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QUINTESSENTIAL CONCEPTS OF ABSRM AND ITS SIGNIFICANCE IN R & D ANALYSIS

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ABSTRACT

It is important that animals are aware of the handler's presence before attempting to restrain them, particularly if the animal is initially asleep. In general, the greater the phylogenetic disparity of the antigen and the experimental animal, the greater will be the immune response the antigen induces. The degree of pharmacological response produced should be reproducible under identical conditions. Bioassay might measure a different aspect of the same substance compared to chemical assay. Spectroscopy is the branch of science concerned with the investigation and measurement of spectra which is produced when matter emits electromagnetic radiation. In 2- (or multi-) dimensional NMR spectroscopy many sequences are employed to provide additional information that are not obtainable from one-dimensional spectra. Research methodology is a careful, systematic, patient study and investigation in some field of knowledge undertaken to establish principles / policies. Every true experimental design must have research hypothesis at the core of its structure, as the ultimate aim of any experiment.

KEYWORDS: 2-Dimensional NMR spectroscopy, Phylogenies, Chemical assay, Patient study, Research hypothesis.

ANIMAL HANDLING

Introduction

The techniques used to handle animals vary slightly according to different species, however many of the general principles are the same. While handling animals, gentle approach is advisable. It requires care, patience and a technique. The adoption of these techniques will help to minimise stress for the animals and also helps to reduce the risk of bite injuries to the handler. It is important that animals are aware of the handler's presence before attempting to restrain them, particularly if the animal is initially asleep.[1]

Objective

- To comply with the Animal Welfare Ordinance and avoid mishandling of animal in research.
- To provide basic concepts of animal handling technique to new animal user.
- While offering concepts and techniques to animal user, comments are encouraged from experienced animal users which results to enrich knowledge in the field of laboratory animal research.

1) Handling Of Rabbit

- pick up the ears and loose skin which is present at the back of the neck with one hand in a firm grip
- place the other hand under the hind quarter to support the weight and lift gently.
- Never be lifted by ear alone
- Should be placed on a non-slippery surface
- If restraint is required during anesthesia or inoculation, rabbit should be wrapped in a roller towel or placed in a special box.[2,3]

Characteristic Features

- Rectal temperature:- 38.7 -39.1 degrees
- Normal respiratory rate:- 55 per minute
- Pulse rate:- 135 per min
- Gestation period:- 28-31 days
- Weaning age:- 6-8 weeks
- Mating age:- 6-9 months
- Room temp:- 15.5-18.5
- Humidity:- 40 -45 percent
- Weight – adult:- 0.9-6.75 kg

Common Diseases

- Coccidioisis (hepatic and intestinal)
- Pseudo tuberculosis
- Respiratory infections(Snuffles)
- Pneumococci
- Streptococci
- Intestinal infections(mucoid enteritis, Diarrhoea)
- Rabbit syphilis (*Treponema cuniculi*)
- Worms (cysticercus stage of dog tape worm, *Taenia pisiformis)*[4]

Experimental Procedure

Antisera: In certain aspects of immunization practices, which help in producing specific antiserum to substances of biological interest. Many practical considerations must be evaluated in the selection of the animal to be used as the source of antiserum—for example, the amount of antiserum desired, the ease of bleeding the animal, and the cost of purchase and maintenance of the animal. Antigens must be kept out of the circulation of the experimental animal. In general, the greater the phylogenetic disparity of the antigen and the experimental animal, the greater will be the immune response the antigen induces. Because of the variation in antibody response seen in experimental animals, at least two precautions should be taken when establishing an immunization schedule. First, sufficient numbers of animals should be used to assure that even if the proportion of animals producing antibody is low, some of them will produce the antibody desired. Second, periodic bleedings should be done during the immunization period and the serum should be examined for the presence of antibody. When the antibody level has reached a plateau, the animal can be exsanguinated or bled successively over a period of days.

Anesthesia: Animals respond in generally predictable ways to individual anaesthetic agents, however, much individual variation from animal to animal can be seen in the depth of response to a given dose. For this reason, most anaesthetic doses are given as ranges across which the ideal dose for each individual is most likely to fall. It should always be remembered that it is easy to supplement an animal with additional anaesthetic agent, but once given, an injectable anaesthetic agent cannot be removed. It is therefore recommended that dosing is done initially at the lower end of the anaesthetic dose range.

