Electrophoretic NMR

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1 Introduction

For more than thirty years nuclear magnetic resonance (NMR) techniques have proved to be among the most sucessful tools in the study of local structures and microdynamics in electrolyte solutions.[1,2] Moreover, NMR spin–echo experiments with pulsed magnetic field gradients (PMFG) have allowed the measurement of an important transport quantity, the translational diffusion coefficient D, for solvent molecules as well as for ionic species. The outstanding advantage of the spectroscopic NMR methods lies in their selectivity, allowing the observation of distinct species in complex systems by using selected nuclei of different elements or signals of the same nucleus but with different chemical shifts, corresponding to distinct molecules or molecular groups. Since electrolyte solutions contain at least three different constituents and are thus typical multi-component systems, it is easy to see why NMR techniques can contribute so much to our understanding of the microscopic properties of these solutions.

Bearing in mind the numerous successful applications of NMR to various problems of the physical chemistry of electrolyte solutions, it is remarkable that until the early 1980s practically all these NMR experiments had been performed on electrolyte solutions at equilibrium,[1,2] which means in the absence of an external electric field and thus in the absence of an electric current. This is astonishing because electric conductivity is the most important and the characteristic property of electrolyte solutions. Moreover, from a theoretical point of view, it is easy to see that in the study of mixtures of macromolecules, e.g. in the field of molecular biology, electrophoretic experiments combined with NMR could offer very promising new possibilities.

The reason why in the literature up to 1980 only two NMR experiments on electrolyte solutions in the conducting state were described[3,4] lies in the fact that serious experimental difficulties are to be expected when an electric current flows in the solution during an NMR experiment, in which the sample is exposed to a strong magnetic Zeeman field B_0. Clearly, there are two main problems, namely undesired additional magnetic fields and heat generation in the sample cell, both arising from the electric current. Further difficulties could be expected to arise from electro-osmosis, from the fact that electrodes have to be placed in direct neighbourhood of the NMR receiver coil, and from mechanical disturbances connected with switching of electric direct currents in strong magnetic fields.

A first attempt to observe the migration of ions in an external electric field by NMR was undertaken in 1972 by Packer and co-workers.[4] However, the above mentioned problems produced unsuccessful experiments. In 1980 the present author and his co-workers examined the feasibility of conducting NMR experiments in the presence of an electric current. First we studied[5] the influence of internal field gradients, caused by the current, on the NMR signal. Encouraged by the results we systematically investigated in the following years the applicability of certain NMR methods to measurements on ionic solutions in the conducting state.[6–10] The general goal of these investigations was the introduction of spectroscopic NMR techniques into the important area of electrochemistry and into its fields of application. Our first and most promising special aim was the measurement of mobilities $u_\pm$ of charged species in liquid solutions by applying known NMR flow measuring techniques. We also intended to examine whether and how magnetic relaxation of nuclei in ions, moving in an external electric field, can be influenced by the migration process.

In 1982 in our laboratory the first successful mobility measurements by ^{1}H NMR were performed on $(C_2H_5)_4N^+$ ions in water,[7,8] demonstrating that such measurements were indeed possible and that the data obtained agreed with results from classical methods. After further technical and methodical developments,[8,9] we showed that ^{7}Li NMR can be used to observe Li^+ mobilities.[9] Finally in 1987 we succeeded in demonstrating that the quadrupolar relaxation of ionic nuclei, e.g.. $^{23}Na^+$ and $^{87}Rb^+$, can be influenced if the ion moves in an external electric field.[10] In the following years two further groups entered this new field of NMR application. Johnson and co-workers[11] conducted mobility measurements for the first time under high-resolution ^{1}H NMR conditions and they introduced the expression 'electrophoretic NMR' (ENMR) for the method. Their group went on to develop interesting experimental and methodical improvements, e.g. a two-dimensional (2D) representation of the ENMR results.[12,13] In 1989 Strange and co-workers[14] finally demonstrated that the new technique can be used successfully for electrophoretic mobility studies on surfactant systems.

Meanwhile the potential of NMR studies in the presence of an electric direct current (DCNMR) had been impressively demonstrated and a new and promising field of NMR thus opened. The basic technology for DCNMR has now been developed, but is not yet commercially available. It should be possible for the instrumental technique to be markedly improved in the future since a number of difficulties still remain. Thus, DCNMR may be regarded as still in its infancy and electrophoretic NMR experiments, as the main application of DCNMR, do not yet have a routine character. It is the purpose of the present review to give a brief introduction to the principles and methods of electrophoretic NMR, to discuss in general the practical problems of DCNMR (and the solutions found so far), and to give an overview on measurements, applications, and possible future developments of this new class of NMR experiments. It should be pointed out that in this article the term 'electrophoresis' is used in a general sense, namely as the term for the migration of all kinds of particles in an electric field.

Manfred Holz was born in Ludwigshafen/Rhein, Germany in 1938. He attended the University of Karlsruhe, where he obtained his 'Diplom' in physics in 1966 under the supervision of G. Laukien. From 1966 to 1970 he worked for the Bruker company, where he was member of the team that developed the first commercial pulsed NMR spectrometers. He then moved back to the University of Karlsruhe to carry out doctoral research under Professor H. G. Hertz. He is now lecturer and 'Akademischer Direktor' at the Institute of Physical Chemistry. His research interests are in the development and application of NMR techniques, such as intermolecular relaxation, NMR diffusion measurements, and electrophoretic NMR, to the study of liquid systems.

2 Principles of DCNMR Experiments

2.1 Measurement of Ionic Drift Velocities by NMR

In an electric field E between two electrodes in an electrolyte solution, ions move with a drift velocity $v_\pm$ along the direction of the electric field. In electric fields of 10 to 100 V cm^{-1} these drift velocities are in the range of 10^{-4}—10^{-2} cm s^{-1}. That means we are dealing with very slow motions, which can be considered as a field-assisted diffusion process. The drift velocity $v_\pm$ is related to the ionic mobility $\mu_\pm$ by:

$$v_\pm = \mu_\pm \cdot E \tag{1}$$

This means that a knowledge of E and the measurement of $v_\pm$ allows the determination of the electrophoretic mobility $\mu_\pm$. If we further measure the electric current I flowing in the electrophoresis cell during the experiment, we obtain an important single ion transport quantity, namely the transference number $T_\pm$ of the ion of interest in strong electrolytes:

$$T_\pm = \frac{cAF}{I} \cdot v_\pm \tag{2}$$

where c is the salt concentration in moles per litre, F is the Faraday constant, and A is the area of cross-section of the cell. The drift velocity of single charged constituents in an electrolyte solution delivers important characteristic quantities of the system of interest.

In the early years of NMR it had been discovered that a translational displacement of particles carrying a nucleus with spin I can be detected by the NMR spin-echo technique, when a linear magnetic field gradient G is applied.[15] The spin-echo is observed at the time 2τ after a $\pi/2$–τ–π radiofrequency (r.f.) pulse pair. Since the NMR precession frequency ω_0 is strictly field-dependent, according to $\omega_0 = \gamma \cdot B_0$ (γ = gyromagnetic ratio; B_0 = magnetic Zeeman field), a translational motion of the spin-carrying particle along an imposed field gradient is connected with a change of the NMR frequency of the nucleus. The displacement of the nucleus in the direction of G during the time 2τ results in a loss of nuclear spin phase coherence, compared with a non-moving nucleus, and in a change of the echo amplitude at 2τ compared with a spin-echo experiment without an imposed field gradient. The physical origin of the displacement may be different. Incoherent translational motions, like translational diffusion, produce a random dephasing of the spin precession during 2τ, resulting in a damping of the echo amplitude $M(2\tau)$.[16] Hence, to the exponential decay of the transverse nuclear magnetization by spin–spin relaxation a second term due to the diffusion is added and we obtain:

$$M(2\tau) = M(0) \exp[-2\tau/T_2 - \tfrac{2}{3}\gamma^2 G^2 D \tau^3] \tag{3}$$

with T_2 = spin–spin relaxation time, D = self-diffusion coefficient.

Coherent motion along the field gradient, e.g. due to plug flow or due to a constant drift velocity, produces for all spins during 2τ the same frequency change, that is the same phase shift with respect to a reference frequency. Consequently, a constant drift velocity causes no dephasing but a *phase shift* of the echo signal, and this phase shift can be easily derived.

