NMR STUDIES OF COLLOIDAL SYSTEMS IN AND OUT OF EQUILIBRIUM

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KTH Chemical Science and Engineering

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NMR studies of colloidal systems in and out of equilibrium

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Abstract

The Thesis describes (i) the development of add-on instrumentation extending the capabilities of conventional NMR spectrometers and (ii) the application of the designed equipments and techniques for investigating various colloidal systems. The new equipments are:

- Novel designs of stopped-flow and temperature-jump inserts intended for conventional Bruker wide-bore superconductive magnets. Both inserts are loaded directly from above into the probe space and can be used together with any 10 mm NMR probe with no need for any auxiliary instruments.
- A set of 5 mm and 10 mm $^1$H - $^{19}$F - $^2$H NMR probes designed for heteronuclear $^1$H - $^{19}$F cross-relaxation experiments in Bruker DMX 200, AMX 300 and DMX 500 spectrometers, respectively.
- A two-stage low-pass filter intended for suppressing RF noise in electrophoretic NMR experiments.

The kinetics of micellar dissolution and transformation in aqueous solutions of sodium perfluorooctanoate (NaPFO) is investigated using the stopped-flow NMR instrument. The sensitivity of NMR as detection tool for kinetic processes in micellar solutions is clarified and possible artefacts are analysed. In the NaPFO system, the micellar dissolution is found to proceed faster than 100 ms while surfactant precipitation occurs on the time scale of seconds-to-minutes. The kinetics of the coil-to-globule transition and intermolecular aggregation in a poly (N-isopropylacrylamide) solution are investigated by the temperature-jump NMR instrument. As revealed by the time evolution of the $^1$H spectrum, the $T_2$ relaxation time and the self-diffusion coefficient $D$, large (>10 nm) and compact aggregates form in less than 1 second upon fast temperature increase and dissolve in less than 3 seconds upon fast temperature decrease.

The intermolecular $^1$H - $^{19}$F dipole-dipole cross-relaxation between the solvent and solute molecules, whose fast rotational diffusion is in the extreme narrowing limit, is investigated. The solutes are perfluorooctanoate ions either in monomeric or in micellar form and
trifluoroacetic acid and the solvent is water. The obtained cross-relaxation rates are frequency-dependent which clearly proves that there is no extreme narrowing regime for intermolecular dipole-dipole relaxation. The data provide strong constraints for the dynamic retardation of solvent by the solute.

**Keywords:** stopped-flow NMR, temperature-jump NMR, cross-relaxation NMR, NMR probe, fluorosurfactant, micellar kinetics, micellar structure, hydration, precipitation, poly(N-isopropylacrylamide), polymer coil, polymer globule, phase separation.
List of papers

**Paper I**
Pavel V. Yushmanov and István Furó
*A rapid-mixing design for conventional NMR probes*
*Journal of Magnetic Resonance* (2005), 175, 264-270

**Paper II**
Pavel V. Yushmanov, István Furó, and Peter Stilbs
*Micellar kinetics of a fluorosurfactant through stopped-flow NMR*

**Paper III**
Pavel V. Yushmanov and István Furó
*A temperature-jump design for conventional NMR probes*
*Submitted to Journal of Magnetic Resonance*

**Paper IV**
Pavel V. Yushmanov, István Furó, and Ilias Iliopoulos
*Kinetics of de-mixing and re-mixing transitions in aqueous solution of poly(N-isopropylacrylamide): A temperature-jump 1H NMR study*
*Submitted to Macromolecules*
Paper V
Lars Nordstierna, Pavel V. Yushmanov, and István Furó
Solute-solvent contact by intermolecular cross-relaxation
I. The nature of the water-hydrophobic interface
Submitted to Journal of Chemical Physics

Paper VI
Lars Nordstierna, Pavel V. Yushmanov, and István Furó
Solute-solvent contact by intermolecular cross relaxation
II. The water-micelle interface and the micellar interior
Submitted to Journal of Physical Chemistry B

Paper VII
Pavel V. Yushmanov, István Furó, and Peter Stilbs
Stopped-flow $^{19}$F NMR studies of surfactant precipitation
Manuscript
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1. Introduction

For centuries, apparent relations between microscopic and macroscopic properties of materials and substances have fascinated and motivated scientists to look into the “microcosm”, hereby stimulating research and development of new experimental techniques. Nowadays a standard tool in chemical sciences, Nuclear Magnetic Resonance (NMR) is a unique detection method capable of revealing in fine detail the properties of matter in bulk. However, the emerging view of the microscopic properties of a system is obviously incomplete without characterization of the internal kinetic processes at the microscopic level. Dynamics of colloidal systems, in particular, are not only of fundamental scientific interest but also play a crucial role in many of their technological applications directly influencing, for example, the effectiveness of a detergent, the stability of pharmaceutical formulations as well as their function in drug delivery systems.

Conventional NMR-based methods such as diffusion\(^1\) or relaxation\(^2\) NMR spectroscopies may reveal various internal kinetic phenomena whose characteristic times are within the micro- (μs) to picosecond (ps) time scale. This span typically covers diffusion-driven processes on the aggregate level or slightly above, and is thereby very important for colloidal science. However, the details of slower (sometimes, indeed, much slower) kinetic modes, typically characterizing various morphological changes in colloidal systems, appear virtually undetectable by conventional NMR techniques. Despite the fact that some of the colloidal structures are metastable, under static conditions the local kinetics is typically reversible. Considered as incoherent microscopic fluctuations these are hard to detect. To make them measurable, the system should be put on an irreversible path. This can, for example, be done by changing rapidly some macroscopic thermodynamic parameters of the system such as temperature, pressure and/or concentration. Kinetics of the induced, e.g., morphological
changes can then be monitored by appropriate NMR techniques generically named kinetic NMR.

This Thesis describes (i) hardware development of experimental setups for kinetic NMR measurements and for cross-relaxation NMR studies and (ii) application of the developed equipments and techniques for investigating various colloidal systems.

Structurally, the Thesis is arranged in the following order:

- NMR fundamentals with a short introduction to relaxation phenomena of nuclear spin systems including heteronuclear dipole-dipole cross-relaxation.
- A classification of and introduction to colloidal systems; conventional theoretical approaches to dynamics of self-assembled colloidal aggregates; a review of, as yet, unsolved problems.
- Conventional NMR and its limited applicability to kinetic studies of colloidal systems; introduction of kinetic NMR techniques.
- The hardware development for (i) concentration-jump NMR experiments, (ii) temperature-jump NMR experiments, (iii) electrophoretic NMR experiments and the construction of $^{19}\text{F} - {^1}\text{H} - {^2}\text{H}$ NMR probes for heteronuclear $^1\text{H} - ^{19}\text{F}$ cross-relaxation experiments.
- Applications of kinetic and cross-relaxation NMR techniques to various colloidal systems, as demonstrated in the enclosed papers and manuscripts.
- Future applications of the developed equipment; a review and proposals for colloidal systems.
2. NMR. Basic principles

Nuclear magnetic resonance can be performed in substances that contain nuclei with non-zero magnetic moments. The nuclear magnetic moment is coupled to the spin angular momentum, the maximum magnitude of which is characterized by the spin quantum number, $I$, as

$$p = \hbar I$$  \hspace{1cm} (2.1)

The value of $I$ is an intrinsic property of a given nucleus with possible spin quantum numbers $I = 0; \ 1/2; \ 1; \ 3/2; \ 2$, and so on. It should be noted that there exists at least one stable isotope with non-zero $I$ for any chemical species. Both the nuclear magnetic moment $\mu$ and the nuclear angular momentum $p$ are vector properties and their relation is defined as

$$\mu = \gamma p = \gamma \hbar I$$  \hspace{1cm} (2.2)

where $\gamma$ is the gyromagnetic ratio which can be either negative or positive and is specific for each isotope. When a magnetic moment is placed in a homogeneous magnetic field, $B_0$, the interaction energy can be written as

$$E = -\mu B_0 = \mu_z B_0$$  \hspace{1cm} (2.3)

where $z$ is assigned to the direction of $B_0$. According to the principles of quantum mechanics, the angular momentum vector cannot assume arbitrary directions. This fact, together with equation (2.2) renders discrete values for the interaction energy

$$E_m = -m\gamma \hbar B_0, \quad m = -I, -I+1, ..., I-1, I$$  \hspace{1cm} (2.4)

where $m$ is the magnetic quantum number. Since the magnetic moment is a dipole property electromagnetic radiation can in first order only excite transitions between neighbouring energy levels ($\Delta m = 1$).
Therefore, the transition frequency becomes

\[ \omega_0 = \frac{\Delta E}{\hbar} = \gamma B_0 \]  

(2.5)

In a real system where there is not just one nucleus in isolation, the whole spin ensemble has to be considered. In thermodynamic equilibrium and in a static magnetic field, the populations of the different energy levels \( n_m \) decrease with increasing of energy according to the Boltzmann statistics

\[ n_m \sim \exp \left[ -E_m / k_B T \right] \]  