Subcutaneous inoculation: Subcutaneous injections are used often to inject anesthetics or to administer fluids for hydration during anesthesia recovery. The subcutaneous route of injection is often abbreviated as SC or SQ. The amount of fluid injected should be limited to volumes that will not overly stretch the skin (which would be uncomfortable) or that will not over-hydrate the animal unnecessarily. Typical volumes injected subcutaneously are in the range of 1 ml or less.

Intra-venous inoculation: Restrain the mice with physical or chemical restraint. Rotate the tail slightly to visualize vein. Disinfect injection site and insert needle (27-30 gauge) into the vein at a slight angle. You will not be able to aspirate, instead inject slowly and watch for clearing of the lumen. Incorrect positioning will result in a slight bulge in the tail. If this occurs, remove needle and repeat process proximal to previous site. Upon completion remove needle and apply pressure to injection site.

Intra-peritoneal inoculation: First locate the point of entry for the needle. The needle will be inserted along this line on the animal's right side and close to the midline. Insert the needle into the abdomen at about a 30-degree angle after disinfecting injection site.. If no fluid is aspirated, you may inject. Withdraw the needle.

Collection of blood: The blood can be collected from cardiac, anterior vena cava / sub calvian vein,jugular vein,cephalic region.[5]

2) Handling Of Mice

- Animal user should take a grip on the middle of the animal's tail with the left hand and gently raise the hind limbs from the floor of the cage
- A mouse should be held in such a position that it cannot turn around to bite.
- Then with the right fore finger and thumb a fold of skin is taken up to the head.
- The animal can now be lifted into convenient position for the animal user to carry out simple inoculation procedures.
- Place the animal on a rough surface and hold it by its tail with right hand, then pick up loose skin at the base of neck with the left forefinger and thumb.
- The right hand is free to pick up the syringe.

Characteristic Features

- Normal temperature:- 37.4C degrees
- Pulse rate:- 120 per minute
- Estrous cycle:- 4-5 days
- Gestation period:- 19-21 days
- Weaning age:- 19-21 days
- Mating age:- 6-8 weeks
- Room temp.:- 20-21
- Humidity:- 50-60 %
- Adult weight:- 25-28 g

Common Diseases

- Salmonellosis
- Ectromelia
- Streptobacillus moniliformis infection
- Miscellaneous virus infection
- Worms(Taenia taenia- formis)[6]

Experimental Procedures

Anasthesia: Animals respond in generally predictable ways to individual anaesthetic agents, however, much individual variation from animal to animal can be seen in

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Intranasal inoculation: inoculation of the drug through nasal/oral route is known as intranasal inoculation.

Collection of blood: The blood can be collected from cardiac, marginal ear vein.

3) Handling Of Guinea Pig

- Place one hand at the back of the animal with thumb behind the shoulder, while the other fingers are forwarded on the opposite side
- Lift the animal gently and support its weight with other hand by placing the palm under the hind quarters.[7]

Characteristic Features

- Rectal temp.:- 37.6-38.9 C degrees
- Normal respiration rate:- 80 per minute
- Pulse rate:- 150 per minute
- Gestation period:- 59-72 days(avg. 63 days)
- Weaning age:- 14-21 days
- Mating age:- 12-30 weeks
- Room temp:- 18.5- 21C degrees
- Humidity:- 45%
- Adult weight:- 200-1000g

Common Diseases

- Pseudo tuberculosis (acute or chronic)
- Abscesses in lymphatic glands
- Respiratory tract infections
- Intestinal infections
- Protozoan disease(Coccidioisis , Toxoplasmosis)

Experimental Procedure

Anasthesia (Pentobarbitone sodium 28mg/kg-body weight): It is a short-acting barbiturate. Pentobarbital can occur as both a free acid and as salts of elements such as sodium and calcium. The free acid is only slightly soluble in water and ethanol. It is used as a veterinary anaesthetic agent.

Subcutaneous inoculation: Subcutaneous injections are used often to inject anesthetics or to administer fluids for hydration during anesthesia recovery. The subcutaneous route of injection is often abbreviated as SC or SQ. The amount of fluid injected should be limited to volumes that will not overly stretch the skin (which would be uncomfortable) or that will not over-hydrate the animal unnecessarily. Typical volumes injected subcutaneously are in the range of 1 ml or less.