Assume we apply a magnetic field gradient along the z-direction, that is $G = \partial B_z/\partial z$. If the nucleus e.g. in an ion moves with constant velocity v along the z-direction, if the electric field E is applied in this direction, the nucleus experiences a time-dependent magnetic field $B(z(t))$, since its position z is a function of time; namely $z(t) = v \cdot t$. From:

$$B(z(t)) = B_0 \cdot z(t)G \tag{4}$$

it follows: $\omega(t) = \gamma B_0 + \gamma G v t = \omega_0 + \gamma G v t \tag{5}$

The frequency difference $\Delta\omega(t)$ with respect to ω_0 is:

$$\Delta\omega(t) = \gamma G v t \tag{5a}$$

This frequency difference leads to an accumulated phase difference $\Delta\phi_{2\tau}$ at the time 2τ, when the echo maximum appears:[17]

$$\Delta\phi_{2\tau} = \gamma G v \tau^2 \tag{6}$$

It is in principle this phase shift which has to be measured in order to determine the velocity v by NMR. The shift causes a cosine modulation of the echo amplitude at 2τ when G or v are changed. Therefore, if both diffusion and a drift velocity control the translational motion, the transversal magnetization $M_v(2\tau)$ is given by:

$$M_v(2\tau) = M(0)\,\cos(\gamma G v \tau^2)\exp[-2\tau/T_2 - \tfrac{2}{3}\gamma^2 G^2 D \tau^3] \tag{7}$$

In modern NMR diffusion and flow studies, pulsed magnetic field gradients (PMFG) are used instead of a steady gradient, as shown in Figure 1 and as introduced and analysed by Stejskal and Tanner.[18] The two gradient pulses with a duration δ, an amplitude G, and a distance Δ are mainly applied for practical experimental reasons. In such a PMFG experiment, equation 7 is slightly modified to become:[4]

$$M_v(2\tau) = M(0)\cos(\gamma G v \delta \Delta)\exp[-2\tau/T_2 - \gamma^2 G^2 D \delta^2(\Delta - \tfrac{1}{3}\delta)] \tag{7a}$$
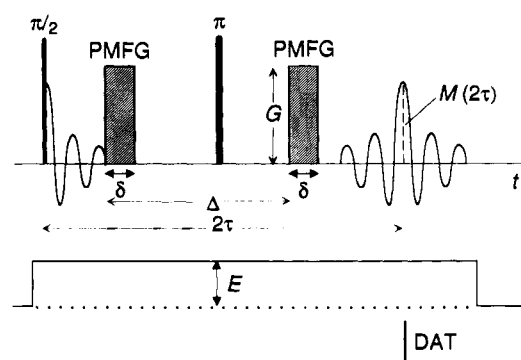

Figure 1 Pulse sequence of the standard pulsed magnetic field gradient (PMFG) spin–echo experiment for the measurement of drift velocities of charged species. $\pi/2$ and π denote the corresponding rf pulses; G is the strength of the magnetic field gradient; E is the strength of the pulsed electric field, and DAT is the data acquisition trigger.

In practical electrophoretic NMR experiments one can determine the ratio

$$M_v(2\tau)/M(2\tau) = \cos(\gamma G v \delta \Delta) = \cos(\Delta\phi_{2\tau}) \tag{8}$$

by measuring $M_v(2\tau)$ in presence of an electric field E ($v_\pm \neq 0$) and measuring $M(2\tau)$ in absence of E ($v_\pm = 0$). For reasons of measuring accuracy the amplitude ratio, or directly $\Delta\phi_{2\tau}$ in equation 8, is commonly determined as a function of one of the quantities δ, G, or v where v is proportional to the applied electric field E and to the current I in the sample.

2.2 Measurement of Relaxation Times in the Presence of an Electric Current

For electrolyte solutions, the case where magnetic relaxation of nuclei residing in ionic species, which move in an external electric field, is changed, has been theoretically analysed for the first time by Atkins and Clugston.[19] These authors considered dipole-dipole relaxing nuclei such as ^{1}H and ^{19}F and a counterion carrying an electron spin. Their calculations revealed that only extremely high electric fields ($\geqslant 10^7$ Vm^{-1}) would produce remarkable changes in the nuclear magnetic relaxation times caused by the change in the dynamics of the interacting partners. The present author pointed out that the *quadrupole* relaxation of ionic nuclei might be the more interesting relaxation mechanism[2,10] for investigations in conducting electrolytes, where at

lower electric fields structural effects could be expected, which could alter the relaxation times.

The experimental method is not of a special nature. The measurement of NMR relaxation times is performed with the known pulse sequence e.g. for T_1 by the inversion–recovery $(\pi-\tau-\pi/2)$ method. The only difference from a common NMR relaxation experiment is that during the time τ, in which the relaxation process is going on, an electrical field is applied between the two electrodes and thus a current flows in the electrolyte solution (Figure 2). Consequently, these relaxation measurements can be made with any probe which is suited for DCNMR experiments.
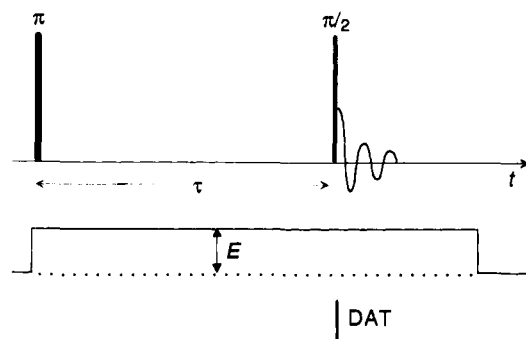

Figure 2 Pulse sequence for a DCNMR T_1 relaxation measurement by the inversion–recovery method. The pulses are denoted as in Figure 1.

3 DCNMR: Technical Requirements, Problems, and their Solutions

We saw that NMR in the presence of an electric current in principle only requires the application of an electric field between two electrodes within the sample cell. Thus for DCNMR experiments a constant current power supply (e.g. 0—500 mA, 0—1000 V) is needed. Since for practical reasons the electric field is only applied for certain time intervals (see Figures 1 and 2) a gateable supply is needed. Gate pulses for the power supply are usually derived from the computer system of the NMR spectrometer, and they are then part of a synchronized pulse sequence consisting of r.f.-pulses, magnetic field gradient pulses, electric field pulses, and data acquisition pulses. For electrophoretic measurements, where the PMFG-technique is applied, it is clear that the NMR probe must be equipped with a field gradient coil system. Modern shielded gradient coil systems[20] are recommended because they avoid problems with magnetic field disturbances due to eddy currents following the gradient pulses, allowing for example short gradient pulse distances Δ and pulses with high gradient strength G to be applied. These two conditions are particularly important if electrophoretic measurements are performed on systems with small T_2-values.

The electrodes used are in most cases made of platinum wires or sheets. If solutions of halides are to be investigated Ag/AgX (X = Cl, Br, I) electrodes are advantageous. More technical and methodical details may be found in the original literature[5—14] and in a comprehensive review on electrophoretic NMR which appeared some years ago.[21] The special problems connected with the presence of an electric current in the sample during the NMR experiments, and the technical solutions of these problems, are now briefly discussed.

3.1 Magnetic Fields Induced by the Current

From classical physics we know that an electric current produces a magnetic field in the plane perpendicular to its direction of flow. Hence, in DCNMR experiments also, additional magnetic field gradients are produced in the sample which can interfere with the Zeeman field B_0. However, as our investigations showed,[5,8,9] the expected difficulties can be overcome in a

relatively easy way. The simplest solution of the problem is to choose as the current direction the direction of the magnetic main field B_0 (z-direction). Consequently, the magnetic field lines produced by the current lie in the x,y-plane. There are no field gradients in the z-direction, which is the relevant direction in NMR, and therefore no signal disturbances can occur. In modern superconducting magnets the probe and the sample tube axis lie in this z-direction and therefore it is in practice very simple to achieve a parallel alignment of current and magnetic field.
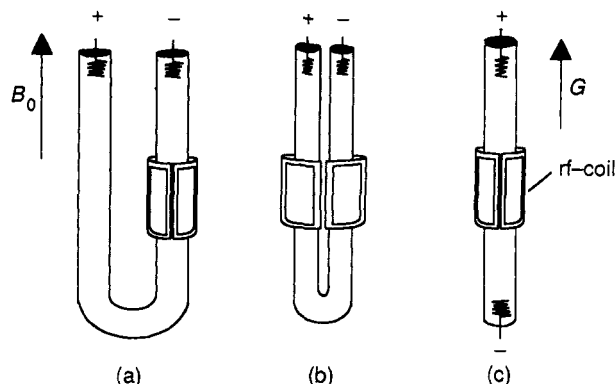

Figure 3 Three basic DCNMR cell geometries, (a), (b), (c), with the corresponding electrode arrangements. The rf coil is a saddle coil as normally used in superconducting magnets. The direction of the Zeeman field B_0 and of the imposed field gradient $G = \partial B_z/\partial z$ is also shown.