(2.6)

where \( k_B \) is the Boltzmann factor and \( T \) is the absolute temperature. Due to unequal populations of levels, the whole system as a macroscopic object is characterized by a nonzero vector of macroscopic nuclear magnetization

\[ M_0 = \gamma \hbar \sum_m n_m m \]  

(2.7)

Without external perturbation, \( M_0 \) must be parallel to \( B_0 \). After a perturbation, this internal equilibrium is re-established by the so-called nuclear magnetic relaxation or NMR relaxation process. This, in general, involves two different but parallel steps. The recovery of the \( M_z \) projection is as the spin-lattice or longitudinal relaxation that describes to dissipation of nuclear spin energy into the thermal bath formed by surrounding matter. Customarily, the time constant characterizing this process is denoted as \( T_1 \). The transverse or spin-spin relaxation with time constant \( T_2 \) is responsible for the disappearance of the transverse component of \( M \) and can be assigned to interactions among the nuclear spins themselves. This is generally a faster process than longitudinal relaxation and may not require energy transfer between spins and surrounding matter.
In general, quantum statistical mechanics is required for calculating the properties and behaviour of a spin ensemble in static and time-dependent magnetic fields. However, even a classical approach introduced by Bloch captures the most basic elements of NMR spectroscopy. Thus, the equation of motion of sample magnetization in a magnetic field \( B \)

\[
\frac{dM}{dt} = \gamma [M \times B] \tag{2.8}
\]

can be derived from equations (2.2), (2.7) and classical electrodynamics. The common solution of such an equation is a magnetization vector that precesses around \( B \) with an angular frequency \( \omega = \gamma B \). With only \( B_0 \) present, frequency \( \omega \) becomes identical to \( \omega_0 \) derived in equation (2.5) for a single nucleus.

It is known from the theory of quantum transitions that the magnetic component of an electromagnetic field \( B_1 \) oscillating with an angular frequency \( \omega \approx \omega_0 \) must be directed perpendicularly to \( B_0 \) to be able to induce transitions between different energy levels of the spin system. The influence of such a field on the behaviour of the macroscopic magnetization vector can be also described within the classical approach. This is done by introducing a reference frame which rotates around the z-axis by an angular frequency of \( \omega_0 \) and solving again equation (2.8) in the new frame with \( B = B_{\text{eff}} \) defined as

\[
B_{\text{eff}} = B_0 + B_1 + \frac{\omega}{\gamma} = B_1 + \frac{\Delta \omega}{\gamma} \tag{2.9}
\]

where \( \Delta \omega = \omega - \omega_0 \). Hence by analogy to the obtained precession around \( B_0 \), the magnetization vector simultaneously precesses around \( B_{\text{eff}} \) in the rotating frame with an angular frequency of \( \omega_{\text{eff}} = \gamma B_{\text{eff}} \). This outcome allows us to calculate the perturbation of the magnetization vector caused by a short \( B_1 \) pulse.
If its duration is $t_i$ and $B_{\text{eff}} = B_1$ (i.e., $\Delta\omega = 0$), the magnetization will be rotated by an angle

$$\Theta = \gamma B_1 t_i. \quad (2.10)$$

Assuming initial thermal equilibrium, the $B_1$ pulse tips the magnetization by an angle $\Theta$ with respect to the $z$ axis. Since only a precession of transverse component of $M$ gives an observable NMR signal, a pulse with $\Theta = \pi/2$, (i.e., the so-called 90° pulse) produces an NMR signal with largest initial amplitude.

Since relaxation processes are not considered above, a complementing set of equations is required to describe them. The exact form of the relaxation equations depend on the various modes of molecular dynamics and on the type and magnitude of microscopic spin interactions within the spin ensemble. Although these equations can be very complex, providing several relaxation pathways by which the nuclear spins may interact with each other and with surrounding lattice, they describe the recovery of the longitudinal component of magnetization vector ($T_1$ process) and the disappearance of transverse one ($T_2$ process). These recoveries may be exponential or multiexponential.

The factors influencing spin-lattice and spin-spin relaxation can be elucidated via quantum mechanics. The particular relaxation pathways depend on the full nuclear spin Hamiltonian of the system. For dipole-dipole interaction of two non-identical spins $I$ and $S$ this becomes

$$\hat{H}_{dd} = \frac{\gamma_I \gamma_S \hbar^2}{r^3} \left[ (\mathbf{i} \cdot \mathbf{S}) - \frac{3(\mathbf{i} \cdot \mathbf{r})(\mathbf{S} \cdot \mathbf{r})}{r^2} \right] \quad (2.11)$$

where $\mathbf{r}$ is the distance vector connecting the two point dipoles $\mu_1 = \gamma_I \hbar \mathbf{I}$, and $\mu_1 = \gamma_S \hbar \mathbf{S}$. Since the dipole-dipole interaction described by $\hat{H}_{dd}$ is
usually much smaller than $\hat{H}_0 = -\gamma_1 \hbar \hat{I}_z B_0 - \gamma_2 \hbar \hat{S}_z B_0$ which characterizes the interaction of the spins with $B_0$, the theory of time-independent perturbation can be used for deriving the energy levels and the corresponding spin eigenfunctions. The kinetic equations written for populations of the obtained energy levels also reflect the behaviour of macroscopic magnetization. Thus, the so-called Solomon equations will be valid for our two-spin system:

$$\frac{d\langle I_z \rangle}{dt} = -\rho_i (\langle I_z \rangle - I_0) - \sigma_{is} (\langle S_z \rangle - S_0)$$

$$\frac{d\langle S_z \rangle}{dt} = -\rho_s (\langle S_z \rangle - S_0) - \sigma_{is} (\langle I_z \rangle - I_0)$$

(2.12)

where $I_0$ and $S_0$ are the thermal equilibrium values of macroscopic longitudinal spin polarization, $\rho_i$ and $\rho_s$ are individual longitudinal spin relaxation rates, and $\sigma_{is}$ is the so-called cross-relaxation rate. This latter parameter describes the transfer of magnetization between two spin systems coupled by dipole-dipole interaction. This transfer can, under suitable circumstances, be manifested by a remarkable increase of the longitudinal magnetizations, which is the so-called Nuclear Overhauser Effect. Since cross-relaxation, in general, strongly ($\sim r^{-6}$) depends on the distance between the two interacting spins, it can yield important structural information.
3. Colloidal systems

The term “colloid” which means “glue” in Greek was introduced in 1861 by Thomas Graham to describe “pseudosolutions” of various sols in water. Almost a century later, it has been found that dissolved polymers that have at least one molecular dimension greater than 1 nm exhibit many features of classical colloidal solutions. Hence, these systems are often called “lyophilic” colloids that may be thermodynamically (in contrast to kinetically) stable.

A polymer chain dissolved in an excess of solvent has many degrees of freedom and can fold in different ways. There are three distinguishable types of chain folding. In a globular state the chain folds back on itself to minimize polymer – solvent contact. The effective radius of the polymer globule is equal to \( N_p^{a=0.6} \), where \( N_p \) is the degree of polymerization. A stiff linear conformation of polymer chain is often dictated by the formation of local helical structures. In this case, the length of the structure increases linearly with \( N_p \). As a third option, a polymer chain may form a less well-defined (random) coil. In this state, the chain may adopt many different conformations the average of which can be characterized by various parameters the most common of which is the radius of gyration \( R_g \)

\[
R_g^2 = \frac{\sum_{i=1}^{p} |\mathbf{r}_i - \mathbf{r}_{cm}|^2}{N_p},
\]

where, for a homopolymer, \( \mathbf{r}_i \) stands for the position of the \( i \)th segment and \( \mathbf{r}_{cm} \) is the location of the centre of mass.

Which chain conformation the polymer adopts depends specifically on the polymer-solvent interaction and on external macroscopic parameters such as temperature, pressure and concentration. After a temporary perturbation of any of these parameters, the original chain conformation can be re-established.
The kinetics of this process may vary between extremely broad bounds, from instantaneous conformational changes to year-long metastable states, and following its time course can yield important information.

Another family of colloidal particles consists of self-assembled “association” colloids formed by amphiphilic (surfactant) molecules. Among various self-assembled structures intrinsic to different surfactant concentrations in the solution, the micellar aggregates are typically the first to appear when the surfactant concentration exceeds the so-called critical micellar concentration, CMC\textsuperscript{10}. The heterogeneous character of a micellar solution above CMC is well represented by a typical aggregate size distribution shown on Figure 3.1

![Figure 3.1](image)

*Figure 3.1 The average equilibrium concentration $A(N)$ of surfactant molecules residing in species with aggregation number $N$.\*

This type of size distribution is provided by thermodynamic calculations for fairly dilute micellar systems\textsuperscript{11}. The shape of distribution curve suggests that:

- The micelles are not monodisperse, so that the micellar region is characterised by a mean (most probable) aggregation number $\bar{N}_{\text{mic}}$ and a width of the distribution curve $\delta_{\text{mic}}$.\*
• Not only monomers \( (A_1) \) may be present in the bulk solution but also dimers, trimers, etc.

• Micellar and bulk monomer (or oligomer) states of surfactant molecules typically co-exist.

• There is a low equilibrium concentration of surfactant species with aggregation numbers in the intermediate region between proper micelles and monomers.

