The signal intensities of the picture elements ("pixels") that form the image of the slice are

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primarily dependent on the proton density and the relaxation times T_1 and T_2 in the selected volume elements of the object. Differences in these parameters lead to a high contrast image. As T_1 and T_2 are influenced differently by the interactions between eluent and mobile phase, the band profile becomes visible. As a sample we chose the diethylenetriaminepentaacetic acid–gadolinium complex, Gd(DTPA). This compound, in which seven unpaired electrons in Gd^{3+} strongly accelerate the relaxation times of the mobile phase by means of electron–nucleus interaction, resulting in high contrast images, is a widely used contrast agent in medical MRI[5]. As the mobile phase we used water–acetonitrile (80:20) because we found water to offer the best contrast for imaging the columns. With this eluent and modified silica gel, Gd(DTPA) has a capacity factor (k') of 0.05, which indicates weak interactions between the solute and the stationary phase.

EXPERIMENTAL

NMR

Experiments were performed on a 1.5-T Magnetom (Siemens, Erlangen, F.R.G.) whole-body system. The imaging technique was a 2D-FLASH pulse sequence[6]. The parameters were as follows: echo time, 10 ms; repetition time, 22 ms; flip angle, 30°; slice thickness, 5 mm; pixel matrix 256 × 256; spatial resolution, *ca.* 1 mm; and imaging time for one image, 6 s. The chromatographic column was positioned horizontally in the head coil and the images were recorded in the horizontal plane as central longitudinal slices through the column (see Fig. 1).

Chromatography

We injected 400 μl of a 10% aqueous solution of Gd(DTPA) (Magnevist; Schering, Berlin, F.R.G.) using a Merck (Darmstadt, F.R.G.) Lobar six-port valve

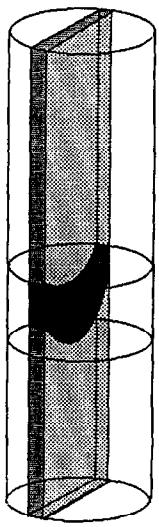
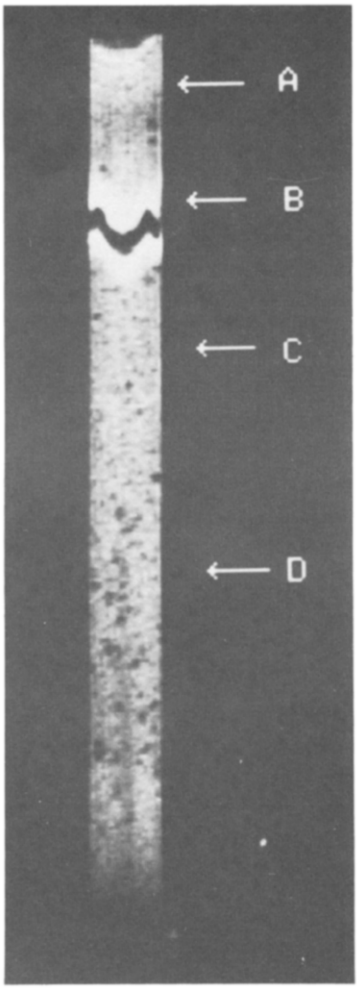


Fig. 1. Schematic diagram of the imaging slice selected in the column.



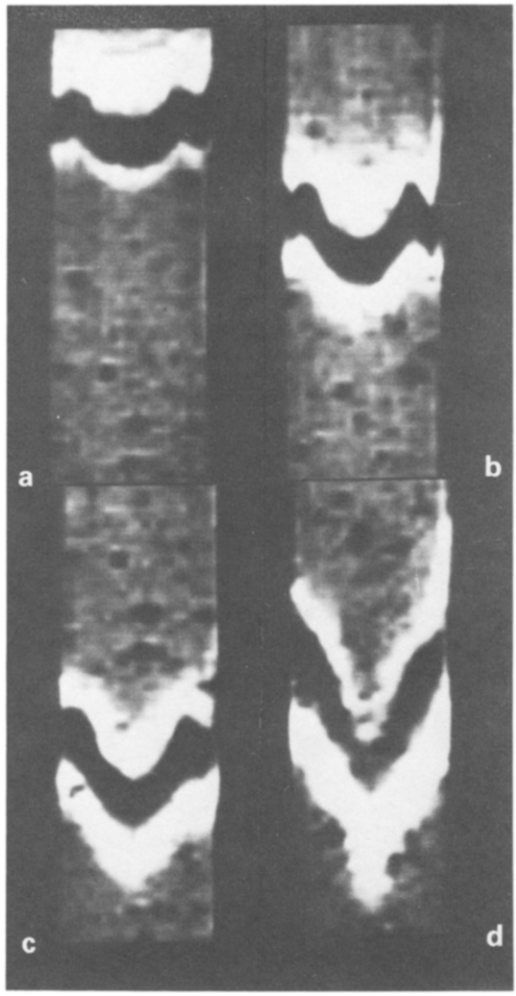


Fig. 2. MR image of the complete column.

Fig. 3. Four magnified images of the band profiles at different states of elution.

injector onto a Merck Superformance preparative glass column (300 × 26 mm I.D.) packed with reversed-phase silica gel (LiChroprep RP-8, 40–63 μm). The mobile phase was water–acetonitrile (80:20, v/v).