In Figure 3 three general electrophoretic cell geometries are shown together with the corresponding electrode positions and r.f. saddle coil arrangements. In all these cells the current I flows parallel to B_0. However, we recognize that there are important differences. In the two U-tube-type cells in Figure 3(a and b) the current I flows parallel to B_0 (and in the direction of the applied gradient G) within the r.f. coil region, but in the range of the knee of the U-tube the direction of I is perpendicular to B_0. Thus the disadvantage of cells (a) and (b) lies in the fact that z-field gradients can act within the coil volume, where the NMR signal is generated. This undesired effect may be reduced if the knee of the U-tube cell is not too near to the r.f. coil. The advantage of cells (a) and (b) is that both electrodes are in the upper part of the cell and are vented to the atmosphere, a fact which is important if the electrodes are gassing and bubbles occur. In order to minimize the induced field gradients, the best solution is the cylindrical cell (c), but gassing of the lower electrode has to be avoided. Finally, one has to keep in mind that the electrical connections to the electrodes should be kept, as far as possible, parallel to the B_0 direction. It should be pointed out that the cell shown in Figure 3b is an example of the counterflow technique, previously developed by my group,[7] where within the measuring volume, the sample coil, the current flows in both directions. A disadvantage of the counterflow arrangement is the loss of information with respect to the sign of the mobilities.

If electromagnets must be used or if from a theoretical point of view[19] an ionic flow perpendicular to the B_0 field is to be observed, internal field gradients in z-direction will be induced. However, this problem can be solved. As shown in a previous work these induced gradients are linear[5] and, as demonstrated experimentally,[8] the current through the electrophoretic cell can be led through shim coils outside the sample in such a way that a self-compensating system is achieved, where the external gradients from the shims compensate the internal gradients.

In summary, the problem with the additional magnetic field gradients in a DCNMR experiment is practically solved and today is of minor importance. However, in a general context it should be pointed out that field gradients produced by a current in the sample may be utilized, for example if current distributions in complex conductors are the subject of interest.[2,5]

Meanwhile, NMR imaging experiments with the aim of mapping electrical circuits have been successfully commenced.[22]

3.2 Problems Caused by Resistive Heating, Mechanical Disturbances, and Electro-osmosis

Serious problems in electrophoretic NMR can arise from heating of the sample by the electric current. There are two possibilities for reducing resistive heating: (i) The use of pulsed electric fields. This means that the current is applied only during the actual measuring time 2τ in the spin-echo experiment or during the time interval τ in a T_1 experiment. (ii) The use of an efficient cooling system.

The quantity $\Delta\phi_{2\tau}$ (see equations 6 and 8) is proportional to $v_\pm$. Thus, for reliable measurements of mobilities, high $v_\pm$ values and therefore high electric fields E are desirable. On the other hand, without effective cooling the temperatures increase ΔT by resistive heating is proportional to E^2. Therefore one must find a compromise with respect to these two effects. In practice, electric fields of about 1—100 V cm^{-1} are applied for a duration from 50 ms up to ca. 1 s, depending mainly on T_1 and T_2 of the nucleus under observation. The cell volumes used are typically 0.5—5 cm^3. Depending on the conductivity and the heat capacity of the sample, theoretically the temperature in the cell can increase by ca. 0.01 to 1 °C under the above conditions during one current pulse.[10,21] Since in most cases signal accumulation is performed, accumulation of resistive heating can also result. These facts clearly reveal the importance of the thermostatting system in practical DCNMR.

A temperature rise has two consequences. First, through the temperature dependence of translational molecular motions in liquid systems the mobilities and relaxation times are changed. Secondly, a much more serious problem can arise through convection. Any macroscopic motion in a liquid system (convection, vibrations, or shock waves) can cause dramatic measuring errors in diffusion or mobility measurement by the spin-echo method. The reason lies in the fact that with ionic mobilities in the range of $u_\pm = 5 \times 10^{-8}$ m^{2}s^{-1}V^{-1} for example, typical velocities of $v_\pm \sim 10^{-5}$ ms^{-1} are measured and thus with $\Delta = 300$ ms in a PMFG experiment an actual displacement of the ionic species of 3 μm is measured! This figure clearly demonstrates why convective motions or mechanical disturbances must be strictly suppressed.

In order to stabilize the solution against 'macroscopic' motions like convection, agar–agar and some other gelling agents were tested and successfully used in aqueous solutions by my group.[7,8] The investigation showed for example that for $(C_2H_5)_4N^+$ ions in aqueous solutions with 1 wt% agar an obstruction effect of only 1% is observed. Since, as we will see, most mobility and diffusion measurement are performed relative to a standard system under identical stabilization conditions, the obstruction effects can be neglected at reasonable agar concentrations. We recently succeeded in applying a porous sinter glass as stabilizer for a non-aqueous electrolyte system.[23] However, this stabilizer can only be used if no high resolution spectra are required. Johnson and co-workers[21] proposed using fibrin, a material with large pores, which might be ideally suited for medium-sized proteins. However, it should be emphasized that a number of successful measurements, for example in microemulsions with low conductivities, have been performed in free solution without a stabilizer,[21,24] showing that with low currents (0—10 mA) the above mentioned problems are markedly reduced.

We saw that mechanical disturbances can also cause serious problems in ENMR. One source of those disturbances is DC pulses, which are switched on and off during the experiments. If the electrical connections to the electrodes and electrodes themselves are not exactly parallel to the B_0 field, strong forces can act on these components and therefore the DC switching can produce shock waves in the solution under investigation. Special care has to be taken therefore rigidly to fix these electrical connections at the probe. The same is true for the gradient coils

and their connecting wires. The effect of small vibrations of the electrodes alone is avoided by the gel stabilization, but shocks arising from the other electrical DC connections can only be eliminated by a good mechanical stability of the whole probe system.

A further possible difficulty for electrophoretic NMR measurement can come from electro-osmosis. If the walls of the electrophoretic cell are untreated and therefore charged in the presence of an electrolyte, this electrokinetic effect has to be taken into consideration. Then, as discussed in detail elsewhere,[21] in most electrophoretic experiments and also in ENMR, a diffuse layer, including the solvent, flows along the cell walls and generates a counterflow in the centre of the cell. Similar problems to those encountered with convection can then result – but by using stabilizers these electro-osmosis effects can also be circumvented. Johnson and He[21] described how to solve the electro-osmosis problem in non-stabilized solutions by carefully coating the cell surface with a polymer like methylcellulose. In this way, in their ENMR experiments in free solutions, the electro-osmosis flow could be reduced to zero. Thus this problem may also be regarded as basically solved.

Finally, we come to a special problem in connection with DCNMR *relaxation* studies. Macroscopic movements in the solution caused by convection or electro-osmosis can also play a role in measurements of the relaxation time T_1. As can be seen from Figure 3 for example, in DCNMR cells there is always electrolyte solution above and below the r.f. coil volume. This fact has to be taken into account if relaxation measurements are performed. The first π or $\pi/2$ pulse in the inversion-recovery or saturation-recovery pulse sequence, respectively, which changes the population of the Zeeman levels, acts only inside the r.f. coil volume. Thus, for example, after a $\pi/2$-pulse, the nuclear magnetization inside the coil volume is saturated, but outside it is not. If during the relaxation delay τ in a $\pi/2$–τ–$\pi/2$ sequence, convection or electro-osmosis transports species of interest from outside the coil volume into this volume, unsaturated magnetization flows into the r.f. coil and is detected by the second $\pi/2$-pulse, resulting in an apparently shorter relaxation time T_1.[10,25] This effect can cause considerable measuring errors, if the relaxation time T_1 is relatively long and therefore long τ-values have to be applied. With a typical coil length of 1 cm and a convection velocity of 0.25 cm s^{-1}, a T_1 of 50 ms is decreased by 3% and a T_1 of 500 ms by 23%.[26] These effects have been experimentally observed and it has also been shown that this undesired influence on the NMR T_1 measurement can be suppressed by using a porous sinter glass,[26] as described above for the ENMR experiment. In DCNMR relaxation experiments, two glass filter discs at the immediate upper and lower ends of the coil are sufficient to eliminate inflow and outflow of magnetization by convection. It should be mentioned that in every DCNMR experiment, due to the drift velocity in the electric field, magnetization transport into the coil and out of the coil does occur. However, at the electric fields normally applied the drift velocities are so small that this effect can be neglected.

