(12) UK Patent Application (19) GB (11) 2 126 727 A

(21) Application No **8225695**
(22) Date of filing **9 Sep 1982**
(43) Application published
28 Mar 1984
(51) **INT CL³**
G01N 24/08
(52) Domestic classification
G1N 508 512 523 525
533 545 546
(56) Documents cited
None
(58) Field of search
G1N
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(54) **Method and apparatus for imaging substances in biological samples by nuclear magnetic resonance**

(57) A method of determining the distribution in a sample, of non-proton nuclei having a magnetic moment, comprises subjecting the sample to a magnetic field, irradiating the sample with RF radiation at a proton magnetic resonance frequency, deriving a first NMR signal, indicative of electro-magnetic absorption of the sample at the proton magnetic resonance frequency, deriving from the sample a second such NMR signal at the proton resonance frequency in the presence of RF radiation at the nuclear magnetic resonance frequency of the said non-proton nuclei so as to decouple protons in the sample from the said non-proton nuclei, and applying an imaging technique to produce an image indicative of the spatial variation of the difference between the said first and second signals. Imaging may be performed on the difference between the two NMR signals, or on each NMR signal followed by subtraction of the images. The method can be used to trace how a ^{13}C. labelled material introduced into a patient, and its breakdown products, become distributed.

GB 2 126 727 A

SPECIFICATION

Method and apparatus for imaging substances in biological samples by nuclear magnetic resonance

Nuclear Magnetic Resonance (NMR) is based on the phenomenon that when a substance is subjected to a static magnetic field B and an oscillatory electro-magnetic field of angular frequency ω then a condition of resonance occurs when $\omega=\gamma$ B, where γ is the gyromagnetic ratio for a particular nucleus present in the substance. The resonance can be detected and is indicative of the presence of a particular element comprising that nucleus.

Where the material includes complex molecules the localised fields produced by molecular electrons have a screening effect which causes identical nuclei in different chemical or molecular environments to resonate at slightly different frequencies, that is to say the value of B is slightly modified for the same nucleus in different environments. This effect is known as the chemical shift. The magnitude of such shifts is very small but it can often be detected and can be used for distinguishing between different compounds containing the same element.

The nuclear magnetic resonance technique may be used only on nuclei which have a magnetic moment, and this places severe restriction on the circumstances in which the technique can be used. Examples of nuclei having magnetic moment are ^{1}H, ^{31}P and ^{13}C.

In recent times, great interest as been expressed in utilising nuclear magnetic resonance in conjunction with imaging techniques, to form a two or three dimensional representation of the distribution of particular materials in a sample. Various imaging techniques that use NMR have been proposed in which a specimen is subjected to non-homogeneous magnetic fields varying in time or space, for example by superimposing a time-varying magnitude field component or a linear magnetic field gradient on the main magnetic field, to induce localised resonances in parts of the specimen and thus enable an image to be built up based on the presence of a particular nucleus. Examples of such prior art proposals are the following techniques:—

(1) Projection-reconstruction procedures, e.g. P.C. Lauterbur (Nature, *242*, 190 (1973)),

(2) Selective excitation methods, e.g.:—

(i) Garroway A.N., Grannel P, and Mansfield P., J. Phys. C., *7*, L457 (1974),

(ii) Lauterbur P.C., Dulcey C.S., Lai C.M., Feiler M.A., House W.V., Kramer D.M., Chen C.N. and Dias R., Proc. XVIII Ampere Congress (eds. P.S. Allen, E.R. Andrew & C.A. Bates), Amsterdam: North Holland, P27 (1974),

(iii) Hutchison J.M.S., Proc. 7th L.H. Gray Conf., Chichester: Wiley p135, (1976)),

3) Modifications of 2D Fourier Transform NMR techniques, e.g.:—

(i) Kumar A., Welti D. and Ernst R.R., Naturwissenschaften *62*, 34 (1975),

(ii) Kumar A., Welti D. and Ernst R.R., J. Magn. Reson., *18*, 69 (1975) and

(4) Fonar techniques, e.g.:—

Damadian R., Minkoff L., Goldsmith M., Stanford M. Koutcher J., Physiol. Chem. Phys., *8*, 61 (1976).

A more complete list of imaging techniques useful in NMR may be found in Phil. Trans. Roy. Soc., *B289* (1980).

It can be seen from the above references that imaging techniques are themselves very well known, and the present specification should be considered to incorporate by reference the disclosures of all of the above listed documents.