Intraperitoneal inoculation: First locate the point of entry for the needle. The needle will be inserted along this line on the animal's right side and close to the midline. Insert the needle into the abdomen at about a 30-degree angle after disinfecting injection site. If no fluid is aspirated, you may inject. Withdraw the needle.

Collection of blood: The blood can be collected from cardiac, anterior vena cava / sub calvian vein.

Bio Assay

Definition: It is defined as Estimation of the concentration / potency of a substance by measuring its biological response in living systems i.e., Observation of pharmacological effects on [1] living tissues, or cells [2] microorganisms [3] animals

Indications For Bioassay

- Active principle of drug is unknown
- Active prodrug cannot be isolated, e.g. insulin, posterior pituitary extract etc.
- Chemical method is either
  - not available
  - if available, too complex,
  - insensitive to low doses e.g. Histamine can be bio assayed in microgram conc.
- Unknown Chemical composition, e.g. long acting thyroid stimulator.

Chemical composition of drug differs but has same pharmacological action e.g. cardiac glycosides isolated from different sources, catecholamines etc.

Principles of Bioassay

- Active principle to be assayed should show the same measured response in all animal species
- The degree of pharmacological response produced should be reproducible under identical conditions [Eg: Adrenaline shows same rise in BP in the same species under identical conditions: wt, age, sex, strain / breed etc]
- The reference standard must have its activity to the principle for which the sample is being bio assayed.
- Activity assayed should be the activity of interest.
- Individual variations must be minimised.
- Bioassay might measure a different aspect of the same substance compared to chemical assay [Eg testosterone & metabolites]

Types Of Bioassays

- [1] Quantal Assays [Direct endpoint]
  – Elicits an 'All or None' response in different animals
  Eg. **1**) Digitalis induced cardiac arrest in guinea pigs
  2) Hypoglycaemic convulsions in mice.
  3) Digitalis induced head drop in rabbits
  4) Calculation of LD50 in mice or rats
- [2] Graded Response Assays [mostly on tissues]
  – Graded responses to varying doses
  – Unknown dose response measured on same tissue

3 Point Assay [2+1 Dose Assay]

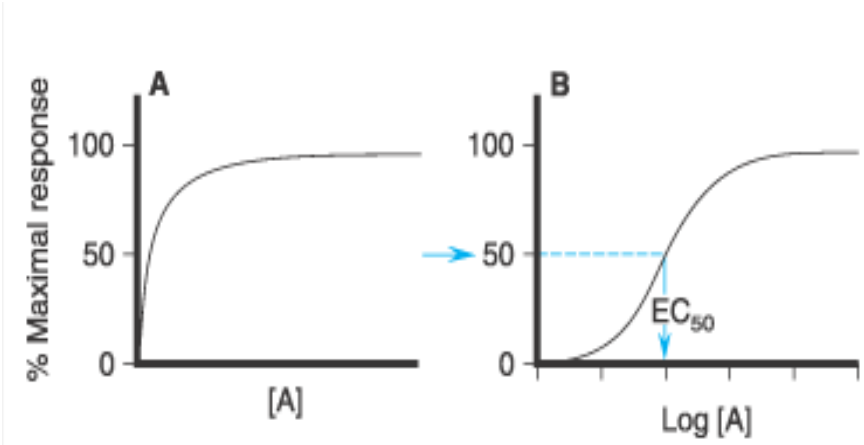
- Fast & convenient
- Procedure [Eg Ach bioassay]
  – Log dose response [LDR] curve plotted with varying conc of std Ach solutions and given test solution
  – Select two std doses s1& s2 [in 1:2 dose ratio] from linear part of LDR [Let the corresponding response be S1, S2]
  – Choose a test dose t with a response T between S1 & S2
  – Record 4 sets data [Latin square: Randomisation reduces error] as follows
- s1 s2 t
- t s1 s2
- s2 t s1
- s1 s2 t
  – Plot mean of S1, S2 and T against dose. Calculate
  – Log Potency ratio [M] = [(T –S1) / (S2-S1)] X log d
  [d = dose ratio]