3.3 DCNMR Cells

In Figure 3 we saw the possible basic geometries of DCNMR cells. Three cells in practical use today are now described. In the early days of DCNMR, U-tube cells with counterflow were preferred[7—9,11,12], but now the tendency is towards sample cells of cylindrical geometry (Figure 3c). In Figure 4a a cell is shown, designed by Coveney et al.,[24] which has easily detachable electrodes. These are Pt-blacked electrodes, in order to prevent gas evolution. For reduction of electro-osmosis effects the perspex tube has a glass sleeving. This electrophoresis cell has been used in connection with a custom-built probe. Another interesting technical solution, by Morris and Johnson,[27] is shown in Figure 4b. It is a concentric cylindrical electrophoresis chamber having the advantage that both electrodes are in the upper part of the cell and thus vented to the atmosphere. The actual sample of interest is in the inner tube, separated by a gel
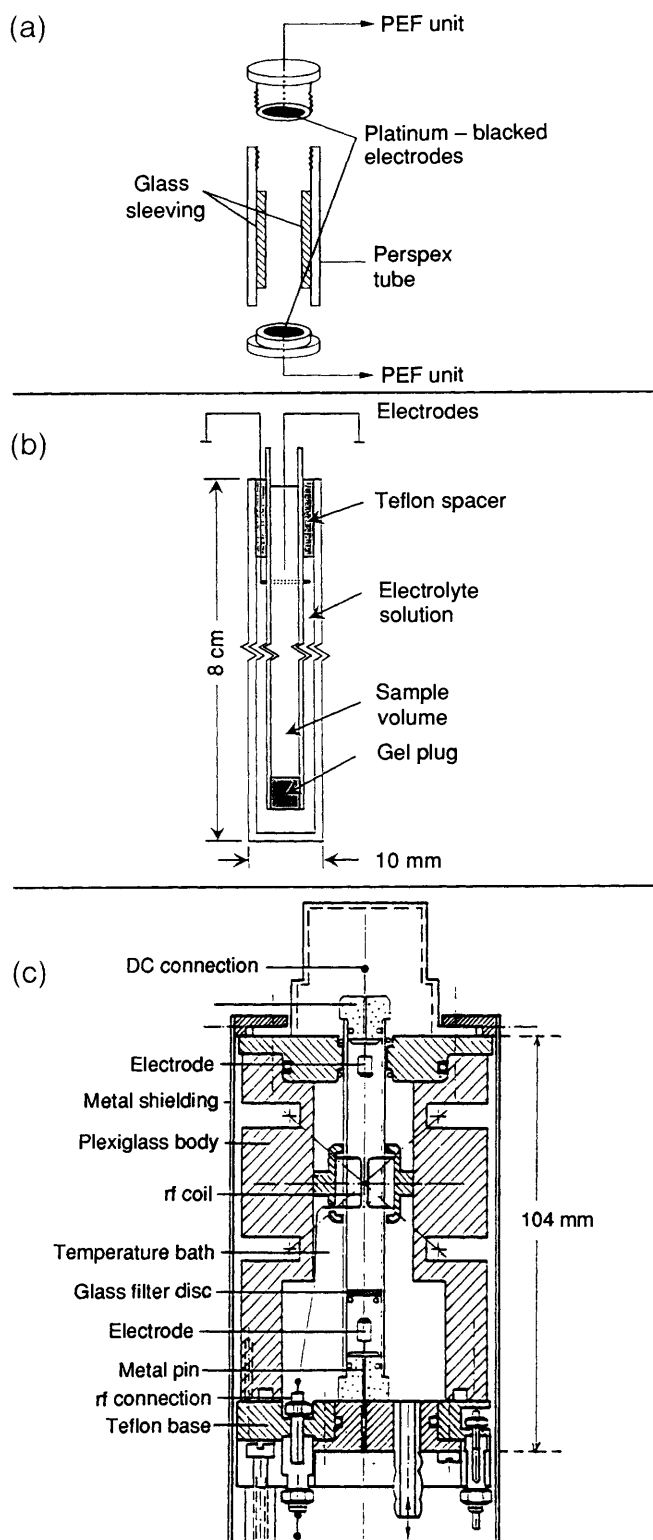
(a)

PEF unit

Platinum – blacked
electrodes

Glass
sleeving

Perspex
tube

PEF unit

Electrodes

(b)

Teflon spacer

Electrolyte
solution

8 cm

Sample
volume

Gel plug

10 mm

(c)

DC connection

Electrode

Metal shielding

Plexiglass body

rf coil

104 mm

Temperature bath

Glass filter disc

Electrode

Metal pin

rf connection

Teflon base

Figure 4 Examples of the ENMR cells in practical use.
(a) ENMR cell, by Coveney et al.,[24] made of a perspex tube of 8 mm diameter. The electrodes are Pt discs of 3 mm diameter. 'PEF unit' means the pulsed electric field generator.
(Reproduced by permission from *Mol. Phys.*, 1992, **75**, 127.)
(b) Concentric ENMR cell by Morris and Johnson[27] made of glass. The inner tube is held by a Teflon plug with ventilation holes. The o.d. of the inner tube is 3—5 mm.
(Reproduced by permission from *J. Magn. Reson.*, 1993, **A101**, 67.)
(c) Probe-head insert for DCNMR, consisting of a Plexiglas body on which the gradient coils are mounted. The electrophoresis cell in the centre is made of glass and is surrounded by a temperature bath liquid. (The coolant inlet can be seen at the bottom). The cell is easily exchangeable.
(Reproduced by permission from *J. Magn. Reson.*, 1993, **A105**, 90.)

plug from a salt solution in the annulus between the tubes. This salt solution, with high conductivity, serves simply as an electric conductor. Despite the fact that just below the gel plug the current flows perpendicularly to the B_0 direction, magnetic field gradients in the z-direction are not to be expected since the outer electrode has the shape of a ring and therefore in the x,y-plane currents appear in all directions compensating for the induced magnetic field gradients.

This cell design has the further advantage that it might be the best suited for use in commerical NMR probes for cryomagnets. However, there is also a disadvantage. The filling factor is relatively poor, since the sample of interest fills only the volume of the inner tube, and this probably restricts its application to electrophoretic ^{1}H NMR, where normally no serious sensitivity problems are to be expected.

The third example (Figure 4c) is a special probe insert for DCNMR experiments which is in use in my laboratory and which has recently been described in detail elsewhere.[28] A Plexiglass body carries the gradient coils and also allows inspection of the electrophoresis cell under working conditions.The cylindrical cell is made of glass and is easily removable for filling and cleaning purposes – the electrodes are fixed on detachable plastic plugs. An important aspect of this cell arrangement is that it is located in a liquid temperature bath. The coolant is pumped through the interior of the Plexiglass body and results in a direct and effective cooling of the electrophoretic chamber. Temperature gradients along the cell axis are also completely avoided.

All three cells shown in Figure 4 allow the mobility sign of charged species to be obtained from the direction of the phase shift $\Delta\phi_{2\tau}$.[9,24,27]

4 Experiments and Applications

4.1 Which Nuclei can be used in ENMR?

The measuring principle of ENMR is, as we saw, based on the spin-echo experiment. The higher the maximum $\Delta\phi_{2\tau}$ values which can be measured in an experiment, the greater the accuracy of the method. We recognize from equations 6 and 8 that large τ and Δ values, respectively, are the most important conditions for successful ENMR measurements. In a standard spin-echo experiment (see Figure 1) the transverse relaxation time T_2 determines the intensity of a spin-echo at 2τ (also see equation 7), hence the longer the T_2 the better are the conditions for ENMR. One can estimate that under the extreme narrowing condition, where $T_1 = T_2$ is valid, a value for T_1, $T_2 > 50$ ms is required. This means that in diamagnetic solutions all spin-1/2 nuclei, which relax by the dipole–dipole interaction (e.g. ^{1}H, ^{13}C, ^{19}F, ^{29}Si, ^{31}P, ^{205}Tl), are nuclei which could be utilized in ENMR experiments. Of course, among these spin-1/2 nuclei, ^{1}H plays an outstanding role owing to its NMR sensitivity and because of its general importance in chemistry and life sciences. Nuclei with $I > \frac{1}{2}$ relax almost exclusively by quadrupolar relaxation, which is a strong relaxation mechanism, and these nuclei often have very short relaxation times. However, there remain a number of possible candidates for ENMR experiments and we mention here ^{2}H, ^{7}Li, ^{23}Na, ^{27}Al, ^{35}Cl, and ^{133}Cs. It should be emphasized though that the relaxation times of these nuclei can vary strongly depending on the electronic surroundings of the nucleus. With a high symmetry of the charge distribution around a quadrupolar nucleus we can expect longer relaxation times. For example ^{7}Li in Li$^+$, with an electronic noble gas configuration, has a much longer relaxation time than ^{7}Li in a covalently bound Li atom.

