Visualization of Chromatography Columns by N.M.R. Imaging

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Three-dimensional n.m.r. tomography at 80 MHz has been used to image the location of copper(II) ions on the 'Chelating-Sepharose-6B' adsorbant of an ion-exchange chromatography column; the spin–lattice relaxation rates for water on the column are: free water, 5×10^{-1} s^{-1}; water–gel, 6×10^{-1} s^{-1}; water–copper(II), 16 s^{-1}.

Apart from the behaviour of substances containing a chromophore which can be observed directly, most information concerning the performance of chromatography columns has been derived[1] from the final separation achieved. We now demonstrate the feasibility of using n.m.r. tomography (Zeugmatography[2]) to follow the distribution of substances on a chromatography column whilst it is being eluted. The method is illustrated here by the adsorption of aqueous copper(II) ions on a 70 × 5 mm column of 'Chelating-Sepharose-6B,' in which the bis(carboxymethylamino) moieties that provide the loci for metal-binding are coupled to Sepharose-6B *via* a 12-atom long hydrophilic spacer arm.[3]

The imaging method is an extension of the spin-warp procedure[4] that produces a single data matrix, $S(t_x, t_y, t_x)$ which encodes a three-dimensional, volume-image of the entire column. The raw data have three time dimensions, which correspond to the frequency co-ordinates, f_x, f_y, f_z, which in turn reflect the three spatial co-ordinates, d_x, d_y, d_z. The x and y spatial co-ordinates are phase encoded by the imaging gradients G_x and G_y, whose magnitudes are varied in a systematic fashion.[5] For N incremental values of the gradients, the magnitudes vary from $-(N-1)/2$ to $(N-1)/2$ and the spin-echo is formed by reversal of the z gradient. Signals are collected for different combinations of G_x and G_y. The first Fourier transformation produces a series of 'slices' along the z-direction. The appropriate physical location (d_z) of each slice is chosen by selecting the appropriate value of f_z. Double Fourier transformation of the corresponding two-dimensional data set $S(t_x, t_y)$ produces a slice-image, $S(f_x, f_y)$, showing the projection of the distribution of spins in the plane $S(d_x, d_y)$. These slices can have any physical orientation with respect to the column.

Our home-built imaging device is based on a 1.9 Tesla Oxford Instruments solenoid with a room temperature bore of

300 mm. The n.m.r. console is a Nicolet NT-300 unit fitted with a Nicolet 1280 computer and a 293C pulse programmer operating with imaging software developed at the University of British Columbia.[6] This software enables any combination of magnetic-gradient and radiofrequency pulses to be incorporated into a single sequence. That used here, illustrated in Figure 1, includes a 180°-pulse which is used in conjunction with the variable delay-period (P_1) to locate the magnetization of the nuclear spins along the negative z-direction, as in the conventional inversion-recovery pulse sequence;[7] however, in this case the familiar read-pulse is replaced by the imaging sequence itself.