Under equilibrium conditions the distribution curve remains constant. Upon any deviation from equilibrium, relaxation processes re-establish a new distribution curve and, therefore, new molecular distribution among states and aggregates. Experimentally\(^{12}\), this equilibration process is characterised by two relaxation regimes operating on time scales that differ by as much as three orders of magnitude. Since all used detection methods provide only indirect information about actual kinetic processes in micellar solution, those were instead rationalized in various theoretical frameworks. In the well-known theory of Aniansson et al\(^{13, 14}\), a fast relaxation process, often characterized by a single time constant \( \tau_1 \), is assigned to the re-establishment of equilibrium distribution of molecules in micellar and monomer states, whereas a slower relaxation (\( \tau_2 \)) is indicative of stepwise disintegration and formation of micelles. In later works\(^{15}\), the slow process was simply (but incorrect) attributed to the micellar life time. More sophisticated approaches\(^{16, 17}\) point to the existence of more than two dynamic regimes for the aggregate size distribution to evolve toward equilibrium. The extension of these depends of the amplitude of initial deviation from equilibrium. This is in agreement with experiential observations\(^{18}\) of the relaxation times being dependent not only on the surfactant type but also on the amplitude of perturbation and on the method by which system was driven from equilibrium. Therefore, the kinetics in micellar solutions is still an open issue in demand of new experimental and theoretical approaches.
4. Kinetic NMR methods

In application to colloidal systems, various NMR techniques are inherently useful because nuclei in a surfactant or a polymer molecule in different environments generally give different NMR signals. For example, micellization changes the shielding constant for nuclei in the surfactant tail and, therefore, affects their NMR frequencies. Observing only a frequency shift but no line splitting for coexisting micellar and monomer states, one can directly classify the kinetics of exchange of single surfactant molecules between those two states. The magnitude of the experimental frequency shift also contains information about the average amount of surfactant molecules residing in micelles and as monomers (Paper 2). This is a unique feature of NMR in comparison with other detection methods such as conductivity, turbidity and X-ray scattering, all of which have previously been used in kinetic experiments in micellar solutions.

Whereas the influence of micelle formation on the NMR signal is relatively minor, anisotropic crystalline or liquid crystalline phases exhibit a non-vanishing dipole–dipole and/or quadrupole (for nuclei with spin \( I>1/2 \)) broadening and splitting of NMR spectra. Hence, NMR is very sensitive transformations into and from such phases.

Information about the state of interface between surfactant aggregates and the solvent can be in many cases obtained by cross-relaxation experiments. Since the large surface areas of entities is an intrinsic property of colloidal systems, interface properties are of vital importance for colloidal science. Although relaxation and diffusion measurements may also provide information of this kind, only the cross-relaxation method is capable to provide quantitative structural relations between solutes and solvents.

However, most results obtained in conventional NMR experiments relate to the equilibrium state of a colloidal system at a given temperature, pressure and chemical composition. To access the kinetics
of phase transitions and other kinetic processes in micellar and polymer solutions, it should, indeed, be very useful to study the evolution of NMR parameters under non-stationary conditions\textsuperscript{24}. For such investigations, initial non-equilibrium states can be established by various approaches, among which temperature-jump, pressure-jump and concentration-jump methods are the most common ones. As follows from thermodynamic principles, the free energy of transition ($\Delta G$) for each different control parameter is determined by different factors. Hence, each method above may generate its own unique evolution and, therefore, is equally important for kinetic investigations.

Despite its obvious advantages, using NMR as a detection tool under non-equilibrium conditions has a few shortcomings even when some of the instrumental challenges (see below) are solved. Hence,

- information about material properties cannot be acquired at an arbitrary speed. In particular, this limitation stems from the pulse performance of the instrumentation that produces the initial condition for evolution and from the longitudinal and transverse relaxation properties of the investigated system.

- NMR does not provide instantaneous recording of material properties but is ultimately limited by the time scale defined by (on the reciprocal scale) the magnitude of change in the observed NMR variables. If evolution during signal acquisition itself cannot be neglected, the interpretation of the NMR signal is complicated\textsuperscript{24, 25} and different from that for static measurements.

**The single-pass approach**

A typical pulse sequence for a single-pass kinetic NMR experiment designed for serial recording of evolution of the NMR spectrum is shown on Figure 4.1. The evolution initiated during period $t_j$ is studied by recording a series of subsequent responses of the spin system obtained by 90° pulses.
Figure 4.1 The pulse sequence for a single-pass recording of a series of NMR spectra.

Hereby, the whole evolution is scanned in a single pass, i.e., a single jump from the equilibrium state. As shown in Chapter 2, the 90° pulse of the $B_1$ field applied orthogonally to $B_0$ causes the macroscopic longitudinal nuclear magnetization vector to be tipped into the transverse plane. This transverse magnetization precesses thereafter by its characteristic Larmor frequency and induces a signal, the so-called free induction decay (FID) as schematically indicated in Figure 4.1. Since transverse magnetization relaxes to zero with its characteristic decay time $T_2$, the length of the FID is also characterized by $T_2$. Subsequent pulses to provide new FIDs and, thereby, new NMR spectra can only be applied if both transverse and longitudinal magnetization components relaxed back sufficiently close to equilibrium. For a 90° pulse experiment this repetition (equilibration) time is

$$t_r \geq 5T_1 \geq 5T_2.$$  \hspace{1cm} (4.1)

However, $t_r$ is not only a function of $T_1$ and $T_2$ but also the initial deviation from equilibrium. Hence, faster repetition and thereby higher resolution for the time evolution is available with pulses shorter than 90°. A shorter pulse provides also a smaller NMR signal and, therefore, a compromise between signal intensity and time resolution must to be found.
The multi-pass approach

This method uses the fact that the duration of time between the end of the jump and the first scanning pulse can be selected without restriction by spin relaxation. The only limiting factor is instead the recording delay $t_d$ that depends on the construction of the jump apparatus (see Chapter 6). The corresponding pulse sequence designed for monitoring fast (with respect to $T_1$) kinetics is shown in Figure 4.2.

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**Figure 4.2** A multi-pass kinetic NMR experiment for studying fast kinetics.
The system is taken out of equilibrium $N$ times, and the $N$ separate evolutions for different $t_f' = [t_d \ldots 5T_1]$ are recorded. Subsequent $m$th spectra in the different series are then recorded at the times $T = t_f' + m \cdot 5T_1$. Data from the $N$ separate jumps is then placed in one kinetic curve so that the effective repetition time becomes $N$ times smaller than that in Figure 4.1. This approach, however, is not applicable or limited if (i) the substance has a hysteresis in its evolution, or (ii) in concentration-jump experiments in which case $N$ sets of fresh solutions are required. If the NMR signal is very small, the same pulse sequence but with constant $t_f$ values can be used. The obtained $N$ sets of equivalent evolution points can be then summarized to improve the signal-to-noise ratio of the kinetic curve by factor $\sqrt{N}$.

**Kinetic NMR relaxation and diffusion measurements**

The examples above illustrate recording the evolution of NMR spectra. Spectral parameters such as shape, intensity, and chemical shift may contain sufficient information but it is sometimes important to investigate the evolution of other parameters such as the relaxation times $T_1$ and $T_2$ or the self-diffusion coefficient $D$. These parameters may reveal information about molecular motions and interactions of surfactants or polymers in various colloidal phases.

Since the conventional approaches used in static NMR experiments are typically time consuming, various innovative pulse sequences were proposed. Another possibility pursued here is to use conventional pulse sequences, but with one set value of that parameter that is the experimental variable in the original sequence. Hence, the obtained signal intensity will be multiplied by a factor that depends on the applied pulse sequence and on the molecular property the sequence is intended to measure.

Examples are

- $I(t, T_2) = I(0) \exp\left(-t_{eff} / T_2(t)\right)$ (4.2)
where the detection pulse sequence is either a spin echo or a spin echo train with total transverse decay time set to $t_{\text{eff}}$.

- $I(t, D) = I(0) \exp(-kD(t)G^2)$

where the signal from stimulated echo with a given gradient strength $G$ is recorded.

Note that in both cases the variation of the intensity with the kinetic evolution time $t$ can be obtained either by the single-pass or multi-pass sequences shown in Figures 4.1-2 above. The optimum values for timing or gradients have to be found in static experiments.

As concerning diffusion studies, the pulsed-field-gradient double stimulated echo (PGDSTE) sequence was found to be more suitable for kinetic experiments because of its insensitivity to motional and convective artifacts\textsuperscript{28}. The corresponding pulse sequence for a single-pass kinetic NMR experiment designed for recording of evolution of the diffusion coefficient is shown in Figure 4.3.