With spin-1/2 nuclei, e.g. ^{1}H, residing in molecules with a high molecular mass such as proteins, the molecular correlation times τ_c in aqueous solution are typically 10^{-8}–10^{-9} s and therefore the extreme narrowing condition $\omega_0 \cdot \tau_c \ll 1$ is no longer fulfilled. This results in a shortening of T_2 and consequently $T_1 > T_2$ is valid. T_1 might then be one order of magnitude longer than T_2, and T_2 can be too short to achieve the required Δ-value in the conventional spin-echo experiment. However, there is a

modified spin-echo technique, 'the stimulated-echo (STE) technique' which can be applied in this case.[21,29] In this well-known NMR pulse sequence, magnetization is stored for a time in the range of T_1 by two $\pi/2$ pulses and after that it is detected relatively shortly after a third $\pi/2$ pulse. Therefore the measuring parameter Δ is only limited by the longer relaxation time T_1.[21]

Despite the fact that there is a considerable number of nuclei that might be utilized for ENMR, the only successful experiments conducted so far have been with ^1H, $^7Li^+$, and $^{133}Cs^+$. This may be regarded as a further indication that ENMR is still in its infancy.

Up to now in this section we have spoken solely of *electrophoretic* NMR. The situation with respect to the above given conditions for T_1 and T_2 is different for DCNMR *relaxation* measurements, where quadrupolar relaxing ionic nuclei are of interest.[2,10] In these experiments the absolute values of T_1 and T_2 do not play such a restrictive role as in ENMR experiments.

4.2 Measuring Methods

The practical NMR diffusion measurements and NMR flow measurements are of a very similar nature because in both cases the spin-echo is observed in a PMFG experiment. They differ only in the dependence of $M(2\tau)$ on the gradient strength G or the pulse length δ, respectively, for v and D, (see equation 7a). Therefore the simplest ENMR experiment is analogous to a standard NMR self-diffusion experiment, where the dependence of $M(2\tau)$ upon δ or G is measured. Because the effective gradient G acting over the sample volume is very sensitive to the sample position and geometry, the absolute value of G is normally not known with high accuracy. For reliable and precise NMR diffusion and velocity measurements it is therefore recommendable to measure relative to a reference sample.[28,30]

For the determination of absolute mobilities in an ENMR experiment, a knowledge of the acting electric field E in the NMR coil volume, the actual measuring volume, is required. This can be obtained by mounting two Pt probe electrodes in the cell just above and below the NMR coil.[7,9] Using a current-stabilized power supply as the electric field generator so that the value of the current I flowing in the cell is known then allows the accurate determination of transference numbers $T_{\pm}$.

In the standard ENMR experiment $M(2\tau)$ is observed in a measuring sequence for every G or δ value, with and without the applied electric field, giving the amplitude ratio from equation 8. Since we also obtain in this procedure the quantity $M(2\tau)$ in absence of the current, these data can be evaluated with respect to the translation diffusion coefficient D. This fact reveals an interesting advantage of ENMR, since if we measure the mobility $\mu_{\pm}$ of an ion, we can always gain, as a 'by-product', the equilibrium transport quantity $D_{\pm}$, which delivers further valuable information on the system of interest.

Since in ENMR a phase shift $\Delta\phi$ is the quantity of interest, the pure *amplitude* measurement of $M_v(2\tau)$ in the standard spin-echo experiment can be replaced by measuring procedures where the *phase shift* $\Delta\phi_{2\tau}$ is determined more directly. If simple NMR instruments are used, in an 'off-resonance' experiment the phase shift can be directly determined from a phase shift of the time-domain echo-signal,[9,13] a method which has certain advantages over amplitude measurements. With a FT-NMR system it is better to make the phase-shift measurement, after Fourier-transformation, in the frequency domain,[23,24] since the signal-to-noise ratio is better in the frequency domain. Moreover, in solutions with a number of different ionic species containing the same nucleus, the FT-NMR experiment allows the simultaneous mobility measurement of the various charged species, under the condition that their chemical shift difference is resolved. As an illustration of these direct $\Delta\phi_{2\tau}$ measurements as a function of gradient pulse length δ, we show in Figure 5 data of $^7Li^+$ drift velocities in aqueous solution at different LiCl concentrations and electric fields E.[9] A simple slope comparison yields the $v_{\pm}$ values of interest. The above procedures can also be used with stimulated echoes.
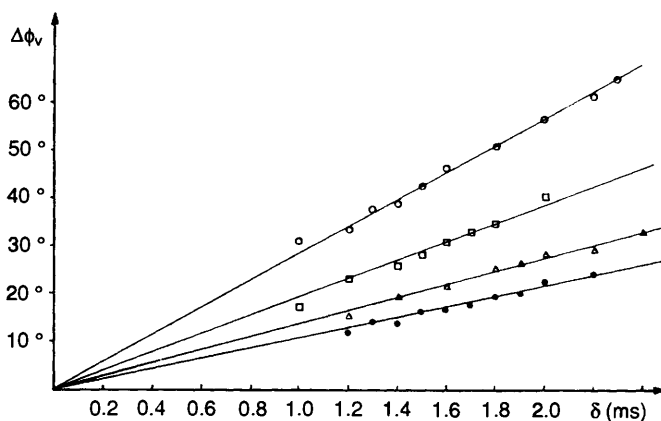
Figure 5 Experimental data of $\Delta\phi_{2\tau}$, the phase shift of the $^7Li^+$ spin-echo signal, as a function of PMFG duration δ in a standard spin-echo experiment as shown in Figure 1. System: LiCl in water. The slopes of the straight lines are proportional to the drift velocities $v_{\pm}$ at different salt concentrations. (○: 0.15 M LiCl, E = 12.5 V cm^{-1}; □: 0.2 M LiCl, E = 9.3 V cm^{-1}; △: 0.3 M LiCl, E = 7.1 V cm^{-1}; ●: 0.2 M LiCl + 0.1 M CsCl, E = 6.4 V cm^{-1})

(Reproduced by permission from *J. Magn. Reson.*, 1986, **69**, 108.)

From the relation $\Delta\phi_{2\tau} = \gamma Gv\delta\Delta$, it can be seen that in ENMR with high-γ nuclei, particularly with ^1H, relatively large values of $\Delta\phi_{2\tau}$ can be achieved. Thus, varying the drift velocity $v_{\pm}$ by increasing the electric field (increasing the current I), the value of $\Delta\phi_{2\tau}$ can be varied up to a value of 3 to 4 π and the cosine modulation of the FT NMR signal intensity can be evaluated. In Figure 6 an example from the work of Saarinen and Johnson[11] is given, where the $(CH_3)_4N^+$ drift velocity has been observed for the first time in a high resolution experiment. Note that the resonance line of the stationary solvent HDO does not show the cosine modulation and thus moving and non-moving species can be easily differentiated.
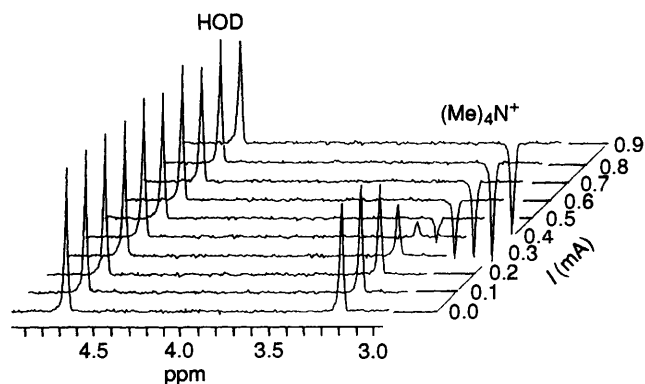
Figure 6 The first ^1H ENMR experiment under high resolution conditions. The dependence of resonance lines of HOD and $(CH_3)_4N^+$ upon the current I, and hence drift velocity v, is shown.

(Reproduced by permission from *J. Am. Chem. Soc.*, 1988, **110**, 3332.)