By means of a Bischoff (Leonberg, F.R.G.) Model 2200 HPLC pump a flow-rate of 2.0 ml/min was generated, which resulted in a pressure of 2 bar. Gd(DTPA) was detected with a Kontron (Zürich, Switzerland) Uvikon 720 LC UV–VIS detector at 215 nm. The dead volume was determined by a separate injection of uracil[7].

The elution lasted 25 min, within this time we were able to record 256 images, which were combined into a video film[8].

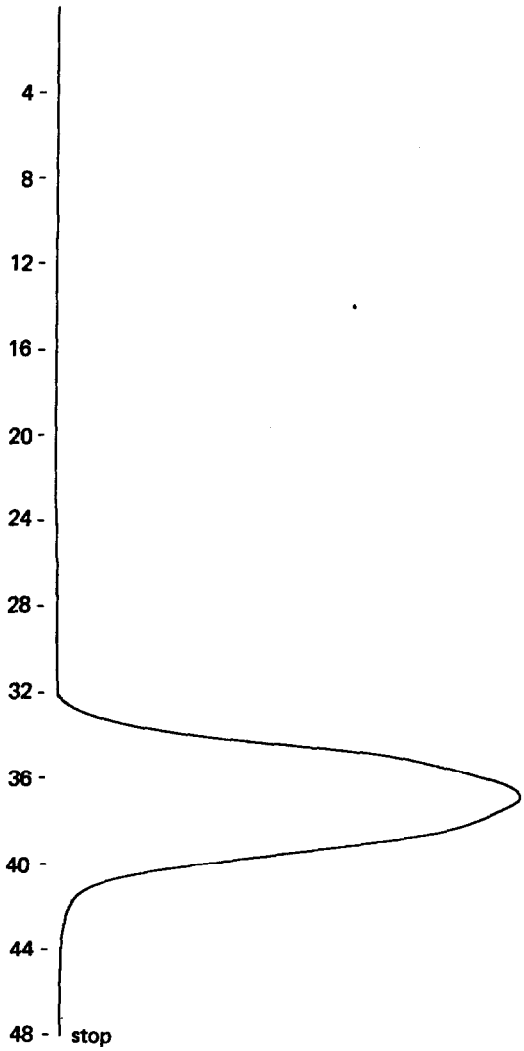


Fig. 4. Chromatogram from Gd(DTPA). Glass column (300 × 26 mm I.D.); flow-rate, 2.0 ml/min; mobile phase, acetonitrile–water (20:80); detection, 215 nm. Retention times are in min.

RESULTS

Fig. 2 shows the whole column when about one third of the elution time had elapsed. The column appears to be homogeneous, with some dark spots (diameter between 0.5 and 4 mm), which we interpret as regions that failed to be adequately wetted. The diminished contrast at the lower end of the column is due to the column exceeding the imaging range of the head coil. The chromatographic band is clearly visible. Magnified images of four selected elution profiles are shown in Fig. 3A–D. The positions of the bands on the column are indicated with the corresponding letters in Fig. 2. Fig. 4 shows the chromatogram. It is noteworthy that the band profiles were similar with flow-rates of both 0.5 and 20.0 ml/min.

DISCUSSION

The appearance of Gd(DTPA) as a black band with white edges is the result of a concentration phenomenon: phantom studies, using aqueous solutions of different Gd(DTPA) concentrations, revealed that in those regions where the concentration of Gd^{3+} is very high, the protons of surrounding liquid relax so rapidly that they are no longer detectable by MRI and the signal from this volume is eliminated. In those areas where the Gd^{3+} concentration is lower, the relaxation of these protons is slightly accelerated in comparison with uninfluenced protons . In FLASH imaging this results in a brighter signal. This effect, which is in fact the basis for the use of Gd(DTPA) as a medical contrast agent, allows an approximate estimation of the sample concentration on the column.

The interpretation of the elution profile reveals first the so-called wall effect[9,10]. This effect had been studied theoretically[11] and in practice after elution by examining the peak shapes on the chromatogram. Here it was possible for the first time to study the peak on the fly. The wall effect is mainly attributed to steel columns[12], but it also appears in glass columns. Unfortunaly, with this imaging technique we are restricted to the observation of non-metal columns and are therefore not able to compare this effect on both types of column. The wall effect is explained by a lower packing density and thus higher flow velocity in the vicinity of the walls, leading to an inverted V shaped profile. This profile, however, cannot develop completely in our system: the sample is observed to move most rapidly through the centre of the column. This effect can be ascribed to the higher temperature at the core of the column. The reason for this might be the heat of adsorption[13] of the solute on the stationary phase or the heat of friction caused by the viscosity of the solvent and pressure drop on the column[7,14,15]. The column material cannot transfer the heat rapidly enough to the walls and this thermal inertia reduces the viscosity of the mobile phase thus causing an increase in flow velocity.

Both effects counterbalance in the upper part of the column, leading to the characteristic M-shaped profile, after which the thermal effect prevails the band finally leaving the column heavily V-shaped. In addition, the band becomes broadened and more blurred on its way owing to inevitable diffusion.

Despite this band profile, the chromatogram reveals an almost symmetrical Gaussian peak. This is indicated by the calculation of the asymmetry factor:

$$T = b_{0.1}/a_{0.1} = 0.92$$

Although we have not derived any concrete figures from these first results, it is obvious that MRI offers new possibilities for basic investigations in liquid chromatography, for example to study the effects of temperature, diffusion, flow velocity and packing methods. With the help of faster imaging techniques, at present under development, we hope to be able to investigate faster elutions and systems with higher pressures in the near future.

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