The abilities of NMR spectroscopy in researching of living tissues

In NMR experiments, like in others spectral researching, we gain information through exposure on conversion's velocity of microparticles from one state to other energy state.

According to energy law, energy state can change at expense on absorption or emanation EMF.

In our case, conversion with spin's orientation changes from across field to against field will cause radiofrequency absorption. In other conversions RF will be emanated.

EMF's energy E(v) defined by frequency v and intensity. However, in discrete interactions with microcosm objects, field's energy represented by partial carriers of EMF(E = hv, field particles) and number of such carriers.

As we see, EMF energy represented as sum of partial carriers, using Plank constant.

In resonance experiments we can stimulate spin conversion from one energy state to another, by exposing to the nucleus, placed in constant magnetic field B_0, with the EMF in resonance frequency v_0.

Resonance could be observed in case of equality of energy conversion ΔE and quantum energy dE(v) (EMF carriers): $h * \nu_o = (\mu/I) * Bo$ (13)

So we obtain main nuclear magnetic resonance equation: $\nu_o = (\mu/hI) * Bo$ (14)

Here in brackets we have gyromagnetic ratio – one of main adjustments. Gyromagnetic ratio γ defines resonance frequency v for some isotope in concrete magnetic field. For example, for protons in spherical ampoule with water gyromagnetic ratio was defined as $\gamma_p = 42, 576\ 375\ (13)$ MHz/Tl.

Resonance frequency, that could cause conversions and orientation changing for water hydrogen nucleus in magnetic field with induction $B_0 = 1$ Tl, is $v_0 = 42\ 576\ 375$ Hz

Alternating fields with such frequency are usually generated in special radio-frequency coils, prototype systems are located inside such coils.

Spectral researching mode

Initially NMR effect was used as analytical method for researching in molecular physics and chemistry, because of dependence nucleus resonance frequency from molecule's electronic structure.

Electronic surround of nucleus in molecule decreases external field B_0 on σB, where σ – shielding constant for current group of equivalent nucleuses. Local field on i-th nucleus B_i becomes lesser than used: $B_i = B_0(1 - \sigma_i)$ and resonance frequency is decreased too: $\omega_i = 2\pi\nu o_i = \gamma_i B_i = \gamma B_0(1 - \sigma_i)$.

Displacement of resonance absorbing frequency under influence of electronic surrounding calls chemical shift(δ). Absolute value of resonance frequency displacement depends on value of field B_0, that's why chemical shift measures in non-dimensional ppm:

$\delta_i = (\omega_i - \omega_{ст})/\omega_0$, where $\omega_0 = \gamma B_0$.

Very simple example of chemical shift can be presented at alcohol molecule.

Here we can see three spectrum peaks, from each covalent bond. First peak is rather big. Second – lesser (as oxygen pulls aside electron cloud), third is the biggest one.. As U can see – spectrums are split.

This effect appears because of influence of neighbour spins through 2 (H-C-D) or 3 (H-C-C-H) covalent bonds. Magnetic field from one magnetic moment changes magnetic field around other magnetic moment, making it to split. Such splitting observed after interaction of non-equivalent nucleuses and nucleuses with different character.

Here we can see three peaks, caused by these 2 protons. Each proton has spin. There is only 4 ways of their orientation. First small peak caused by this orientation, second big includes these two types of orientation. And 4^{th} caused by this orientation. Signal from second bond spit on 4 peaks, as on these 2 protons influence these three protons. As we know, there are 9 ways of their spin orientation. So, first peak caused by this orientation, second caused by all orientations, where two spins look up, third – where look down. Oxygen signal is single because of it property to pull aside electron cloud.

Measure of spin's interaction is the constant of spin's interaction J, which measures in Hertz and defines by the distance between split peaks. It doesn't depend on value of magnetic field.

In protonic MRS in vivo and in vitro, the beginning of scale for chemical shifts is defined as placement of signal from protons of tetramethylsilane(CH3)4Si. This compound is rather inert, its molecule contains 12 chemical equivalent protons with high electronic shielding. Small addition of this substance to blend cause single narrow signal of the beginning of scale in the spectrum in vivo.

Magnetic resonance spectrum is figured as several peaks in chemical shift coordinates. Square under peaks is proportional to concentrate of equivalent protons, that belongs to some molecules located in prototype systems.

So, every signal in NMR spectrum is defined by 2 main parameters: chemical shift and activity, also in several cases it defined by spin interaction.

Hydrogen's spectroscopy has several features. First, there is small variation of proton's electronic shielding for different chemical compounds. Proton's chemical shifts of different compounds located in range of 10 ppm (it means that dispersal of protonic resonance frequency located in range of 10^{-5}). Such narrow range of chemical shift (for example, Fluorine has 200 m.p.) requires high homogeneous magnetic field for achieving acceptable resolution.

So, for providing error of difference registration of proton shielding equal or less than 1% from range of 10 ppm requires homogeneity of magnetic field 10^{-7} or less. It is achieved by special construction of magnetic system that should provide high field homogeneity and high magnetic induction. Except this, all high-resolution NMR spectrometers have quick rotation system for averaging-out field heterogeneity in rotation plane.