A further nice example is given in Figure 7 where the ENMR spectrum of a mixture of ethylendiamine and an amino acid in water is shown,[21] and where two charged species move in the electric field with different mobility. In Figure 8 the cosine peak height modulation of the lines in Figure 7 is plotted, showing the different drift velocities of the two species.

In FT-NMR the time domain signal which is acquired is normally the second half of the spin-echo; the time axis is called the t_2-axis and the Fourier transformation is performed with respect to this axis. The cosine modulation of the signal intensity in electrophoretic NMR described above can also be produced with constant v (constant I) but by an incremented variation of Δ, the gradient pulse distance. If we denote the time interval Δ as t_1, we have a second time axis and we can perform a second
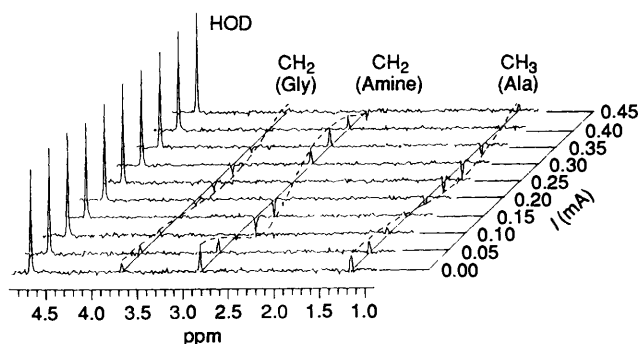
Figure 7 250 MHz ¹H ENMR spectra of a mixture of 1 mM ethylene diamine with an amino acid (1 mM L-alanylglycylglycine) in D₂O. (Reproduced by permission from *Adv. Magn. Reson.*, 1989, **13**, 131.)
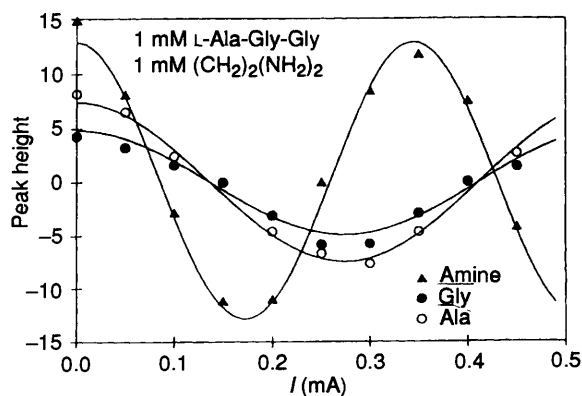

Figure 8 The intensity of the ¹H NMR lines in Figure 7 *versus* current I, showing the different cosine modulation for the amine line and the amino acid lines resulting from their different mobilities.
(Reproduced by permission from *Adv. Magn. Reson.*, 1989, **13**, 131.)

Fourier transformation with respect to t_1, yielding a second frequency axis f_1, and hence a two-dimensional ENMR spectrum can be obtained as in conventional 2D NMR. He and Johnson[12,21] introduced such 2D NMR experiments into the field of electrophoretic NMR. These were important in two respects: NMR spectra could now for the first time be resolved on the basis of electrophoretic mobilities, and the chemical shift dimension could be introduced into electrophoretic studies. The f_1-frequency in the 2D NMR experiment can, with known electric field, be easily transformed in a mobility $\mu_\pm$ since f_1 is directly proportional to $v_\pm$. Morris and Johnson[27] have recently presented ENMR spectra where one axis is the chemical shift axis and the other is the mobility axis, as shown for example in Figure 9. They designated the experiment '*mo*bility-*o*rdered 2D NMR *s*pectroscopy' (MOSY) and implemented a linear prediction analysis of the data with respect to the mobility axis,[27] thus replacing in this dimension the common Fourier transformation and thus avoiding truncation broadening of the observed lines. The authors claim a resolution of approximately one per cent in the mobility dimension.
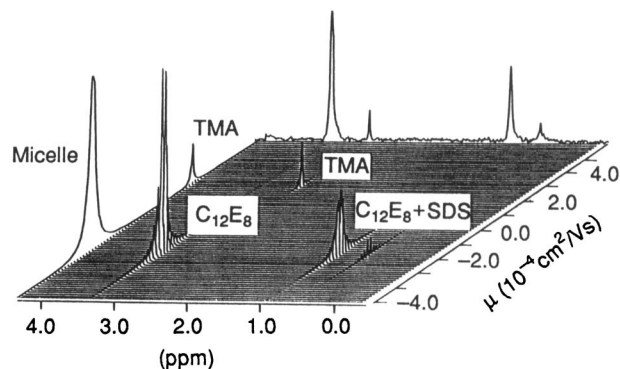

Figure 9 Mobility-ordered 2D ENMR ¹H spectrum of 2 mM $(CH_3)_4^+$ (TMA) in the presence of mixed micelles [1.5 mM sodium dodecyl sulfate (SDS) and 4 mM octaethylene glycol dodecyl ether $(C_{12}E_8)$]. Note the different sign for the mobilities of TMA and $C_{12}E_8$.
(Reproduced by permission from *J. Magn. Reson.*, 1993, **A101**, 67.)

In recent 2D experiments, an incrementation of the amplitude of E is preferred over of an incrementation of Δ, since short and constant Δ-values reduce diffusional broadening.

In ¹H NMR, where homonuclear J-coupling plays a role and where often molecules with relatively high mass are investigated, the stimulated-echo (STE) technique has advantages. In Figure 10 a sophisticated pulse sequence is shown, which includes gradient pre-pulses (recommendable in all PMFG experiments!), and in which a modified STE sequence is applied where instead of the echo a free induction decay after a further $\pi/2$ pulse is observed. The delay T_e is introduced in order to wait for the decay of undesired eddy currents and mechanical disturbances after the last gradient pulse.[31]

2D ENMR experiments hold promise for a wide field of ¹H ENMR applications. The same might be true for ¹⁹F and ³¹P, nuclei with high γ-values. However, where nuclei with low γ-values have to be utilized, for example in the field of inorganic chemistry, the measurable $\Delta\phi_{2\tau}$ values are typically smaller than π, or even $\pi/2$, and therefore standard 1D experiments must be used, as shown for example in Figure 5. But this does mean that there is no limitation with respect to the resolution since often single line spectra, or well resolved spectra of only a few lines, are observed with heteronuclei, as large chemical shifts are typical. As mentioned in Section 3.2, the STE technique is also useful in heteronuclear ENMR if measurements have to be conducted in heterogeneous media, as for example in sinter glass.

4.3 Applications of ENMR

Nuclear magnetic resonance in the presence of an electric current allows the introduction of NMR techniques into the general field of electrochemistry, and therefore also into applied electrochemistry. The DCNMR methods described above are

Figure 10 Modified stimulated echo (STE) pulse sequence for 2D ENMR, after Morris and Johnson.[27] Gradient pre-pulses are used before the three $\pi/2$ rf pulses of the standard STE sequence are applied. The last two $\pi/2$-pulses with the waiting time T_e produce a free induction decay (FID), which is observed.
(Reproduced by permission from *J. Magn. Reson.*, 1993, **A101**, 67.)
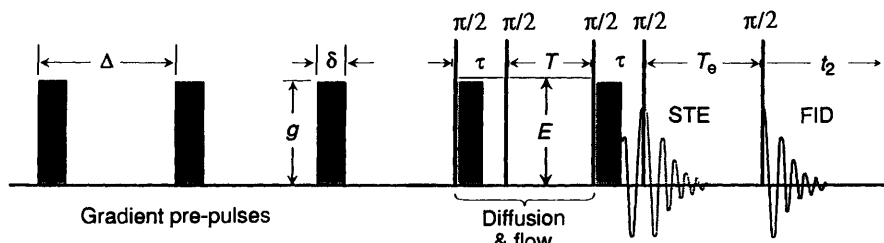
the first steps towards these new NMR applications. Let us briefly summarize the general advantages which are connected with the NMR method. First, NMR is a non-invasive technique and to a large extent independent of the mechanical and optical properties of the system of interest. It can thus be applied to a great variety of systems. Secondly, and perhaps of more relevance, is that by using NMR the selectivity of a spectroscopic method can be included in electrochemical studies. This selectivity on a molecular or atomic level allows the observation of distinct particles in a system of interest. The NMR diffusion and flow measuring techniques described above even allow particles to be distinguished from their physically and chemically identical neighbours by means of the phase of their nuclear spin precession. In this way mobile particles can be distinguishd from immobile particles of the *same* species.