![Figure 4.3 Kinetic NMR diffusion experiment performed with single pulsed-field-gradient double stimulated echo (PGDSTE) detection.](image)

Since all parameters of the pulse sequences are constant, the 1st, 2nd, -$m$th echo signal intensities are different only if the diffusion coefficient is changing. The optimum values for gradient pulse parameters ($G, \delta, \Delta$) have to be found in static experiment; for a given diffusional decay, the most sensitive point is at close to half intensity.
Electrophoretic NMR

Despite of the apparent difference between kinetic and electrophoretic NMR experiments, the latter ones can be also assigned as kinetic NMR methods. This classification is based on the fact that positions or velocities of charged particles are perturbed if a pulsed electric field is applied to the sample. Charged entities in solution are accelerated by the Coulomb force until counteracting viscous forces establish a constant velocity. The effective displacements caused by electric field pulses are then monitored by displacement-sensitive pulsed-field-gradient NMR sequences\(^{29}\). Finally, the magnitude of the electrophoretic mobility is calculated from the obtained dependence of displacement versus the electric field strength.
5. Instrumentation

Concentration-jump NMR and its artefacts

Concentration-jump techniques have gained widespread importance in investigation of kinetic processes in colloidal system\textsuperscript{30-32}. Among all approaches providing fast changes of concentration, stopped flow (SF) arrangements for rapid mixing of two different liquids dominate and excel\textsuperscript{33, 34}.

Combined with NMR, SF shares the capabilities of NMR that are useful in revealing many aspects of the chemical or physical state of matter\textsuperscript{35-38}. Below, our experience concerning stopped-flow NMR is discussed with emphasis on data interpretation and on prudent assessment of experimental artefacts. This experience was gained during the roughly three-years-long process of iterative building-testing-rebuilding that finally resulted in our current apparatus incorporated with a Bruker AMX 300 spectrometer. Since the discussion can not be conducted without referring to the current construction, the design of the SF setup shown on Figure 5.1 is described first.

The stopped-flow apparatus is designed for rapid mixing of two liquids with different viscosities within a wide-bore superconductive magnet by Bruker equipped with a 50 mm inner-diameter room-temperature shim tube. The detection can be made through any 10 mm NMR probe. In contrast to traditional SF setups designed for other detection techniques, the limited sensitivity of NMR requires a rather large volume in the observation chamber (a 0.2 ml U-tube in our setup). This large volume allows us to investigate dilute solutions but increases the transport time required to replace an old mixture in the observation chamber by a fresh one. Since the whole assembly is placed in the region of strong magnetic field traditional electric valves or switches as well as any magnetic devices were not permitted in the construction.
Figure 5.1  Schematic picture of the stopped flow design intended for 10 mm probes for wide-bore magnets.

The individual components of the intended mixture are stored before the experiment in two cylindrical chambers A from where they are driven by pressurised air through valves B when those are open. The mixing takes place in two serially arranged tangential jet mixer blocks F. The mixed liquid streams into the observation chamber located in the sample space of the NMR probe. Thereafter the mixture may continue into an
output chamber C. Pressurized air is constantly applied to the top of chambers A and the stopped-flow experiment is initiated by applying a suitably shaped electric current pulse to solenoid N. It is the Lorentz torque on N in the field $B_0$ that opens valves B. The pulse generator is triggered from a pulse sequence that is carried out by the spectrometer. The mixing ratio of the two liquids is adjusted manually by screws S prior to the experiment.

The performance of a stopped-flow apparatus is typically characterized by the so-called dead time defined as the time taken by the solution to flow from the mixer to a point halfway through the observation chamber. In our apparatus it is 50 -100 ms depending on the properties of investigated materials. Note that this value is large, primarily because of the large volume of observation chamber; the dead time/mixture volume ratio seems to be the same in our instrument and in other designs.

The possible artefacts can be divided into two groups: those common to the stopped-flow technique and those specific to NMR as the detection tool. The first group includes:

1. motional artefacts due to cavitation or long-lived turbulent motion
2. incomplete mixing and poor compositional homogeneity of the final mixture
3. heating of the final mixture due to high enthalpy of mixing

Artefacts a and b are frequently observed and discussed in the literature. In our apparatus, no evidence for cavitation or any long-lived turbulences inside the observation chamber were observed at times >40 ms after the end of driving pulse. However, it was found to be difficult to solve problem b and obtain a satisfying compositional homogeneity of the mixture. Numerous types of mixers were tested, but the only design that performed well even for components with very different viscosities was two tangential Gibson-Milnes mixer blocks placed in series. Results obtained for other types of mixers revealed incomplete mixing inside the observation chamber. In those cases, the
subsequent diffusional homogenization of the mixture looked like slow
dynamics. It should be noted that a single Gibson-Milnes mixer block
did not provide a reliable mixing, either. We suspect that this artefact
might have been a contributing reason behind some observations of slow
dynamics in surfactant solutions. One should also note that NMR is an
excellent technique to reveal compositional inhomogeneity.

Possible distortion of experimental data caused by artefact $c$ is
seldom mentioned. Whereas $a$ and $b$ can be suppressed in a proper
construction, the presence of $c$ depends only on the material properties
of mixture components. When the mixing enthalpy $\Delta H_{\text{mix}}$ is large, a
stopped-flow experiment produces not only a jump in concentration, but
also in temperature. Thus, the system is taken out of equilibrium by
simultaneously changing of two macroscopic parameters that may
counteract or amplify each other. Hence, the subsequent temperature
equilibration process may mask the other kinetics of interest. The
significance of this effect was tested in our instrument applied to the
micellar breakdown kinetics after rapid mixing of a 100 mM aqueous
solution of NaPFO (CMC $\sim$ 31mM) and ethylene glycol. Recent X-ray
scattering stopped-flow studies in a similar system$^{41}$ showed a slow time
dependence of the scattering curve that was attributed to micellar
dissolution kinetics. Whereas a lot of factors may influence the $^{19}$F
spectrum of the mixture, the $^1$H spectrum is insensitive to the state of
surfactant aggregation. However, the shift difference between the $^1$H
hydroxyl and methyl lines of ethylene glycol is temperature dependent
with a $\sim$0.01ppm/K sensitivity. Thus, the temperature inside the
observation chamber after mixing can be precisely monitored as shown
in Figure 5.2. The observed value of initial heating $\Delta T$ is in agreement
with the reported enthalpy of mixing for water-ethylene glycol pair$^{42}$
and the heat capacity of the solution. We found that both the observed
initial heating (from the mixing enthalpy) and subsequent cooling (by
heat loss through the wall of the observation chamber) effects can be
compensated for by appropriate pre-heating and thermostating of the
observation chamber.
Figure 5.2  The temperature inside the observation chamber as derived from the temperature-dependent $^1$H spectra of ethylene glycol. ($\blacktriangle$) no preheating of the observation chamber, ($\vartriangle$) preheating by $\Delta T$.

The results obtained after pre-heating the chamber to the final temperature $T + \Delta T$ (i.e., to 298K in our example with $T = 293$ K for the input chambers) is also shown above on Figure 5.2 When detecting the $^{19}$F signal in the same system, the chemical shift of CF$_3$ line of NaPFO is sensitive to the average quantity of surfactants residing in micellar region. As shown in Figure 5.3, the slow time dependence of chemical shift obtained in the experiments with no preheating of the observation chamber could be easily misinterpreted as a sign of slow micellar dissolution.

Figure 5.3  The chemical shift of CF$_3$ peak in $^{19}$F spectra of NaPFO ($\blacktriangle$) no preheating of the observation chamber, ($\vartriangle$) preheating by $\Delta T$. 
However, the chemical shift remains constant for a preheated observation chamber. This observation illustrates the possibility of heating artefacts. Note that the same experimental outcomes were also obtained for NaPFO – propylene glycol, APFO – propylene (or) ethylene glycol mixtures.

The second group of artefacts consists of:

- **d.** high sensitivity to incomplete degassing of mixture components because of the paramagnetic nature of air bubbles.
- **e.** initial relaxation of NMR signal intensity caused by changing the nuclear spin polarization as liquids are pumped between regions with low (initially, approximately 10 % below the $B_0$ value in the observation chamber) and high magnetic fields.
- **f.** line shape distortions if material evolution is significant during the free induction decay.

The presence of paramagnetic air bubbles in the mixture affects $T_2$ and, therefore, the line shape. However, this influence was found to be significant only for ethanol – water mixtures and could be suppressed by thorough degassing of the water component. As concerning artefact **e** this has always been present in similar NMR experiments and is actually very low in our apparatus where the initial components are stored just 15 cm above the region of the maximum magnetic field.

Conventional interpretations of NMR spectra in terms of concentration are not valid if material properties change substantially and quickly during the FID. Even if the theory for interpreting NMR spectra recorded under such conditions exists, the recorded signal may not always contain sufficient information about the molecular details of evolution. Nevertheless, in our experiments this phenomenon could be neglected.
Temperature-jump NMR and its artefacts

This technique is intended for studying a wide range of temperature-induced phenomena including phase transitions, solvation and chemical reactions. Since colloidal solutions may dramatically change their microscopic or macroscopic states upon temperature change, it would be advantageous to investigate their induced evolution by an NMR spectrometer equipped with a temperature-jump (TJ) setup. The name implies that the temperature of the whole sample should be rapidly changed to the target value and then kept constant. However, in reality the initial heating can lead to temperature gradients that depend on the heat exchange rate within the heated volume and the construction of the apparatus. Additionally, the temperature of the sample may not stay stable during the extent of the performed experiment. The connected artefact may mask the evolution of interest or may erroneously indicate material kinetics even in the absence of that.