Spectrum examples in vivo.

For nucleuses H and C13 in vivo and in vitro as standard use tetramethilselane. For all that proton group, that has chemical shift 1.00 ppm, should show NMR peak with frequency 63Hz if spectrum was written on spectrometer at frequency 63Hz and field 1.5 Tl, or 400Hz on spectrometer at resonance frequency 400Hz.

As an example here we can see spectrum, that was received on phosphorous's nucleuses resonance, stored up from soft tissues of human leg. Signals in spectrum belong to inorganic phosphate, phosphorokreatine and alpha, beta, gamma adenozintriphosfat. Null applied to the most active signal from phosphorokreatine. On the modern devices such spectrums receives by pulse methods. First, numerical array is accumulated at timing scale, after this spectrum is restored by the Fourier transform.

On this picture there is spectrum, registered from human brain tissues on Siemens Magnetom with magnetic field 1,5 Tl, located in San-Francisco. On this spectrum we can see phosphorocreatin, and also spectrums from other chemical substance. Division of spectral lines increases with tumor of magnetic field induction.

Summary shielding effect of group of equivalent nucleuses from local diamagnetic and paramagnetic fields causes Chemical shift for some molecule.

Chemical shift characters for lots of nucleuses were tabulated and calculated.

Some effects should cause increment of spectral line's shift. Such effects as local pH vehicle [vikl], temperature, paramagnetic and ferromagnetic inclusions.

Another NMR-spectrum's feature is spin splitting, which causes to single spectral line splitting on multiplet. Constant J defines value of this splitting. Spin interaction doesn't require magnetic field, so this effect doesn't depends on accepted magnetic field.

If exchange between nucleuses is fast, so it can make it impossible to see splitting. Splitting can be avoided, using double irradiation method. In this method use additional high-frequency field to saturate resonance of those nucleuses which can be split. So, multiplet transforms into singlet. This method calls Destroying spin's interaction method.

In NMR-spectroscopy to increase signal/noise ratio and increase frequency resolution of different spectral lines, use maximum field strength. For maximum use of strong field advantages, it is necessary to raise and support homogeneous of more powerful fields. Though, using small samples this method is useful, but for in vivo researching local variations of magnetic receptivity may prevent from resolution improvements by using strong magnetic fields.

Tooling fills up standard method of field optimization, that consists on assembling of static iron jack and, probably, superconducting compensative coil. It's necessary to pay attention to sample's symmetry, which has an influence on field allocation and lets simply correct any it artifacts. In magnetic systems for analyses of whole body it influence on field allocation is rather large.

Magnetic receptivity (восприимчивость) of sample strongly influence on spectrum, and in some occasions there can be strong fluctuations of local receptivity, which quiet impossible to compensate and which should add large contribution into widening of spectral lines.

These effects especially important in experiments with graft (привитый) living tumor, as it form is not symmetrical and can have large necrosis areas. Nowadays field compensation is executed by length optimization of free decreasing induction signal of protons in sample, or by observation at oscilloscope free decreasing induction, form optimization and maximization of T2 value, or by Fourier-spectrum observation and minimization of spectrum line width. In some systems method of distortion's compensation is pointed as system feature.

Space localization of NMR-spectral research.

Localization is necessary to be sure that signals are emanated from analyzed organ or those regions, where metabolism demonstrates their functions or changes under influence of several factors. Especially it applied to analyses of pathological formations, where it is necessary to take measurements of pathological regions, but not healthy tissues, surrounded it. Problems of measure increases, when measurements are taken not from muscles, but from other biological tissues, such as tumor, which makes signal amplitude lesser than amplitude of muscles signal. Even small group of muscles near tumor adds distortions in spectrums from tumor signals.

In ideal case, using protonic image, we could identify region of tissue with random configuration, and after this could get spectrum from local volume without any distortion.

On practice, no one existing method satisfies demands that are made. Interpretation of measured tumors is too hard also because of heterogeneity [ˌhet(ə)rəudʒəˈniːəti] of tissues, as tumors consist of different kind of cells, which have different stage of genesis and different value of oxygen supply.

Important advantage of localization methods is opportunity of synchronous signal registration from more than one tissue region. It's useful when we need reference spectrum or we are interested in changes in any organ or tissue.

For localization of some surface tissues most simple method is using surface coil. This coil consists of one or several wire-turns and used for registration of signal only from region of sample, bordering to coil turns.

In protonic visualization method surface coils usually used only in receiver-mode, that's why they are electrically unbind from transmitting coils by diodes preventing appearing huge currents in receiving coil, tissues heating and also damaging electronic section of receiver.

However greater extent of space localization achieved, using surface coil as transmitting and receiving. In NMR-spectroscopy it is common work mode. If it possible, coil is constructed in a such way, so it could work at resonance frequencies of different nucleuses.

Image of hydrogen nucleus distribution was received by modified method of "fast" visualization, in which used unselective impulse, so projection image received.