It is clear that the selectivity of NMR is an outstanding advantage in the study of complex multicomponent systems, which are in many cases inaccessible to classical methods. (For example, mobility data of single ionic components in mixed electrolytes are rarely found in the literature.) On the other hand, in molecular biology and in applied chemistry electrophoretic analyses play an important role, and here the combination of electrophoresis with spectroscopic information from NMR is a very tempting approach. Finally, it should be emphasized that owing to the inherent selectivity of NMR, molecular labelling (as required in certain conventional electrophoresis methods) is not needed, and therefore the properties of the system of interest are not affected by the ENMR experiment.

4.3.1 Application with Heteronuclei in Ionic Solutions

Naturally, the first comparisons of ionic mobilities obtained by the novel ENMR method with literature data from classical methods have been made with data from simple binary electrolyte solutions.[7-9] The ions considered were $(C_2H_5)_4N^{+7,8}$ and Li^+ in aqueous solution[9] and the agreement was found to be within 1 to 5%. A first systematic study on ternary electrolyte solutions of simple ions has been performed very recently in my laboratory.[23,28] The systems were $y \times 0.5m$ $LiX + (1 - y) \times 0.5m$ CsX in H_2O ($X = Cl, Br, I$); these are mixtures of lithium and caesium halides at constant ionic strength. Owing to the experimental difficulties associated with the classical methods, it is characteristic that literature data for ionic mobilities, diffusion coefficients, and transference numbers are sparse, even for such a simple mixed electrolyte system as this.

The ENMR measurements were performed by utilizing the $^7Li^+$ and $^{133}Cs^+$ resonance. These two ionic nuclei are very favourable for electrophoretic NMR studies because of their long relaxation times and from a physical-chemical point of view they are interesting species in a mixture, since their ionic diameters differ appreciably. In order to demonstrate the results that can be obtained in such a system, the composition dependence of the ionic mobilities, diffusion coefficients, and transference numbers of the LiBr-CsBr system is shown in Figure 11.

A comparison of the composition dependence of D_+ and μ_+ in Figure 11 shows that the cationic diffusion coefficients, which are equilibrium quantities, change linearly with the composition in the mixed electrolyte, whereas the mobilities of Li^+ and Cs^+, the non-equilibrium quantities, show a non-linear behaviour with opposite curvature. This latter fact indicates that cross-correlation effects influence the ionic mobilities in the ternary system. It is clear that the measurement of single ionic mobilities in mixed electrolytes with more than three constituents should also be easily feasible by ENMR, and this opens new possibilities in the study of mixed simple electrolytes.

Mobility studies in *non-aqueous* systems by conventional methods, *e.g.* the moving-boundary method, are often beset with difficulties. Gel stabilization of non-aqueous systems is often impossible. However, the use of a sinter glass stabilizer has proved to be successful,[23] as demonstrated in Figure 12. In this LiCl in methanol system the mobility of Li^+ could be accurately measured as a function of the salt concentration. It can be seen
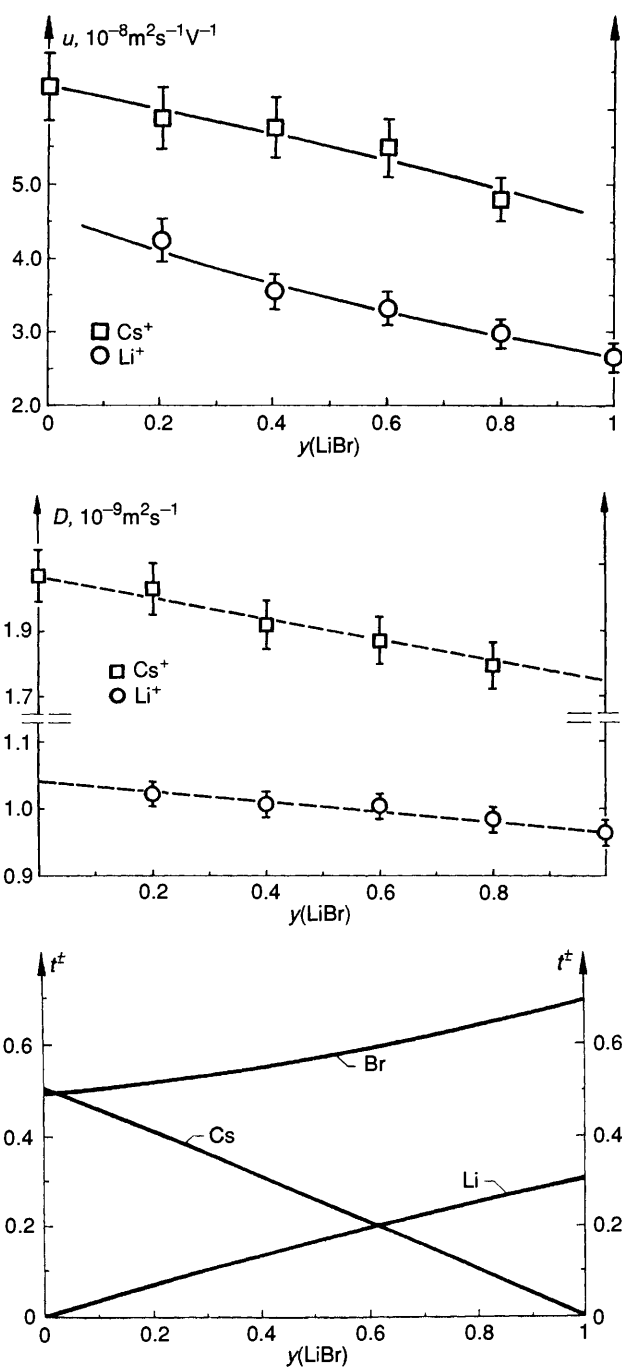

Figure 11 Results of ENMR experiments on a ternary mixture of simple ions at constant ionic strength.

System: $y \times 0.5m$ LiBr + $(1 - y) \times 0.5m$ CsBr in H_2O.
(a) The mobilities v_+ of the cations Li^+ (○) and Cs^+ (□) as a function of the mixture composition.
(b) The translational diffusion coefficient D_+ of Li^+ and Cs^+ in the same system.
(c) The transference numbers $T_\pm$ of the three ionic constituents Li^+, Cs^+, and Br^- in the mixed electrolyte system.
(Reproduced by permission from *J. Magn. Reson.*, 1993, **A105**, 90.)

that the ENMR studies could be performed up to comparatively high salt concentrations and that the agreement with literature data is excellent.

4.3.2 Applications of ^1H ENMR

In electrophoretic NMR, as in conventional NMR, the hydrogen nucleus ^1H plays the most important role. Because of its sensitivity, measurements on charged solutes in the mM con-
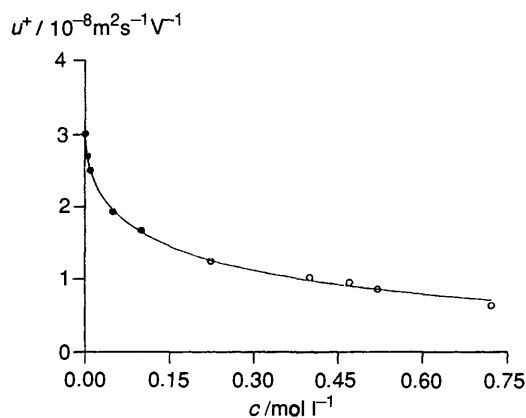
$u^+ / 10^{-8} m^2 s^{-1} V^{-1}$

Figure 12 Dependence of Li^+ mobility in methanol upon LiCl concentration c at 25°C.[23] Open circles: data of ENMR in sinter glass stabilized solutions. Filled circles: literature data, obtained with conventional techniques.

centration range are easily possible and there is a huge number of hydrogen-containing charged species which are of interest in many fields of chemistry and biology.

We saw that the first ^1H ENMR measurements were performed on binary electrolyte solutions of tetraalkylammonium salts. The next application of the technique was to the investigation of mobilities in mixtures of amino acids[21] (see for example Figure 7). It can be stated that ENMR may be applied, without difficulty, to investigations of mixtures of simple ions, amino acids, and peptides, where the proton T_2 values are in the favourable range of some hundred milliseconds.