Consider here an arbitrary function $F(T)$ by which a material property $F$ depends on the temperature. The absence of kinetics in material properties provides that $F(T)$ responds immediately to any change of temperature. Whereas any unknown instability of the target temperature $T(time)$ obviously induces an artificial evolution $F(T(time))$, the influence caused by time-dependent temperature gradients cannot be completely characterized since it depends of the function $F(T)$ itself, the shape of temperature gradient distribution and its time evolution.

For monotonic functions $F(T)$ the effect of the normalized temperature distribution over the sample $W(T)$ and its time evolution can be formally evaluated as

$$
\overline{F} = \int_{\nu} F(T)W(T)dT
$$

(5.1)

where $\overline{F}$ is the observed average property. The target temperature of the whole sample becomes upon the equilibration of the temperature gradient:
which also provides the target value

\[ F_{\text{final}} = F(\bar{T}) \]  

(5.3)

Assuming a linear dependence \( F(T) = a + bT \) with arbitrary coefficients \( a, b \) one can easily show that

\[ \bar{F} = F_{\text{final}} \]  

(5.4)

Hence, for any temperature dependences that are close to linear (that is, their Taylor expansion up to the linear term approximates well the full function) the artefact arising from the temperature gradient is small.

However, in certain NMR experiments one observes not only an average property but the whole distribution \( F(T) \). This is the case when the NMR frequency depends on the temperature and a temperature gradient leads to a line broadening. Even in this case, the average frequency is still time independent if \( F(T) \) is sufficiently close to linear.

A distant and usual situation is when \( F(T) \) is a step function; this is often the case for phase transitions as illustrated in Paper III for the \(^2\)H NMR line splitting across the phase boundary between nematic and isotropic phases of 45 % CsPFO at approximately 315 K. If the average temperature and the temperature gradient is such that the temperatures within the sample spread over both the nematic and isotropic regions, a superposition of both corresponding spectra will be observed. As heat exchange monotonically decreases the temperature gradient, the target temperature to which all parts of the sample will arrive may be either in the nematic or the isotropic region. In whichever case, the observed time evolution of the spectrum would mostly depend on the temperature distribution instead of any actual kinetics of CsPFO. Obviously, this artefact is suppressed if the whole temperature distribution after heating is within a region that corresponds to just one of the phases.
The discussion above illustrates the importance of minimizing the temperature gradient and stabilizing the target temperature. It is also clear that the interpretation of the observed evolution of NMR parameters is easier if one has a good general idea about the response function $F(T)$. These considerations were taken into account during stages of constructing and testing our temperature-jump apparatus (Figure 5.4).

**Figure 5.4** Schematic picture of the probe insert constructed for temperature-jump NMR experiments performed in 10 mm probes for wide-bore magnets.

The setup is intended for the 50 mm inner diameter room-temperature shim tube of a wide-bore superconducting magnet by Bruker. Results of rapid heating can be monitored by any 10 mm NMR probe. Heating is achieved by a powerful RF pulse with 110 – 120 MHz frequency obtained from the standard BLAX 300 amplifier of Bruker. The RF field
is produced by a solenoidal heating coil. The temperature gradient is minimized by appropriate shaping of the heating coil and of the observation chamber, the details of which are presented in Paper III. Note that the RF electronics is built into a spinner body which makes sample insertion easy. The solenoid is connected to the high voltage capacitors placed in the spinner body by well-isolated cables.

The RF design is equivalent to that of tuned RF circuits in NMR probes. By adjusting tuning and matching capacitors, the RF input impedance is matched to the 50 Ω output of amplifier and is tuned to resonance in the 110-120 MHz frequency range. The absence of arcing and the amount of reflected RF power can be controlled by a home-made standing-wave-ratio (SWR) meter installed serially between the amplifier and the apparatus and connected to a Tektronix TDS380 oscilloscope. This arrangement provides the same information as the standard amplifier control board feature of the spectrometer but with a better accuracy.

The type and isolation of all used RF components were set and designed for 300 W maximum RF pulse power. Since the spinner body was constructed from copper, the capacitor array is shielded within. The spinner body, in effect a large capacitor, also provides a “local ground” for the RF heating coil. The shielding and the “local ground” effects are of vital importance in order to suppress deposition of RF noise and RF heating pulses in the NMR probe coil (where the latter can, among other deleterious effects, cause mechanical ringing).

A typical pulse sequence for our temperature-jump NMR experiments is shown in Figure 5.5. This pulse sequence is virtually identical to that shown in Chapter 4. To prevent overloading the RF amplifier, the main heating pulse consists of a train of 10 ms pulses separated by 0.1 ms. The total length of the train depends on the magnitude of the required jump, on the RF losses in the sample (mostly defined by sample conductivity) and on the set RF power. Currently limited by mechanical ringing of the heating coil, the first NMR spectrum of the heated sample can be recorded not faster than 20 ms
after the end of the heating pulse train ($t_f \geq 20$ ms). Short RF heating pulses applied intermittently after the temperature jump compensate for cooling through the wall of the sample chamber and, therefore, thermostate the sample at the obtained temperature during the extent of the performed experiment. Due to the importance of this stabilization, the length of these pulses must be reliably optimized for each new material with a given conductivity. This can be done by monitoring the temperature in the sample chamber; in aqueous samples, this can be easily accomplished by using the water (HDO) signal with its sufficiently temperature-sensitive $^1$H chemical shift as an internal thermometer.

The presented apparatus provides an average heating rate of 50 K/s in the 0.8 – 53 mS/cm conductivity range of the investigated material. Hence, 10-20 K jumps can be achieved in a few hundreds of milliseconds. The initial total temperature spread over the sample was rather large (40-50 %) directly after the jump but equilibrated after 1 s to <5%. Although this provides a limited applicability to materials with fast (<1 s) kinetics certain types of phase transitions are still measurable even below this limit as discussed above. We note that others have achieved a better performance as concerning temperature gradient\textsuperscript{44} by mechanical stirring to speed up heat equilibration. However this method, besides requiring a more complex setup, is obviously not applicable to a wide range of materials like liquid crystals, gels, or shear sensitive polymers.
Suppressing RF noise in electrophoretic NMR

In an electrophoretic NMR apparatus an electric field is established inside the NMR receiver coil by two electrodes connected to a high voltage amplifier. The introduction of RF noise through the extra wiring degrades the signal-to-noise ratio of the experiments. Since that latter parameter is already rather depressed because of the small filling factor of electrophoretic NMR samples, suppressing any noise input on this way is of high importance. There are two different sources that may provide RF noise at the frequencies and bandwidths of typical NMR experiments:

- RF interference which is picked up from the surrounding by the long connecting wires (between the electrophoretic DC amplifier and the probe) working as an antenna.
- RF noise (“noisy ground”) generated predominantly in the digital part of the electrophoretic pulsed power supply (a 500 MHz Pentium II computer used for pulse generation).

The schematic diagram of the RF interference filter is shown in Figure 5.6

![Schematic diagram of the RF interference filter](image)

*Figure 5.6* Schematic picture of the low pass filters used to suppress RF noise in electrophoretic apparatus.
The filter contains two stages, the first one (A) placed in a shielded box inserted halfway between the amplifier and the electrophoretic insert of the NMR probe and the second one (B) placed directly inside this latter insert. Both A and B are of standard design of second-order low-pass filter. All capacitors (500 pF from Elfa) rated for 2 kV maximum voltages; inductances $L$ (0.1 mH ferrite-cored coil) were scavenged from faulty NMR amplifiers; home-made inductances $l$ (10 μH) contained 100 turns coiled on a 2 mm ceramic tube. Due to the required double polarity of DC currents and the related absence of DC ground in the electrophoretic parts, a double coaxial connecting cable (Twinax 2*50 Ω, from Elfa) provided one grounded shield and two “hot” connections.

Stage A suppresses the “noisy ground” effect; the aluminium body of A has a large capacitance and therefore establishes a “local ground” for the braided shield of the Twinax cable. For safety reasons, the shielding must also be connected to the noisy ground of the electrophoretic power supply via a filtering inductance $L$. Stage B suppresses the RF interference that may come from rest of the coaxial cable. The body of B were made of brass. Hence, the filter components within are shielded and a local ground is established. One should note that this two-stage arrangement was necessary because:

- There is no reliable high-capacity ground in and around the electrophoretic insert inside the magnet
- We could not use ferrite–cored inductances at the electrophoretic insert since all ferrites are saturated already in >0.1 T magnetic fields.