4 Point Assay [2 +2 Dose Assay]

- Procedure [Eg Ach bioassay]
  – Log dose response [LDR] curve plotted with varying conc of std Ach solutions and given test solution
  – Select two std doses s1& s2 from linear part of LDR [Let the corresponding response be S1, S2]
  – Choose two test doses t1 & t2 with response T1 &T2 between S1 & S2 ; Also s2/s1 = t2/t1 = 2
  – Record 4 data sets [Latin square: Randomisation reduces error]
- s1 s2 t1 t2
- s2 t1 t2 s1
- t1 t2 s1 s2
- t2 s1 s2 t1
  – Plot mean of S1, S2 and T1, T2 against dose. Calculate
  – Log Potency ratio [M] = [(T1 –S1 + T2 –S2) / (S2-S1 + T2-T1)] X log d [d = dose ratio]

DOSE RESPONCE CURVE

- A curve can be drawn that illustrates the relationship between the dose administered and the observed response. This curve is referred to as the dose-response curve.
- A dose-response curve can be developed form most chemicals. From these curves the threshold level and the relative toxicity of chemicals can be obtained to establish safe levels of chemical exposure.



- The threshold is the dose below which no effect is detected or above which an effect is first observed.
- The threshold information is useful in extrapolating animal data to humans and for calculating a safe human dose for a given toxic substance.

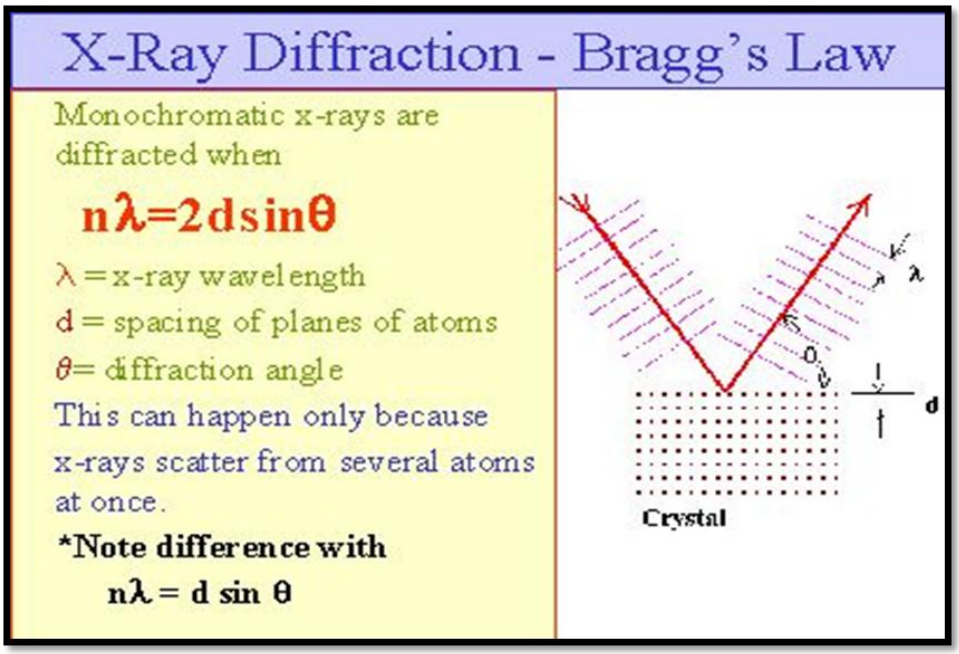
- The threshold dose (ThD0.0) is measured as mg/kg/day. It is assumed that humans are as sensitive as the test animal used. To determine the equivalent dose in man the ThD 0.0 is multiplied by the average weight of a man, which is considered to be 70 kg.

2D & 3D SPECTROSCOPY

Spectroscopy: It is the branch of science concerned with the investigation and measurement of spectra which is produced when matter emits electromagnetic radiation.

Types of Spectroscopy

◊ Emission spectroscopy
◊ Absorption spectroscopy
◊ Nuclear Magnetic Resonance [NMR] spectroscopy
◊ Raman spectroscopy
◊ Fluorescence spectroscopy
◊ Astro-particle spectroscopy



Nuclear Magnetic Resonance [NMR] spectroscopy: It is a technique which is based on the absorption of electromagnetic radiation in the "radio frequency region 4-900 MHz" by nuclei of the atoms. Proton nuclear magnetic resonance (PNMR) spectroscopy is one of the most powerful tools for elucidating the number of hydrogen or proton in the compound.

