These questions are answered by many contributors. If you find any errors, please let me know. Hopefully these short answers will help you with the theoretical questions.

1) **What is the macroscopic magnetization? Draw the scheme.**

It is the sum of all the $z$-components of all the nuclear magnetic moments in a sample ($M_0$). $M_0$ exists because $N_\alpha > N_\beta$.

![Diagram](image)

**Figure 1:** Macroscopic magnetization shown as a sum of vectors.

2) **What is the connection between a nuclear angular momentum and a nuclear spin quantum number? Write down the equation.**

The nuclear angular momentum is given by the nuclear spin quantum number.

$$P = \sqrt{I(I+1)}\hbar$$
3) **What is the connection between a nuclear angular momentum and a magnetic moment? Write down the equation.**

The magnetic moment is proportional to the angular momentum, by the proportionality factor $\gamma$. (The magnetogyric ratio is a constant that varies for each nuclide (isotope of a given element). The value of $\gamma$ determines the sensitivity of the nuclide in NMR, where a higher value means a higher sensitivity.)

$$\mu = \gamma P$$

- $\mu$ = Magnetic moment
- $\gamma$ = Magnetogyric ratio
- $P$ = Nuclear angular moment

4) **What is the Larmor frequency?**

The larmor frequency is the frequency of the precession of nuclear dipoles around the z-axis.

![Diagram](image)

**Figur 2:** $\omega$ indicates the larmour frequency.
5) Does the Larmor frequency depend on the static external magnetic field?

The Larmor frequency \( \nu_L \) is proportional to the magnetic flux density \( B_0 \).

\[
\nu_L = \frac{\gamma \nu}{2} |B_0|
\]

\( \gamma \) = Magnetogyric ratio

\( B_0 \) = Magnetic flux density

6) Which values can a nuclear spin quantum number have?

0, 1/2, 1, 3/2, 2, 5/2 …

7) The \(^{14}\text{N} \) nucleus has the nuclear spin quantum number:

1

8) How many values can the magnetic quantum number have?

The magnetic quantum number \( m \) can have the following values: \( m = I, I - 1, \ldots, -I \)

A sum of \((2I+1)\) different values of \( m \).

9) Write down possible values \( m \) can have for the proton. Draw the scheme.

A proton has spin 1/2, the sum of different values of \( m \) is therefore \((2 \cdot 1/2 + 1) = 2\), which gives \( m = I \) and -I, ergo +1/2 and -1/2.

10) Write down possible values \( m \) can have for a nucleus with \( I=1 \). Draw the scheme.

+1, 0 and -1.
11) **What is the directional quantization?**

Directional quantization is the (number of possible) orientations of the angular momentum and the magnetic moment in the magnetic field.

12) **Explain α- and β-spin states of spin-1/2 nuclei. Draw the scheme.**

Due to directional quantization only certain angles of the precessing nuclear dipole are allowed. α-spin is with positive magnetic quantum number (m = + 1/2) and β-spin with negative (m = -1/2).

13) **Write down the equation for energy difference between two adjacent energy levels of magnetic dipole in an external magnetic field.**

\[ \Delta E = \gamma \hbar B_0 \]
14) What is the saturation?

Saturation is when the N-populations are equal (N_α = N_β). The population is greater in the lower energy level, and this leads to the transition from the lower to the higher level to become the dominant process and is therefore observed signal. The process of moving up in energy require absorption of energy, while the transition down requires emission. Under saturation the absorption and emission are equal in intensity and cancel each other out, and thus no signal is observed. Used for broadband decoupling, and selective removal of signals.

15) Which transitions are allowed by the spectroscopic quantum mechanical rules?

Both transitions from lower to upper energy level due to energy absorption and from upper to lower due to emission of energy are allowed. The magnetic quantum number m can only change by a value of 1.

16) What is the pulse?

The pulse is the radiofrequency pulse that excites all nuclei of one species simultaneously. It contains a band of frequencies symmetrical about the center frequency ν_1, with a fixed duration τ_P (usually several µs).

17) What happens with microscopic magnetizations after the 90° pulse?