Indeed, the signal-to-noise ratio was worse when only stage B was used. In contrast, we detected no extra noise with the electrophoretic insert with full filter set inserted in an NMR probe. Without the filter set, one experienced a 3-4 times higher noise level.
Development of NMR probes for heteronuclear $^{1}\text{H}-^{19}\text{F}$ cross-relaxation experiments

The tunable frequency range of the broadband (BB) channel of standard NMR probes, typically present in any NMR laboratory, is limited to low frequencies, usually 10-60 % of the corresponding proton frequency. On other hand, the $^{1}\text{H}$ channel is typically selective with a narrow range of tunable frequencies. Hence, NMR probes suitable for NMR experiments with simultaneous access to $^{19}\text{F}$ (at 94 % of the resonance frequency of $^{1}\text{H}$) and $^{1}\text{H}$ nuclei are not widespread. This chapter describes our approach, in form of designs and constructions, to solving this problem and provide just such probes for $^{1}\text{H}-^{19}\text{F}$ cross-relaxation experiments at several different spectrometers, a Bruker DMX200, an AMX300 and a DMX500.

Among all existing designs of multinuclear probes, the double-coil construction of the RF part dominates and excels. In such a design, two saddle-shaped coils are placed coaxially, the internal of which tuned to the frequency of the “sensitive” nuclei (typically either $^{1}\text{H}$, or BB) and the external doubly tuned to the frequencies of decoupling and lock (usually $^{2}\text{H}$) nuclei. The angle between the two corresponding $B_1$ fields is arranged to be as close as possible to 90º to avoid unfavourable inductive coupling between the two coils. In contrast, another RF designs with a single coil were also proposed\(^47,48\), in particular for $^{1}\text{H} - ^{19}\text{F}$ probes. Because of the closeness of $^{1}\text{H}$ and $^{19}\text{F}$ frequencies the single RF coil participates simultaneously and with comparable efficiencies in two separate circuits tuned and matched to the corresponding frequencies. Although simple, this approach can suffer from several shortcomings such as a high level of coupling between the two RF channels and limited possibility for a $^{2}\text{H}$ lock channel. This latter limitation cannot be accepted in cross-relaxation experiments that use difference detection and therefore demand a superior stability of the magnetic field.

Hence, we relied on double-coil designs as described below; the standard schematics is shown in Figure 5.7
Figure 5.7  Schematic diagram of the $^{19}$F-$^1$H-$^2$H NMR probe.

The components participating in $^{19}$F, $^1$H, and $^2$H tuned circuits are given in “**BOLD**” “Cursive” and “*lower-case cursive*”, respectively. Adjustable capacitors with superscripts “T” and “M” are responsible for tuning and matching of RF circuits to the required frequencies and 50 $\Omega$ resistance. Superscripts “R” and “E” denote chip capacitors that supplement and close the parallel resonance circuits and influence thereby both tuning and matching. Capacitors $C_E$ and $C_E$ placed nearby the RF coils also suppress the influence of connecting wires on the filling factor of the coils and improve RF efficiency.

Because of the close frequencies arranged in a limited space, the $^1$H and $^{19}$F channels interact. To decrease their mutual coupling, the angle between the $B_1$ fields of the $L_{\text{int}}$ and $L_{\text{ext}}$ coils has to be precisely set to $90^\circ$. How well this can be done depends, among other things, on the
directional distribution of \( B_1 \). Also for this reason, this distribution should be as low as possible.

The \(^2\text{H}\) lock channel is connected to one of the main RF circuits (\(^{19}\text{F}\) in the figure) which is thus doubly tuned. Like a filter, the \( l-c \) circuit decouples the two involved channels. Despite this, the presence of inductance \( L \) decreases the efficiency of main circuit. Hence, a smaller inductance of \( L \) is favourable for the efficiency of the lock channel but unfavourable for \(^{19}\text{F}\) one.

Hence, the main difficulty in constructing the probes was with optimizing all parameters and searching for appropriate geometries of \( L_{\text{int}} \) and \( L_{\text{ext}} \) coils. Since we had several different sets of NMR frequencies and, therefore, different optimal inductivities for the RF coils, we could not use a single design of \( L_{\text{int}} \) and \( L_{\text{ext}} \), respectively. The selected coil sets are shown in Figure 5.8 and Table 5.1:

\[ \text{Figure 5.8} \quad \text{Schematic pictures of the RF coils.} \]
### Table 5.1  The list of used component values.

<table>
<thead>
<tr>
<th>Spectrometer</th>
<th>Sample</th>
<th>Internal coil</th>
<th>External coil</th>
<th>Lock channel</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DMX 200</strong></td>
<td>5 mm</td>
<td>A, $C_E=5$ pF</td>
<td>B, $C_E=7.5$ pF</td>
<td>Connected to internal coil</td>
</tr>
<tr>
<td></td>
<td>10 mm</td>
<td>B, $C_E=5$ pF</td>
<td>B, $C_E=4.7$ pF</td>
<td>Connected to external coil</td>
</tr>
<tr>
<td><strong>AMX 300</strong></td>
<td>5 mm</td>
<td>A, $C_E=2.2$ pF</td>
<td>B, $C_E=2.2$ pF</td>
<td>Connected to internal coil</td>
</tr>
<tr>
<td></td>
<td>10 mm</td>
<td>B, $C_E=2.2$ pF</td>
<td>B, no $C_E$</td>
<td>Connected to external coil</td>
</tr>
<tr>
<td><strong>DMX 500</strong></td>
<td>5 mm</td>
<td>B, no $C_E$</td>
<td>C, $C_D=3.3$ pF</td>
<td>Connected to internal coil</td>
</tr>
</tbody>
</table>

The table also contains information about the lock channel and capacitances $C_E$ and $C_D$ (the latter is not shown on the Figure 5.7). All mentioned parameters were found experimentally by a home-built sweep-frequency instrument and then adapted in real NMR experiments. This sweep-frequency instrument includes a sweep generator (used only up to the 300 MHz frequency), a Tektronix oscilloscope and an RF bridge. For higher frequencies (up to 500 MHz) a home-built sweep generator (see part of the design on the cover) was constructed. The NMR probes themselves were constructed from surplus “General Electric” probes, the body size and temperature stabilisation part of which were mechanically adapted for Bruker wide-bore (DMX 200 and AMX 300) and standard-bore (DMX 500) magnets and temperature control units respectively.
6. Conclusions

Paper I

A new approach for constructing stopped-flow NMR tools intended for conventional Bruker wide-bore superconductive magnets is presented in this paper. In many ways, the design is a patchwork of compromises among the many conflicting demands on a fast-mixing NMR setup.

The apparatus is loaded directly above the probe space and can be used together with any 10 mm NMR probe. One advantage of this arrangement is the absence of long transfer lines that saves sample and suppresses motional and spin polarization artefacts.

While traditional stopped-flow setups\textsuperscript{32-37} use plungers to drive and stop the flow, pressurized air and rapid electrically-driven valves are used for this purpose in our construction. To be close or open, these valves exploit the Lorentz torque exerted by the magnetic field on a current-leading-coil. In contrast to more conventional arrangements, our approach does not require precise and low-friction syringe-plunger fitting. On the other hand, the volumes of mixed liquid components are controlled less precisely. The obtained reproducibility of mixing ratio over separate experiments was in the order of a few percent. Hydrodynamic factors influencing the reproducibility and the general performance were also investigated.

The chosen volume of the U-tube-shaped observation chamber (0.2 mL) reflects a compromise between access to fast kinetics in dilute (~10 mM) surfactant solutions and the so-called dead time of the stopped-flow apparatus. In our construction, the latter becomes typically 50-100 ms depending on the actual conditions.

Among all problems that arose during designing and testing the apparatus, the optimal construction of the mixing chamber prevailed. In this context it is worth to note that the actual mixing quality is seldom controlled in a typical stopped-flow experiment. Here, using NMR as a detection tool provides us with a superior method to do
Conclusions

just this. Hence, the compositional homogeneity of the obtained mixtures in our apparatus was controlled by recording conventional NMR spectra that contained lines with strongly concentration-dependent resonance frequencies. The following pairs of liquids with different viscosities were selected and used for monitoring the mixing quality:

- H₂O and ethanol (96 %), where the exchange rate of water and ethanol hydroxyl protons is strongly dependent on the ethanol concentration. The corresponding part of the ¹H spectrum may be either split into two lines or a single line with a narrow range of continuous transition between these two extremes.

- H₂O and ethylene glycol (99 %). Similarly, the shift and the width of the water/hydroxyl line of the mixture depend on the composition. Note that the two liquid components had rather different viscosities.

- aqueous solutions of D-alanine (0.5 M, 99 %) with pH = 6 and pH = 14, respectively. Here the chemical shift of the methyl resonance strongly depends on the pH.

In all these tests, there was no sign of incomplete mixing (local or large scale) if two serially arranged tangential jet Gibson-Milnes^40 mixing blocks served as mixing chamber. Due to their high mixing enthalpies, heating artefacts were found in the water–ethanol and water – ethylene glycol mixtures. This problem has to be counted as a serious hidden potential artefact in stopped-flow experiment in many colloidal systems. A method based on an appropriate pre-heating of the observation chamber was proposed and tested to suppress this artefact.
Conclusions

Paper II

The kinetics of micellar dissolution and transformation in aqueous solutions of sodium perfluorooctanoate (NaPFO) were investigated by our stopped-flow NMR instrument. Previous stopped-flow studies\textsuperscript{49} by time-resolved small-angle X-ray scattering (SAXS) detection indicated a slow (~10 s) kinetics in the scattering curve that was assigned (on the basis of the classical Aniansson theory) to slow kinetics of dissolution. In contrast, we found no evidence of any slow kinetics.