Principle of NMR

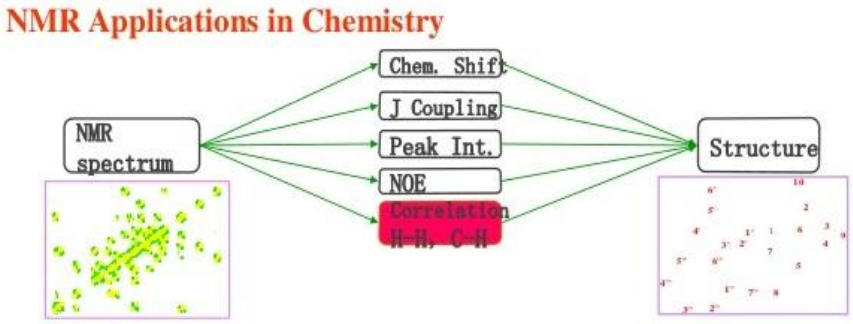
1. The theory behind NMR comes from the spin of the nucleus and it generates a magnetic field. Without an external applied magnetic field, the nuclear spins are in random directions.
2. But when an external magnetic field [B_0] is present, the nuclei align themselves either with the field of external magnet or against the field of external magnet.
3. If an external magnetic field is applied, an energy transfer [ΔE] is possible from ground state to excited state.
4. When the spin returns to its ground state level, the absorbed radiofrequency energy is emitted at the same frequency level.
5. The emitted radiofrequency signal gives the NMR spectrum of concerned nucleus.[8]

NOTE: The emitted radiofrequency is directly proportional to the strength of the applied force.

$$\upsilon = \frac{\gamma B_0}{2\pi}$$

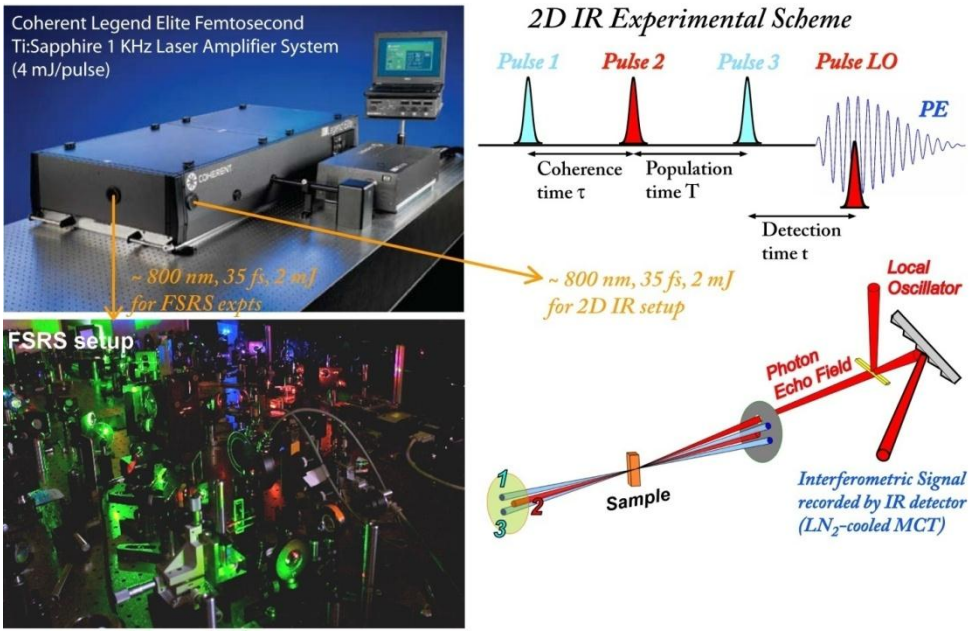
B_0 = External magnetic field experienced by proton.

γ = Magnetogyric ratio [ratio between nuclear magnetic moment & angular moment].