However, the most interesting applications of ^1H ENMR are expected in the important field of supramolecular chemistry; most examples of applied ENMR come from this branch of chemistry. Coveney et al.[14,24] have observed the mobilities of charged micelles, namely the anionic sodium dodecyl sulfate (SDS) and the cationic cetyltrimethyl ammonium bromide (CTAB) in water. In these charged surfactant systems the electrophoretic mobilities at various concentrations were measured (see Figure 13), allowing extrapolation to the critical micelle concentration, where for SDS $\mu_- = 4.49 \times 10^{-8}$ $m^2 V^{-1} s^{-1}$ and for CTAB $\mu_+ = 3.58 \times 10^{-8}$ $m^2 V^{-1} s^{-1}$ were found at 25°C.[24] These values agree within 1% and 2%, respectively, with Dye tracer measurements. In the same work,[24] the mobility of SDS was measured after the ionic environment of the micelles had been changed by the addition of salt. Mixtures of surfactants have also been studied. An impressive example of a 2D mobility-ordered spectrum of negatively charged micelles
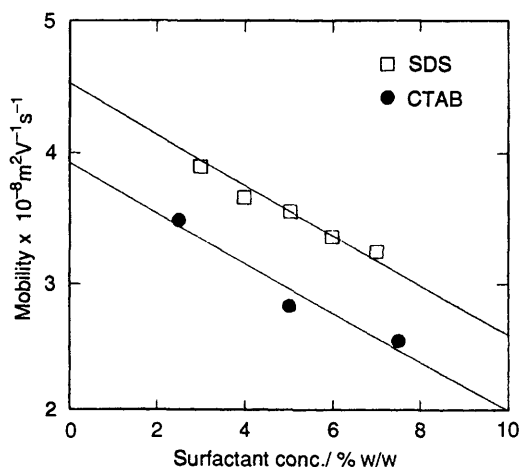

Figure 13 Mobilities of charged micelles in water measured by ENMR as a function of surfactant concentration. SDS: sodium dodecyl sulfate, CTAB: cetyltrimethyl ammonium bromide.
(Reproduced by permission from *Mol. Phys.*, 1992, **75**, 127.)

in a mixture with positively charged tetramethylammonium ions has already been given in Figure 9. In both the examples of micelle studies given here, the information about the sign of the mobility has been derived from the corresponding phase shift direction.

The power of electrophoretic NMR in *microemulsion* systems has been demonstrated by Johnson and co-workers.[21] In microdroplets of oil in water (o/w) it allows the drift velocity of surfactants and of the hydrocarbon in the droplets to be compared. These interesting applications have been extensively reviewed in the literature.[21] An example is given where modern multiquantum (MQ) NMR is combined with electrophoretic techniques in a MQ ENMR experiment.[21] This might offer certain advantages, namely simplification of spectra by multi-quantum filtering and increased $\Delta\phi_{2\tau}$ values, which are important when low mobilities are of interest. However, MQ ENMR suffers from sensitivity problems.

Finally, studies on phospholipid vesicles are further interesting examples of the application of electrophoretic NMR. These systems consisting of a phospholipid shell around an aqueous inner region may serve as models for biological cells. ^1H signals can be observed from the shell and from the molecules entrapped within. A question of interest here is the charge and size distribution of these polydisperse systems. This polydispersity means that for the same type of species there is a velocity distribution $g(v)$ in the electric field. Under certain conditions this velocity distribution can be derived from electrophoretic NMR data, as shown by Johnson and He[21] and as demonstrated practically in a 2D STE NMR experiment.[29] In this connection it would be interesting to compare the distribution of the translational diffusion coefficients $g(D)$, which is solely dependent upon size and mass, with $g(\mu)$, the mobility distribution, which depends additionally upon the charge distribution.

ENMR measurements can be made of slowly diffusing supramolecular systems in a rapidly diffusing solvent such as water and in the presence of other small molecules by the incorporation of 'diffusion filters' as used in modern NMR spectroscopy. By using relatively strong magnetic field gradient pulses, the signals from the highly mobile components in the mixture are strongly quenched and the signals from slowly diffusing particles can be monitored without problems of overlap.

4.4 Relaxation of Quadrupolar Nuclei in Migrating Ions

The area of application for DCNMR relaxation studies is different from that just discussed for ENMR and up to now there has only been one publication[10] in this field. This kind of DCNMR represents an attempt to contribute to the basic understanding at a microscopic level of the mechanism of motion of ions in liquid solutions under the influence of an external electric field. It seems to be possible to obtain information about the behaviour of solvent molecules around the migrating ions and about the relative motion of ions. The first experiments on quadrupolar ionic nuclei ($^{23}Na^+$, $^{87}Rb^+$, and $^{35}Cl^-$) showed that there are indeed several systems where the quadrupolar relaxation of these ionic nuclei is changed when the ion migrates in the electric field.[10] The relaxation rates in the presence of a current I, $(1/T_1)_{I \neq 0}$ and in the absence of I, $(1/T_1)_{I=0}$ have been measured, allowing the determination of the ratio $R_I = (1/T_1)_{I \neq 0}/(1/T_1)_{I=0}$. In Figure 14 the behaviour of this relative relaxation rate is given as a function of the current I, which means as a function of the drift velocity, for $^{23}Na^+$ in acetonitrile and in methanol. R_I can increase or decrease, and this behaviour has also been found in other systems. The increase of the relaxation rate is explained[2,10] by a deformation, and thus by a symmetry distortion, of the solvation shell of the migrating ion, e.g. as discussed theoretically in terms of a 'kinetic depolarization effect'.[32] Such a structural symmetry distortion increases the electric field gradient at the nuclear site and would increase the relaxation rate. The decrease observed in methanol can be interpreted as a result of a decreasing ion–ion contribution to the quadrupolar relaxation rate[2] by an alteration of the
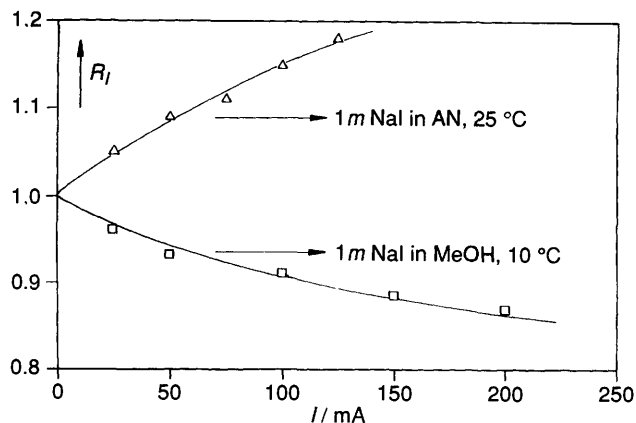
Figure 14 ^{23}Na$^+$ relaxation rate ratio R_I as a function of the applied current I in solutions of $1m$ NaI in acetonitrile (AN) and in methanol (MeOH).

(Reproduced by permission from *Chem. Phys. Lett.*, 1987, **142**, 492.

cation–anion pair correlation function in the electrolyte solution, when electric field forces are acting on the ions. In other words, the latter effect would then reflect the asymmetry of the ion cloud according to the Debye–Hückel theory.

5 Concluding Remarks and Outlook

It is hoped that the present review has shown that DCNMR, a relatively young and promising NMR technique, offers new possibilities for investigating and analysing complex fluid systems with charge-carrying species, such as simple ions, charged macromolecules, and charged supramolecular systems. Up to now, because of the technical problems that have been described, DCNMR in general and electrophoretic NMR in particular have not found extensive application, although it has been shown that many very interesting applications are feasible. The main reason for this may be that purpose-designed equipment is not yet commercially available. If so, a most interesting future development will be a DCNMR cell which can be easily incorporated into conventional NMR probes. As a result of the recent development of NMR 'gradient accelerated spectroscopy' (GRASP), more commercial probes are now equipped with gradient coils and therefore in the near future it may be easier to perform ENMR experiments with conventional instrumentation.

With respect to future applications, it is expected that ^{1}H ENMR will be used to investigate the wide area of polyelectrolytes and colloidal systems. An interesting general application of ENMR could lie in its use as a 'mobility filter', *i.e.* NMR signals from charged species could be identified when an electric field is applied and the charged particles migrate in this field.

We saw that heteronuclear ENMR can be performed with a number of nuclei and it is clear that the future development will also go in this direction. The systematic investigation of mix-

tures of simple electrolytes has just begun and will surely be continued.

The combination of DCNMR with NMR imaging methods is very tempting and has been proposed previously.[2,21] The future developments of DCNMR relaxation studies cannot be judged so easily. In the reviewer's opinion, generally very interesting information can be expected; the interpretation of quadrupolar relaxation data is, however, a difficult task.[2,10] It is clear that more experimental data are required in order to judge the full power of DCNMR relaxation studies. The application of alternating currents, leading to ACNMR relaxation experiments might be a very interesting approach, in particular if high frequencies could be used which would modify the microdynamics in electrolyte solutions in a distinct frequency range of interest.

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