The imaging techniques described above as applied in biology and medicine have hitherto been almost exclusively directed to the detection of resonance of the proton or ^{1}H nucleus. Biological material contains large amounts of hydrogen, substantially all of which is the isotope ^{1}H, and thus the technique is relatively sensitive for protons. In order to successfully carry out an imaging experiment, it is desirable to be able to select from the NMR spectrum a single sharp peak which is widely spaced from any neighbouring peaks in comparison with the field gradient required to perform the imaging. Because all biological material contains very large quantities of protons however, it is in general not possible to follow, using conventional imaging techniques, the progress of individual metabolites in a biological system, for example in the human body.

If the chemical environment of a proton in a molecule includes an atom having a magnetic moment, then the proton magnetic resonance signal derived will be split into a multiplet. For example, the spectrum of a proton adjacent to a ^{13}C atom will in general be split into a doublet, and the splitting can be detected, provided that the magnetic field is sufficiently homogeneous (i.e. homogeneous to at least 1 in 10^{6}).

A number of workers have shown that it is possible to obtain a large increase in specificity of proton NMR spectra by utilising a ^{13}C-labelled substrate and observing the difference between proton spectra in the presence and absence of ^{13}C decoupling fields. An example of such a proposal is put forward in a paper by L.O. Sillerud, J.R. Alger, and R.G. Shulman, (Journal of Magnetic Resonance 45, 142—150 (1981)). In this paper, the Authors show that it is possible to differentiate selected proton resonances very easily from background resonances, and thereby distinguish signals obtained from a labelled compound, for example glucose, alanine, or glycerol, to enable kinetic studies to be carried out on metabolism of such compounds, in particular in yeast.

We have now discovered that, by combining a decoupling technique such as that used by Sillerud, Alger, and Shulman with an imaging technique it

is possible to form an accurate picture of the distribution of labelled metabolites in living systems, and to follow the way in which the distribution of such metabolites changes with time.

According to a first aspect of this invention, there is therefore provided a method of determining the distribution of non-proton nuclei having a magnetic moment, for example ^{13}C, in a sample, which method comprises subjecting the sample to a magnetic field, irradiating the sample with radiofrequency radiation at a first frequency, corresponding to a proton magnetic resonance frequency, deriving a first signal, indicative of electro-magnetic absorption of the sample at the proton magnetic resonance frequency, deriving from the sample a second signal indicative of electro-magnetic absorption of the sample at the proton resonance frequency in the presence of electromagnetic radiation at a second frequency, corresponding to the nuclear magnetic resonance frequency of the said non-proton nuclei, and being such as to decouple protons in the sample from the said non-proton nuclei, and applying an imaging technique to produce an image indicative of the spatial variation of the difference between the said first and second signals.

The method of the invention is based on the familiar INDOR principle used in conventional high resolution NMR. It can be used to provide an image of a sample, for example a portion of the body of a human patient, with the sensitivity normally associated with proton NMR, but the chemical selectivity which could be obtained if ^{13}C could be imaged directly. The method can therefore be used with great chemical specificity, to determine the progress of a particular metabolite through the various organs of the body.

Preferably, the method is carried out by introducing into a sample a quantity of a chemical compound labelled with ^{13}C. By a conventional INDOR process, for example that described by Sillerhud, Alger, and Shulman, a single sharp peak can be obtained by a differencing technique, whereby a proton signal derived only from those protons coupled with the labelled ^{13}C atom is produced, by subtraction of signals obtained in the presence and absence of a decoupling RF field at the ^{13}C resonance frequency. At a magnetic field strength of approximately 2 Tesla, a suitable proton (first) frequency is approximately 80 MHz, the ^{13}C decoupling (second) frequency being approximately 20 MHz.

When carried out on a human patient, the method can be used, for example, to determine the way in which a particular labelled substance, which may be given by mouth or injected, is metabolised, and the way in which the distribution of the substance, and its products after breakdown, are distributed between various internal organs of the body. By careful application of the second radiofrequency signal, individual carbon-atoms may be followed through various metabolites.

The imaging may be carried out either by first forming a difference signal corresponding to the difference between the proton resonance in the presence and absence of the decoupling radiation, and then using the difference signal in an imaging experiment. Alternatively, an image may be formed by a conventional proton imaging technique in the absence of decoupling radiation, and the second image formed whilst decoupling radiation is applied. The two image signals may then be subtracted to form the difference image.

The invention also provides apparatus for determining the distribution in a sample of non-proton nuclei, which apparatus comprises means for generating a magnetic field, means for supporting a sample in the magnetic field, means for generating in the sample a radiofrequency electromagnetic field at a first frequency, corresponding to a proton magnetic resonance frequency in the magnetic field, means for generating in the sample a radiofrequency electromagnetic field at a second frequency, corresponding to the nuclear magnetic resonance frequency of a non-proton nucleus, to decouple protons present in the sample from the said non-proton nucleus, means for deriving from the sample first and second signals, indicative respectively of electromagnetic absorption of the sample at the proton magnetic resonance frequency, in the absence and presence respectively of the said second frequency electromagnetic radiation, means for producing a spatial variation in the magnetic field or electromagnetic field, and for determining the spatial variation in the difference between the said first and second signals, to determine the distribution in the sample of the said non-proton nuclei.