A small fraction of the microscopic magnetizations (nuclear angular momenta P) are now processing around the z-axis in phase coherence. This results in a macroscopic magnetisation in the y’ direction. On top of this the number of angular momenta in alpha state is equal to that in the beta state, which results in the disappearance of the macroscopic magnetization in the z direction. (See Figure 1-12, page 12) Phase coherence; see Figure 1-14, page 14 in the textbook.
18) **What is the relaxation?**

Immediately after a pulse the spin system will start to revert to its equilibrium state. This is relaxation. Relaxation occurs as transverse (spin-spin) and longitudinal (spin-lattice). The dipoles will dephase, reducing $M_y$ until zero, restoring their random distribution in precession around the $z$-axis. $N_\alpha$ and $N_\beta$ return to their equilibrium state, gradually increasing $M_z$. (See textbook Figure 1-14, page 14)

19) **What is the purpose of the Fourier transformation?**

Its purpose is to transform the time domain spectrum, the FID, recorded during analysis into readable data in the frequency domain.
20) **What is FID (free induction decay)?**

It is the decay of the transverse magnetization, which is detected by the NMR apparatus. It is often the detected in the \( y' \)-direction. (Textbook Fig 1.14, 1.15, 1.16)

21) **Write down the expression showing the dependence of the S/N ratio on the number of scan.**

The signal/noise-ratio is proportional to the number of scans performed.

\[
S:N = \sqrt{NS}
\]

- \( S:N \) = Signal to noise ratio
- \( NS \) = Number of scans

22) **What is the acquisition time?**

The time used to obtain the FID per scan (time of relaxation between each pulse).

23) **Give a list of main parts of a pulse NMR instrument.**

- Magnet: Cryomagnets capable of a frequency up to 900 MHz in protons. (21,14 Tesla, Hz is relative to the nuclei in question)
- Probe-head: Where the sample is inserted
- Transmitter: Radiofrequency generator and frequency synthesizer. Also capable of pulse production.
- Receiver: Radiofrequency voltage proportional to the transverse magnetization
Mᵥ is induced in the receiver coil.

Computer: Digitizes the information and (Fourier) transforms it to readable data.

24) What is the chemical shift?

The effect of the magnetic field (Bₑff) on a specific molecule is always less than the applied field (B₀). This is due to shielding effect (σ) on the nuclei which is analyzed. As nuclei are shielded differently due to their molecular environment they will give separate resonance signals in the spectrum, creating a specific chemical shift for a specific nuclei. The different Larmour frequencies of the nuclei result in different chemical shifts.

25) What is the scalar coupling?

It is the interval between the two signals of a doublet. It’s the splitting of a signal due to interactions through the chemical bonds. J coupling.

26) What is the zero-order spectrum?

It is a spectrum containing only singlets.

27) What is the first-order spectrum?

It is a spectrum containing multiplets which follow the multiplet rules, meaning that the shape of the multiplets are of consequent size and shape (1:1, 1:2:1, 1:3:3:1 etc. . . Pascals’ triangle.) and where the frequency interval between nuclei is large compared to the coupling constant (Δν >> J).

28) What is the spectrum of higher order?

It is a spectrum containing multiplets which does not follow the multiplet rules. The condition Δν >> J is not met, altering the intensity ratios within the multiplets (no longer following Pascals’ model) causing additional lines to appear in the spectra and making it more complex.

29) What are the ¹³C satellite spectra?

These are signals observed due to coupling between proton and ¹³C . Observed as very small doublets evenly spaced around the proton signal. In proton NMR ¹³C satellites are usually 1,1 % of the intensity of the main signal, due to the low abundance of ¹³C .
30) What are integrals?
The area of each signal. In $^1$H NMR the integrals corresponds with the relative amount of nuclei with the same chemical shift. Integrals from $^{13}$C NMR is generally not accurate due to NOE.

31) Which are the reasons for integration of $^{13}$C spectra being not very common practice?
Due to the low sensitivity of $^{13}$C spectra each shift is made of few data points. Which makes each shift different and the integration unreliable simply because of the resolution of the spectras. The system usually does not have time to restore equilibrium between two pulses. Because the range of carbon shifts is so large, there is a considerable frequency difference between the RF pulse and the larmour frequency of a majority of the carbons in the sample, leading to lower intensities. Finally the NOE distorts everything when you use broadband decoupling of protons. (See the four points in 1.6.3.2, page 35 for further details.)

32) The schemes below (Figures a, b and c) show pulse sequences of three double-resonance NMR experiments. For each of them answer:

(a) Which experiment is in the matter?
a = Inverse gated decoupling, b = Broadband $^1$H decoupling, c = Gated decoupling

(b) What is achieved by this experiment?
a: All proton coupling is removed but no NOE is built up. (no amplification/distortion)
b: All proton coupling is removed. NOE amplification allows for faster experiment.
c: C-H coupling is observed while some NOE amplification is retained.
33) **Which types of relaxation you know?**

- Spin-lattice/Longitudinal relaxation ($T_1$): Relaxation is applied in field direction.
- Spin-spin/Transverse relaxation ($T_2$): Relaxation perpendicular to field direction.

34) **Through which mechanisms does relaxation occur?**

**$T_1$:**
- Dipole-dipole
- Spin-rotation
- Chemical shift anisotropy
- Scalar coupling
- Electric quadrupolar
- Interactions with unpaired e- in paramagnetic compounds

**$T_2$:**
- Magnetic field inhomogeneity

35) **What is the correlation time, $\tau_C$?**

Corresponds roughly to the interval between two successive reorientations or positional changes of the molecule.