The evolution of the micellar solution was monitored through $^{19}$F NMR chemical shifts and transverse relaxation times $T_2$, both measured as a function of time after quick stopped-flow dilutions of micellar solutions of NaPFO with water. Since among the $^{19}$F lines it is the CF$_3$ line that is most sensitive to the state of surfactant aggregation, we recorded the time dependence of the corresponding spectral range of NaPFO and the transverse relaxation time $T_2$ of that line. Since we have a single CF$_3$ line, the molecular exchange of NaPFO between micellar and aqueous environments must be fast with $\tau_{\text{mon}} \ll 100 \, \mu s$. Hence, the measured chemical shift $\delta$ is given by the population average

$$
\delta = \frac{c_{\text{mic}}}{c_{\text{mic}} + c_{\text{mon}}} \delta_{\text{mic}} + \frac{c_{\text{mon}}}{c_{\text{mic}} + c_{\text{mon}}} \delta_{\text{mon}}
$$

(6.1)

with $c_{\text{mon}}$ and $c_{\text{mic}}$ denoting the surfactant concentrations in monomer and micellar states, respectively.

Various proportions of dilutions and initial concentrations of NaPFO were tested. Irrespective of the selected conditions, the obtained chemical shift and thereby the average intermolecular environment of the CF$_3$ groups changed immediately upon mixing without any trace of slow (> 100 ms) dynamics. This means (within limits set by the experimental precision) that the relaxation of both $\delta_{\text{mon}}$ and $\delta_{\text{mic}}$ to the new equilibrium values is rapid. Additionally, from the measured time-independence of the $T_2$ values after dilution we conclude that the
micelles do not significantly change their average size and/or shape at > 100 ms after the concentration jump.

In summary, the obtained results show rapid, <100 ms dissolution and/or transformation of NaPFO micelles. This is in agreement with the results obtained for hydrogenated surfactants with comparable chain hydrophobicity, but is in contradiction with SAXS-detected stopped-flow studies\textsuperscript{49} of the same material. Apart from systematic error, this discrepancy might perhaps be explained by the complementary nature of NMR and SAXS as detection tools. In this context, one should note that the slow kinetics observed by SAXS for a 10-fold dilution to 50 % of CMC is difficult to reconcile with any existing theory: since no micelles are present at the final concentration, where is no “narrow passage”-like bottleneck as introduced by Aniansson\textsuperscript{13}. This conclusion should remain valid even when one considers that the Aniansson theory may not be directly applicable for experiments where the system is driven far from equilibrium.
Paper III

A new construction intended for rapid heating of conductive samples within any standard 10 mm NMR probe is presented in this paper. Existing temperature-jump setups use laser\textsuperscript{46, 50} or microwave volume\textsuperscript{44} heating that require rebuilding of the NMR probes and auxiliary equipments. In our construction, the volume heating is achieved by dielectric and inductive losses of an RF field within the conductive material\textsuperscript{51}. This field is produced by a solenoidal coil wound around the sample and connected via a tuning/matching scheme to the output of a conventional RF amplifier. The temperature gradient within the sample volume is minimized by shaping the coil and the observation chamber.

As one disadvantage, we obtained a significant field inhomogeneity and resulting spectral broadening caused by inclusion of the heating coil in the sample space. The efficiency of the apparatus was tested by recording temperature-sensitive NMR spectra of (i) 0.2 M aqueous solution of K\textsubscript{3}Co(CN)\textsubscript{6} in H\textsubscript{2}O and (ii) 50 \% aqueous solution of ethylene glycol with adjusted (by dissolved NaCl) conductivity. The following performance parameters were attained:

- the average heating rate was 50 K/s for the 0.8 – 53 mS/cm conductivity range.

- the initial temperature spread over the sample was large, 40-50 \%, directly after the jump but equilibrated within a second to <5\%.

- heating by dielectric losses predominated in the investigated conductivity range.

The performance of the design was also illustrated by temperature-jump \textsuperscript{2}H NMR experiments in the lyotropic liquid crystalline mixture of cesium perfluorooctanoate and \textsuperscript{2}H\textsubscript{2}O. We observed a quick (<500 ms) phase transition between the nematic and isotropic phases in that system.
Conclusions

Paper IV

The kinetics of the coil-to–globule transition and intermolecular aggregation of a poly (N-isopropylacrylamide) solution in D\textsubscript{2}O are investigated by our temperature-jump NMR instrument. Both states of the polymer were first characterized by recording (in static NMR experiments) the temperature dependences of \textsuperscript{1}H spectra, the $T_1$ and $T_2$ relaxation times and the self-diffusion coefficient $D$. The obtained dramatic decrease of spectral intensity upon crossing the lower critical solution temperature (LCST, ~306 K) is attributed to the collapse of the initially highly mobile PNIPAM coils into tight and rigid globules, whose broad NMR signal is undetectable in high-resolution detection mode. Hence, only the signal from a fraction $P$ of mobile PNIPAM chains remains narrow and measurable in relaxation and diffusion experiments. The polymer relaxation times and the self-diffusion coefficient do not change in a stepwise manner at the LCST; instead, the slope of their temperature dependence changes markedly. These data allow us to calculate the lower size limit of >10 nm for the intermolecularly aggregated globules at few degrees above the LCST.

The kinetics of spectral intensity, $T_2$ and $D$ were investigated upon temperature jump from 300 K to 312 K. The immediate decrease of spectral intensity (see Fig. 4a in Paper IV) shows that the rigid globular state with equilibrium concentration $1-P$ appears very quickly, <1 s after crossing the phase transition temperature. In contrast, data for $T_2$ and $D$ (Figs. 4b and 4c) are time dependent: the $T_2$ of the mobile phase increases (by approximately 60-70 %) for a long time, 30-40 seconds, after the jump while $D$ decreases (by approximately 20-30 %). These two apparently contradicting observations show that:

- the reorientational mobility of the polymers in the mobile phase increases as time passes after the temperature jump.
- the translational mobility of those polymer decreases during the same time.
Since the observed parameters are attributed not to the aggregates but only to the presumably shorter mobile PNIPAM chains, the molecular details of the whole kinetic process remain less characterised. However, the kinetic observations for the mobile chains are distinctive and may point to some sort of slow reorganization/redistribution of individual chains among and within the globular and mobile states and/or to a weak association between the shorter chains and the large aggregates.

Cooling the sample quickly from 312 K to 295 K, an immediate jump in NMR parameters within the ~3 s experimental time resolution was found. This means that, as NMR spectroscopy sees it, starting from a globular state, the system swells into distinct coils within a few seconds.

The presented experiments also illustrate a methodological point: during our investigation, our temperature-jump setup could be readily (with a transfer time of a minute) used in combination with several NMR probes and, hence, could detect not only the evolution of more conventional NMR parameters but also that of the self-diffusion coefficient.
The main aim of this study was to explore the frequency dependence of the intermolecular $^1$H – $^{19}$F dipole-dipole cross-relaxation rates between the solute and the solvent in aqueous solutions of (i) ammonium perfluorooctanoate (APFO) or (ii) trifluoroacetic acid (TFA). The obtained intermolecular cross-relaxation data were evaluated to provide information about the water – hydrophobic interface.

The investigation has been performed using carefully designed $^1$H – $^{19}$F experiments and our new $^1$H-$^{19}$F–$^2$H probes developed and constructed as described in Chapter 5. Since the whole range of our spectrometers (DMX200, AMX300, DMX500) is now equipped with the above-mentioned probes, it was possible to perform the experiments at three different magnetic fields. The experiments provided cross-relaxation rates $\sigma_{HF}$ between water and APFO and TFA, respectively.

Both sets of the obtained $\sigma_{HF}$ data are clearly and significantly frequency (field) dependent that provides a clear demonstration of the lack of extreme narrowing for intermolecular dipole-dipole relaxation. This latter finding may sound surprising since it is in apparent contradiction with the small size and fast tumbling of APFO, TFA, and water with rotational correlation times far below 100 ps.

The observed frequency dependences were analyzed and explained in the framework of a theory originating from a long line of contributing researchers and their co-workers (Torrey-Hwang-Freed-Ayant-Fries-Halle). This theory that takes correctly into account the long-time tail of the diffusional autocorrelation function reveals that intermolecular dipole-dipole interactions and the cross-relaxation they cause are far more complex than the intramolecular ones. Thus, the conventional concept of a spectral density function based on results obtained for isotropic tumbling and intramolecular interactions and with a well-defined motional correlation time $\tau_R$ is not valid in this case.

Using a more complex novel relaxation model, various motional modes of the whole ensemble of interacting spins are taken into account:
the translational-rotational motion of a spin pair whose members reside in two distinct molecules and the non-uniform mobility of solvent molecules closest to solute. One drawback of the model, itself a very rough representation of the actual molecular system, is that the spectral density functions and therefore the $\sigma_{HF}$ rates depend on model parameters that are more than the number of experimental points. Several model parameters can be judiciously fixed with the help of, e.g., translational self-diffusion and intramolecular cross-relaxation experiments. Nevertheless, it is only allowed parameter ranges that can be extracted from such data.