The sample support is preferably adapted for supporting a human patient, with at least a portion of the body of the patient, for example the trunk, in the magnetic field, whereby the distribution of labelled substances in the patients internal organs may be determined. The radio-frequency source at the second frequency is preferably phased locked to the means for generating the radiofrequency field at the first frequency. The patient support may take any conventional form, for example a bed or chair in the vicinity of the magnet, to enable the desired part of the body to be located in the field.

The method of the invention has a wide range of applications in the field of medicine and diagnosis, and in addition may be used in mapping of labelled compounds in tissue samples, for example in autopsy, Spin-echo techniques may preferably be utilised in the imaging method, for example the echo planar method, as described by P. Mansfied, A.A. Maudsley, and T. Baines (J. Phis. E., *9*, 271 (1976)).

Care must be taken when carrying out *in-vivo* measurements to avoid any harmful effects of heating, induced by the radiofrequency irradiation. However, the technique as a whole is

reasonably safe to use *in-vivo* particularly as compared with X-ray imaging techniques.

Claims

1. A method of determining the distribution of non-proton nuclei having a magnetic moment, in a sample, which method comprises subjecting the sample to a magnetic field, irradiating the sample with radiofrequency radiation at a first frequency, corresponding to a proton magnetic resonance frequency, deriving a first signal, indicative of electro-magnetic absorption of the sample at the proton magnetic resonance frequency, deriving from the sample a second signal indicative of electromagnetic absorption of the sample at the proton resonance frequency in the presence of electromagnetic radiation at a second frequency, corresponding to the nuclear magnetic resonance frequency of the said non-proton nuclei, and being such as to decouple protons in the sample from the said non-proton nuclei, and applying an imaging technique to produce an image indicative of the spatial variation of the difference between the said first and second signals.

2. A method as claimed in Claim 1 wherein the non-proton nuclei are ^{13}C nuclei.

3. A method as claimed in Claim 2, wherein the magnetic field strength is approximately 2.0 Tesla, the first frequency is approximately 80 MHz, and the second frequency is approximately 20 MHz.

4. A method as claimed in any one of the preceding Claims, wherein the sample comprises a ^{13}C labelled compound.

5. A method as claimed in any one of the preceding Claims, wherein the sample is a living organism.

6. A method of determining the fate of a metabolisable substance in a living organism, which method comprises introducing into the organism a quantity of the substance which has been labelled with non-proton nuclei having a magnetic moment, and determining the distribution in the organism of the labelled substance by means of a method as claimed in any one of Claims 1 to 3.

7. Apparatus for determining the distribution in a sample of non-proton nuclei, which apparatus comprises means for generating a magnetic field, means for supporting a sample in the magnetic field, means for generating in the sample a radio-frequency electromagnetic field at a first frequency, corresponding to a proton magnetic resonance frequency in the magnetic field, means for generating in the sample a radiofrequency electromagnetic field at a second frequency, corresponding to the nuclear magnetic resonance frequency of a non-proton nucleus, to decouple protons present in the sample from the said non-proton nucleus, means for deriving from the sample first and second signals, indicative respectively of electromagnetic absorption of the sample at the proton magnetic resonance frequency, in the absence and presence respectively of the said second frequency electro-magnetic radiation, means for producing a spatial variation in the magnetic field or electromagnetic field, and for determining the spatial variation in the difference between the said first and second signals, to determine the distribution in the sample of the said non-proton nuclei.

8. Apparatus as claimed in Claim 7, wherein the means for generating a radiofrequency field at the second frequency is phase-locked to the means for generating a radiofrequency field at the first frequency.

9. Apparatus as claimed in Claim 7 or Claim 8 wherein the means for supporting the sample is adapted for supporting a human patient with at least a portion of the body of patient in the magnetic field.

10. Apparatus as claimed in Claim 9 wherein the sample support is adapted for supporting the patient in a position for determining the distribution of a labelled substance in the patient's internal organs.

11. A method of determining the distribution in a sample of non-proton nuclei substantially as hereinbefore described.

12. Apparatus for determining the distribution in a sample of non-proton nuclei substantially as hereinbefore described.

Printed for Her Majesty's Stationery Office by the Courier Press, Leamington Spa, 1984. Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.