36) **Describe in 5 steps the inversion-recovery experiment for quick estimation of $T_1$ relaxation time constant.**

A series of $^{13}$C spectra with pulse sequence $180^\circ_x - \tau - 90^\circ_x$ - FID while simultaneously $^1$H BB decoupling.

The $180^\circ_x$ pulse turns the macroscopic magnetization in the -z direction after which it starts to relax and turn towards the -y' axis. The amount of relaxation is controlled by $\tau$, the time between the two pulses. Low $\tau$ means a short relaxation time which means that the magnetization vector will not have time to relax past the -y' axis, meaning a negative signal. Conversely, a long $\tau$ means long relaxation and a positive signal. At one time, $\tau = \tau_{\text{zero}}$, there is no signal and $T_1 = \ln(2)/\tau_{\text{zero}}$.

37) **What is the effect of the pulse field gradient on transverse magnetization?**

The pulsed field gradient will set up a linear magnetic field gradient along the sample tube, meaning that every nuclei will feel a slightly different magnetic field.
This affects the frequency with which they precess around the z-axis in the rotat-
ing frame of reference. After a time of about 1 ms, all the nuclei will have spread
out, completely cancelling the transverse magnetization.

It is possible to recover the transverse magnetization by inducing another field
gradient in the opposite direction for the same period as the first.

38) **What is achieved by the SPI (selective population inver-
sion) experiment?**

In the SPI experiment, one selectively transfers the magnetization from one nucleus
to another, typically $^1$H to $^{13}$C in order to improve the sensitivity of the carbon
nucleus. In a $^1$H spectrum $^{13}$C satellites are often seen, and these are the tran-
sitions targeted in the SPI experiment. The transfer of magnetization is achieved
by a selective 180 degree pulse for the sensitive nucleus ($^1$H). This inverts the po-
pulations of one of the $^{13}$C satellites, which transfers the magnetization from the
proton to the carbon, leading to a much larger signal in $^{13}$C NMR.

39) **What is the difference between the APT (attached proton
test) and the DEPT experiments?**

In the APT CH$_3$ and CH gives positive signals, while the CH$_2$ and quaternary
carbon gives negative signals. The DEPT experiment can be divided into three
subspectra, one for primary (CH$_3$), one for secondary (CH$_2$) and one for tertia-
ry (CH) carbons by using different pulse-field gradients. Quaternary carbons are
not visible, but can be found by comparing the three subspectra with the $^1$H-BB
decoupled $^{13}$C-spectra and identifying the peaks missing.

40) **The figure below shows two magnetization vectors in x',y'
plane. Draw on the same picture the effect of 90° $y'$ pulse on
them (draw their new positions).**

Textbook Fig 8.3, page 191. The vectors rotate clockwise around the axis of the
pulse. A 90° $y'$ pulse will rotate both vectors 90° around the y'-axis, leaving them in
the y',z-plane.
Figur 7: Vectors A and B are flipped 90° into the y', z plane.

41) Draw the basic spin-echo pulse sequence and show its effect on an AX spin system by using Bloch’s vector model.

$90^\circ_{x'} - \tau - 180^\circ_{x'} - \tau - \text{echo 1} - \tau - 180^\circ_{x'} - \tau - \text{echo 2} - \tau - 180^\circ_{x'} - \text{echo 3}$...

Figur 8: Spin-echo pulse sequence.

Vector model: Textbook Fig 7.4 C (a-h).
42) What is the purpose of the polarization transfer experiments?

It enhances the signal for insensitive nuclei and nuclei with negative magnetogyric ratios. This is achieved by transferring the polarization from an insensitive nuclei.

43) Draw the scheme of energy levels of the heteronuclear two spin system AX.

Fig. 8.8, page 200.

44) In the reverse INEPT experiment, it is necessary to apply phase cycling. Why?

Reverse INEPT is used to look for weak $^{13}$C satellites and the main signal will still be clearly visible. Phase cycling is used to suppress the main signal, leaving only the satellites. Furthermore, the two signals from reverse INEPT are of different intensity to begin with. This is caused by the different lengths of the $M_H$ vectors. Phase cycling equalizes the two signals.

45) What information is available from the TOCSY experiment?

Coupling between protons and other nuclei over long distance, not only neighboring nuclei. Will show coupling if there is a complete chain of coupled protons. Used typically to decide which protons are situated on different monomers in a polysaccharide chain.

46) What is shimming?

Adjustments for optimizing the field homogeneity.
47) Why is it necessary to use deuterated solvents for NMR experiments?