Those ranges and the qualitative data behaviour permit characterization of the water-hydrophobic interface in the two investigated molecules in terms of two parameters: (i) the number of water layers dynamically retarded by solute, and (ii) the retardation factor within these layers defined as the ratio of the translational self-diffusion coefficients in this layer and in bulk.

Results obtained for APFO and TFA are reproduced in Figure 6.1 (identical to Figure 8 in Paper V).

![Figure 6.1](image.png)

**Figure 6.1.** The retardation factor obtained by fitting the theoretical model (see text) to the frequency-dependent cross-relaxation rates in Fig. 6, for TFA (●) and APFO (■). In case of ≤2 hydration layers, the APFO data cannot be fitted.

As it was found, our model could not fit at all the APFO data for ≤2 hydration layers irrespective the value of the retardation factor: the model function could reproduce neither the overall magnitude nor the
Conclusions

slope of the experimental cross-relaxation rates. Importantly, this also means that the two involved parameters of the model are uncoupled. In other words, intermolecular cross-relaxation seems to have a remarkable and unique property: it may distinguish between the extent and the degree of dynamic retardation of the solvent by the solute.

Another model-dependent conclusion is that the hydration level of the CF$_3$ groups in APFO and TFA are different. Irrespective whether it is the extension or the degree of retardation that differs between APFO and TFA, the different nature of water-hydrophobic interface follows from the strikingly different slopes of frequency dependences of the obtained cross relaxation rates.
Paper VI

Heteronuclear $^{19}\text{F} - ^1\text{H}$ NMR cross-relaxation techniques are exploited to study the solute-solvent contact in aqueous micellar solution of NaPFO. The paper also develops the methodological and theoretical approaches for evaluating the frequency-dependent intermolecular cross relaxation rates that were introduced in Paper V.

The cross relaxation rates for each group of fluorines residing in the surfactant chain were measured in DMX 200, AMX 300, DMX 500 spectrometers using our new $^1\text{H} - ^{19}\text{F} - ^2\text{H}$ probes developed and constructed as described in Chapter 5. Because the surfactants are in fast exchange between monomeric and micellar states, these quantities are environmentally averaged. The contribution of monomers was subtracted by using the results of Paper V.

The obtained micellar cross-relaxation rates $\sigma_{HF}^{\text{mic}}$ decrease toward the end of surfactant chain that manifests a decreasing contact between the solvent and groups deeper down on the hydrophobic chain. The influence of spin diffusion (intramolecular $^{19}\text{F} - ^{19}\text{F}$ cross-relaxation $\sigma_{FF}$ along the chain) on the obtained data was negligible as shown by evaluating the experimental $\sigma_{HF}$ and $\sigma_{FF}$ values within the framework of three-spin Solomon equations.

The spectral density function introduced in Paper V was thoroughly revised and adapted for the case of micellar solutions. As there, the parameters characterising solute-solvent contact were evaluated in framework of this model. Hence, the experimental cross-relaxation rates for all fluorines could be simultaneously fitted only if one or two retarded layers of surface water were considered. The obtained retardation factors were 13 or 5, respectively. The average distances between the fluorines and micellar surface were also evaluated yielding the distance distribution in Figure 6.2 (identical to Figure 4 in Paper VI).
The obtained monotonically increasing trend is in agreement with some degree of internal mobility. Another important finding is the direct contact between water molecules and fluorines in the C(2)F₂ group for which water and fluorine nuclei seem to be separated by a distance that corresponds to their van der Waal’s radii.
This work was initiated by our recent finding of fast (<100 ms) kinetics of dissolution of micelles in aqueous solution of NaPFO in H₂O (Paper II). Theories predict and experiments indicate that the presence of salt in ionic micellar solutions may retard the different kinetic processes. It is also well known that the amount of surfactant monomers in solution is gradually decreasing with increasing amount of added salt.

All experiments were performed by our home-built stopped-flow apparatus (Paper I) in combination with a Bruker AMX 300 spectrometer. The investigated materials were 100 mM aqueous solutions of either NaPFO or NH₄PFO surfactants. As in Paper II, the evolution of the micellar solutions was monitored through the CF₃ peak in the ¹⁹F NMR spectra recorded as a function of time after quick stopped-flow dilutions of micellar solutions with either NaCl or NH₄Cl solutions. Various concentrations of salts (up to 2 M) were tested. The following results are obtained for NaPFO in combination with NaCl:

a) in a pristine mixture with no pre-existing nuclei, no crystallization occurred on the time scale of minutes with initial salt concentrations up to 1 M. While the system remained in the micellar state, any change of micellar and monomer concentrations happened faster than 200 ms as indicated by the constancy of chemical shift after mixing.

b) with pre-existing crystalline nuclei, complete precipitation occurs over a period of a few seconds for 1 M concentration of added salt. (Figure 6.3, identical to Figure 1 in Paper VII)

c) lowering the salt concentration leads to a significantly slower crystallization process.

The constancy of the micellar-dominated chemical shifts upon decreasing intensity of the ¹⁹F line observed in the Figure 6.3 can not be explained within a two-state quasi-equilibrium model assuming fast exchange of the PFO- ions between monomer and micellar states.
Figure 6.3. The variation of the $^{19}$F NMR spectra of the trifluoromethyl region of 100 mM NaPFO solution after having been mixed by 1 M NaCl solution, with time elapsed after the stopped-flow mixing.

Instead, we consider three exchanging pools in quasi-equilibrium (that is, with slowly varying proportions of ions in the different pools) of PFO-ions: one micellar ($A$ for aggregate), one monomeric ($M$), and one in the forming crystals ($C$)

$$ C \xrightleftharpoons[k_{-1}]{k_1} M \xrightleftharpoons[k_{-2}]{k_2} A. \quad (6.2) $$

This model assumes that there is no direct contact between the micellar and crystalline pools, and the crystals grow by absorbing only free monomers. Within the framework of this model, the concentration of monomers becomes depressed to a lower value if the crystal-monomer quasi-equilibrium is pushed towards the crystals and the rate of micellar dissolution is slow. Hence the micellar-dominated chemical shift is almost unchanged even though the amount of micellized surfactant decreases.

The observed kinetics is strongly dependent on the counterion and on the salt. Repeating experiments with the same concentrations as in Figure 6.3 but with sodium changed to ammonium, we observed a quick (within a few hundreds of milliseconds) formation of a milky sample and a complete disappearance of the $^{19}$F signal. This we ascribe to the formation of large aggregates, perhaps multilamellar vesicles.
7. Future applications

As reported, kinetic NMR experiments can reveal a wide range of kinetic processes in micellar and polymer solutions. Our investigations so far suggest the following future use of the equipment developed.

Stopped-flow NMR

Although, in general, fluorinated surfactants having rigid hydrophobic chains are expected to exhibit a slower dynamics than non-fluorinated ones, we showed that NaPFO does not behave remarkably differently from hydrogenated surfactants. This is probably due to its relatively short chain length of 7 fluorocarbon units.

However, the situation may be completely different for a fluorinated surfactant with longer chains. Recent studies\(^{52}\) reported the presence of massive threadlike micelles and distinct “micellar” and “monomer” sets of peaks in conventional \(^{19}\text{F}\) NMR spectra of tetraethylammonium heptadecafluoroctaneosulfonate (TEAFOS) solutions in \(\text{H}_2\text{O}\). Those split peaks indicate a much slower (compared to that of NaPFO) exchange kinetics between the various states of aggregation of the surfactant molecules. The characteristic times of some of those exchanges may be accessible by our stopped-flow NMR techniques.

SF NMR studies of micellar solutions based on the FOS hydrophobic ion are suggested because:

- Aggregate and monomer peaks are resolved, so kinetics of those two states can be controlled independently.

- Threadlike micellar structures are known to be sensitive to shear-induced perturbations. As we recently found, our SF equipment also offers the possibility to introduce shear-induced perturbation. Shear stress is simply provided by placing identical TEAFOS solutions in both cylinders A and pumping them through the mixer blocks.
• Since LiFOS does not exhibit any split lines in NMR spectra, the comparison of dynamics in LiFOS and TEAFOS micellar solutions is interesting.

However, carrying out stopped-flow NMR experiments with FOS solutions is accompanied by the following potential problems:

(i) Low intensity of the “monomer” peak because of the low CMC value (~ 1 mM) of TEAFOS.

(ii) Very high viscoelasticity of the solution due to presence of threadlike micelles that might influence the homogeneity of the obtained mixtures in concentration-jump experiments.

**Temperature-jump NMR**

Triggered by a temperature jump, the transition between the nematic and isotropic phases of CSPFO (see Paper III) occurs faster than 500 ms. However, much slower and therefore detectable morphological changes may start to appear if both the initial and target temperatures are within anisotropic phases. These slower morphological changes may include re-alignment of the liquid crystalline vector and suppression (or induction) of internal defects. Investigation of these different aspects of the liquid crystalline dynamics provides not only information about the formed structures but also allows us to understand how to control the prepared morphology.
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9. Bibliography