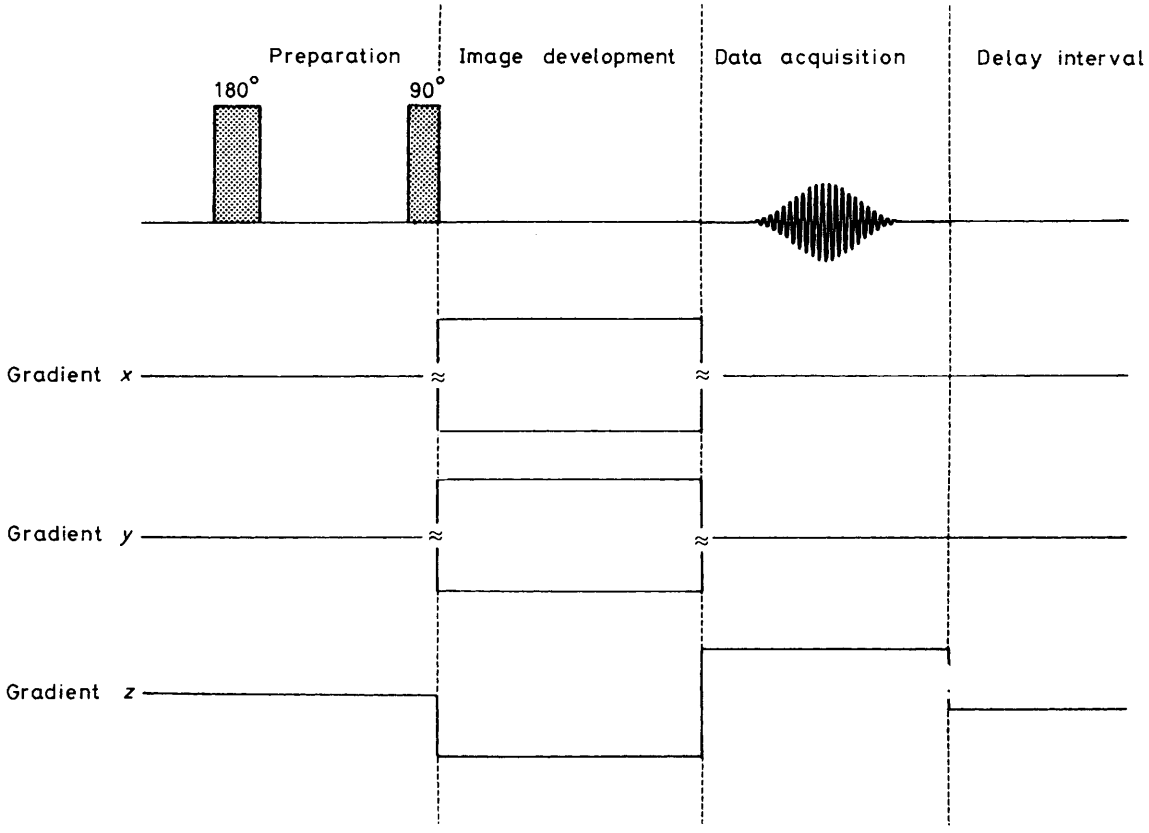

Figure 1. Pulse sequence used for three-dimensional imaging together with the inversion-recovery, preparation pulse.

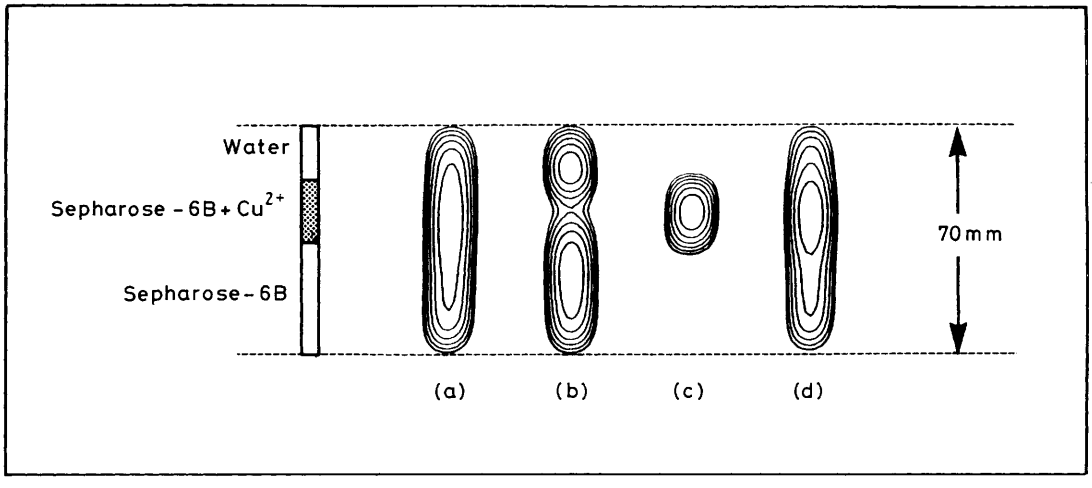

Figure 2. Single slice-images of the chromatography column mounted vertically inside a horizontal bore magnet. (a) The image was obtained using sufficiently long values for P_1 (3 s), that all the protons contributed equally to the intensity of the final image. (b) The image shown had P_1 (1 ms) chosen so that the M_z-magnetization of the water in contact with the copper(II) ions was at its null at the commencement of the data-acquisition sequence. (c) Used P_1 = 300 ms to null all but the water in contact with the copper(II) ions. (d) Shows an image with P_1 = 2 s, indicating partially relaxed water protons. All images were based on four scans, and the total acquisition time for the 16 × 16 × 512 data set was *ca.* 20 min. The gradient increments used were G_x = 0.013 G cm^{-1}, G_y = 0.019 G cm^{-1}, and the maximum value of G_z = 0.20 G cm^{-1} (1 G = 10^{-4} T).

Figure 2 shows single slice-images (0.13 mm thick) taken vertically through the glass column containing 1.5 ml of 'Chelating-Sepharose-6B' on which *ca.* 1×10^{-5} moles of copper(II) ions had been adsorbed.[3] These provide a complementary identification of the region where the copper ions are bound to the adsorbant.

Besides locating the spatial distribution of species it is also possible to characterize some of their properties. This is illustrated here by direct measurement of the spin–lattice relaxation rates (R_1 values) of the water in the chromatography column. That of free water is 5×10^{-1} s^{-1}; that of water in contact with the Sepharose-adsorbant has $R_1 = 6 \times 10^{-1}$ s^{-1}; importantly, the copper(II) ions enhance the R_1 values of water in their near-vicinity to 16 s^{-1}. These data were obtained using a split-ring resonator probe with a 90° pulse duration of 63 μs, using the null-point method.[8]

Given the limited signal-to-noise sensitivity of the n.m.r. method it is clear that direct location of complex organic substances by n.m.r. imaging will always be difficult, and often impossible; fortunately, numerous indirect strategies can be employed. That illustrated here involves the concept of a 'molecular-amplifier'; the image data are encoded in the responses of a probe species present in high concentration, whose properties are directly influenced by interaction with the target molecules, which are themselves present in low abundance. Thus, in the present example, approximately 10^{-5} mol of copper(II) ions bound to the chelating gel produces a three-fold increase in the relaxation of water. Such concepts, coupled with the use of methods to image separately different chemical species[5] offer a challenging array of new opportunities which can be applied to many chemical phenomena which occur in, or on, columns. It has not escaped our attention that some of these, such as enzymatic reactors, have substantial industrial importance.

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