Because the proton signal from non-deuterated solvents will easily overshadow any signals from your sample. In addition, it’s necessary to have nucleus for the locking of the signal. In polysaccharides the deuterated solvent can exchange the protons of the OH-groups with deuterium, making the spectras a lot easier to interpret!

48) Which tasks an NMR probe has to perform?

Transmitting and receiving signals, decoupling, locking, generating field gradients and amplification of signals.

49) Presence of paramagnetic impurities is mostly unwanted for NMR experiments. Why, what is a typical paramagnetic impurity and how it is usually removed from the sample?

Paramagnetic impurities shorten relaxation time and thereby increase band broadening. Nuclei interacting with impurities may also have its shift moved. Dissolved oxygen is a typical impurity and is removed by degassing the sample.

50) When and why are paramagnetic compounds deliberately added to the sample before running an NMR experiment?

It may be used to remove nuclear overhauser effect but also to change the shifts in order to aid spectral analysis. If the molecule contain many similar, overlapping shifts which are hard to discern, addition of paramagnetic compound may chelate with the molecule and change its shifts so that they are discernable.

51) What is locking and what is its purpose?

Ensures the necessary field frequency stability. If there is no resonance condition, the lock unit applies a field correction until resonance is restored.

52) What is the phase correction?

Removes the dispersion component signals.
53) Which requirements a good NMR solvent has to satisfy?

Good solubility of sample, stable, isotope-labeled (deuterated), non-reactive with sample and have low viscosity. Easily removable solvents may be preferred if the compound is to be retrieved after analysis. Traces of water should be avoided as residual HDO can give broad peaks. $^{13}$C-solvents should not overlap with sample.

54) On the scheme of a typical 2D experiment below write on the dashed lines names of the phases.

Preparation - Evolution - Mixing - Detection

![Typical 2D pulse sequence.](image)

55) Using the scheme above explain how the second dimension can be generated.

The difference between 1D and 2D spectra is the the evolution time, $t_1$. This time is variable and it changes the amplitude of the signal peaks. This is similar to, but not the same as the inversion recovery experiment. The result of changing $t_1$ between $t_1 = 0$ and $t_1 = 1/[J(C,H)]$ is an amplitude modulated signal, that is, the resulting signal varies in amplitude from short to long $t_1$ values. This amplitude modulated signal gives the $F_1$ dimension in which peaks are separated by $J(C,H)$. The second dimension, $F_2$, is simply the chemical shift of the peaks in question.
56) The 2D HETCOR and HSQC experiments give practically the same information about the molecule. What is the main difference between them?

The same results but with considerably larger sensitivity in HSQC. HETCOR is recorded on the carbon channel, while HSQC and HMQC are recorded on the proton channel.

57) Can direct C-H connections be seen in an HMBC spectrum?

No. They are suppressed by suitable phase cycles.

58) What information about a compound can be obtained from the 2D INADEQUATE experiment?

It can assign which carbons are connected to each other. Enabling quick identification of which signals are which carbons.

59) If the $W_0$ (zero-quantum relaxation process) is the dominant one, how that affects the NOE?

It reduces the signal intensity. Page 300.

60) What can be said about correlation time for small molecules?

Correlation time for small molecules is smaller as the molecule can move faster.

61) Does the external field $B_0$ influence NOE?

Yes, its signal amplification is dependent upon the magnetic field strength.

62) What improvement offers ROESY in comparison to NOESY?

In NOESY the relative signs of the amplitudes of the cross peaks depend on the correlation time and sometimes the cross peaks may disappear. This effect is removed in ROESY.
63) How close protons must be in order for NOE correlation between them to be detected?
At least less than 5 Å, ideally less than 3 Å.

64) Which interactions NOE is based on?
A continuous saturation of a transition of one nucleus and the system will try to restore the equilibrium through spin-lattice relaxation.

65) What is the purpose of using shift reagents in NMR?
If the molecule contain many similar, overlapping shifts which are hard to discern, addition of a shift reagent may chelate with the molecule and change its shifts so that they are discernable. Similar to paramagnetic compound addition.

66) Is it possible to distinguish enantiomers by NMR?
Not without further action. A chiral lanthanide shift reagent or a chiral diamagnetic reagent may be added to form diastereomers. The different diastereomeric complexes may then be distinguished by their different resonances.

67) What is always the result of the rapid dynamic processes on proton NMR spectrum?
They lead to a simplification of the spectrum.
68) On the figure below NMR signals of two protons exchanging their positions in a dynamic process are shown. How will the increase of temperature affect those signals?

**Figure 11**: Due to a rapid dynamic process, the spectrum shown in B is simplified at higher temperature to spectrum A.

Increase of temperature will lead to one single shift representing both protons. Shift in the middle of the previous shifts. The signals will merge due to the rapid inversion of their positions, making them ‘equal’.