Sensitive of surface coil in receiving mode decreased with the distance. Therefore receiving spectrum depends on coil space variations, multiplied on stimulation level at different depths.

Spectroscopy in rotating frame of reference first was used in Oxford for solving several clinical problems. In this method several signals of decreasing induction are recorded. They are received after sample stimulation by impulses with different length. It cause to changing of local phases of signals, using in this point of sample. Received data can be exposed to Fourier transform. It will perform group of spectrums from region under constant field B1. This method gives deflected complex shape areas, however using huge transmitting coil and small receiving coil; these areas could be made approximately plane and parallel with coil's plane. If necessary changes of phase achieved by changing their length, it can influence on bandwidth of stimulated signals and cause distortions on the border of spectrum.

However method lets to record signals from several planes at once. Hoult proposed selective exitation method in rotating frame (rotating frame selective exitation), in which was used special coil, which creates field B1 gradient, which is analog of linear field B0 gradient, used in laboratorial frame of reference. Field B1 is modulated by phase to create effective field B2, precessed relatively B1 in rotating frame of reference.

Large group of methods are based on field B0 changing by adding gradients. Set of nonlinear gradients of static magnetic field defines configuration of field B0, bounding it by some volume. In this case recording spectrum is sum of homogeneous areas of sample with high resolution and heterogeneous areas with low resolution from the rest part of sample. This wide component can be excluded from resulting spectrum by differentiation and de-escalation. In spite of succeed using of this method, it has some limitations. Such as impossibility of measuring at the border of analyzing region and limits of space displacements of sensitive areas.

Impulse sequence STEAM and PRESS

There are the most common in MRS impulse sequences. Selective RF impulse chooses section. Sequences excitate one by one three perpendicular sections and record signal only from volume, formed by crossing of these three sections especially for choosing area of spectral interest in some limited volume.

STEAM and PRESS use three RF impulses to choose volume of excitation. Main difference between sequences are – using 3 RF impulses with 90dg to proceed stimulated echo by STEAM and using one 90dg impulse with two 180dg impulses to proceed spin-echo by PRESS.

Differences in schemes of excitation lead to differences in sequence sensitive to T2.

STEAM sequence has lesser sensitive to T2. PRESS method gives good localization but also requires attention to T2.

NMR signal suppression from nucleus of water.

Concentration of water in cerebrum tissues excels their concentration in metabolites, which we are interested in. As maximum NR activity in spectrums caused by concentration, so water peak dominates over protonic spectrum if water suppression is not used.

RF impulses are used for nucleus signal suppression. These pulses excite only 50Hz frequency band, concentrated at water frequency peak. RF impulse rotates vector of protonic magnetization from water nucleuses into cross-sectional plane at which works phase gradient.

After such gradient there is no main part of initial vector of water magnetization and signal from water essentially suppressed. However, some relaxation of T1 takes place between the end of pulse and gradient sequences and beginning of receiving data sequence. It means that some value of magnetization vector would be restored and effectiveness of water suppression would be decreased a little.

In order to solve this problem, initial vector makes greater than 90 degree so, there will be no resulted magnetization from water in time. In fact, after three sets of RF impulses and gradients with restore compensation, suppression becomes rather effective.

Also automatic schemes of water suppression are successfully developed. In this case intermediate spectrums from remanence are recorded and analyzed. From such spectrums size of remanent water signal is measured and used for computing rotation angle for third impulse. This process repeats with 1degree step, centered over some angle for final suppression optimization.

Metabolites of brain tissues, available for protonic MRS methods.

Just few most stable brain metabolites are able to be shown at protonic MR spectrums. It caused by typical timing intervals for modern tomographs. Some of these biochemical functions we'll examine.

N-acetylaspartat

Considered, that it is neuronal marker gene[jin]. This NMR signal is most intensive, it also can show contributions from other mixtures, which includes N-acetyl remaining.

Acetylspartat's functions in nerve tissue is not finally defined. Sizeable decreasing of NAA level found in protonic spectrums at meningiome diseased. Possibilities of protonic MRS are rather hig in tumor diagnostic, as there is no acetylspartat in cancerous tumor's cells.

Kreatine.

This compound generates two NMR signals from CH2 and CH3 groups. In brain tissue it percentage is rather high.

Choline.

This signal represents quantity of choline in brain. Function of this metabolite is vitamin generating and regulating of fat exchange.

Mio-inositol

Tumors contains half or lesser of normal concentration. Also it concentration increases at old patients. It's main function is not finally defined.

Glutamat

It figures prominently in exchanging brain processes. Glutamat acid takes part in protein and carbohydrate exchange, stimulates oxidizing process. Increasing of it concentration in blood observed at schizophrenia-diseased.

Glucose – energy source.

Lactate

It concentration increases at hypoxia and ischemia-diseased. Also tumors have high increased concentration of this metabolite. In this case MRS can become very sensitive method for defining intracellular pH level. It is important to know for tumor therapy.

According to aforesaid, functions of chemical compounds, which MRS can define, are little researched. Practically, MRS is a single safe way to discover texture and biochemical processes of brain.