TWO DIMENSIONAL NMR SPECTROSCOPY

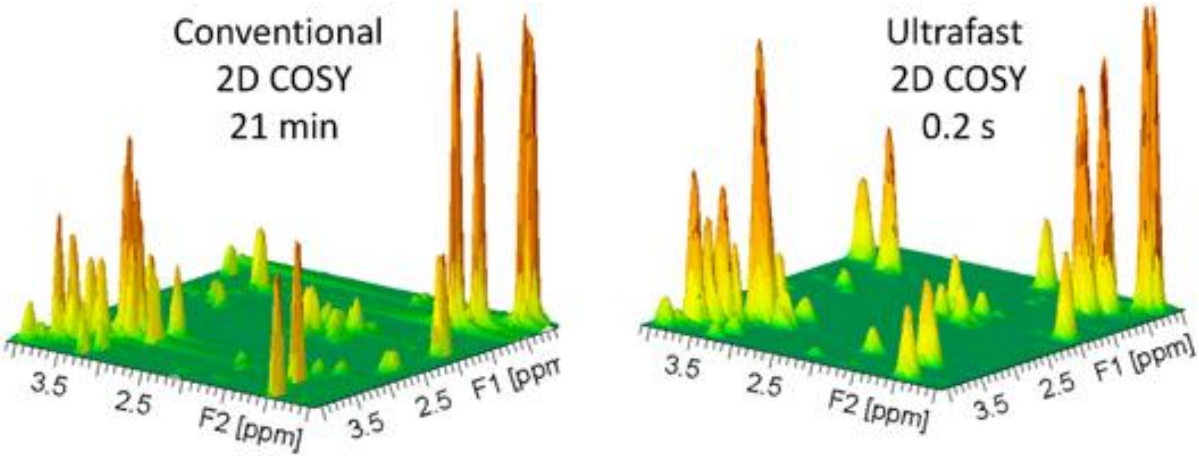
In 2- (or multi-) dimensional NMR spectroscopy many sequences are employed to provide additional information that are not obtainable from one-dimensional spectra. The most useful and commonly used forms of 2D NMR spectroscopy provide correlations between proton signals based on interactions, most frequently J-coupling (H-H. H-C or even C-C) It could also be dipolar coupling (the NOE interaction), rates of chemical exchange (DNMR swapping of spin environments) or relative diffusion rates (DOSY and related experiments).[9]



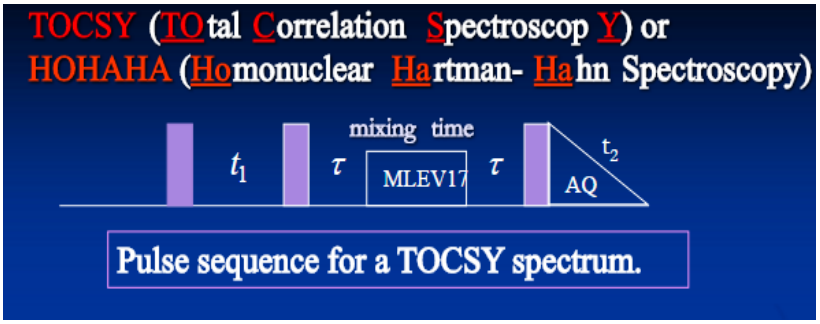
During the experiment, statistical interpretation can be done as it is possible to reach doubly excited states. This results in the appearance of an overtone peak. The harmonicity of a vibration can be read from the spectra as the distance between the diagonal peak and the overtone peak. One obvious advantage of 2DIR spectra over normal linear absorption spectra is that they reveal the coupling between different states. Spectral interpretation can be successfully assisted with developed theoretical methods.

The most common types of 2D experiments are listed below

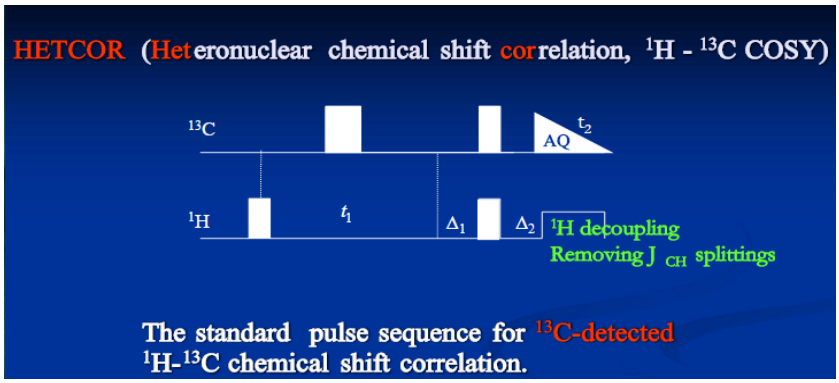
COSY: Homonuclear correlated spectroscopy. Correlation between protons that are coupled to each other. There are many modified version of the basic COSY experiment: DQF-COSY, COSY45, LRCOSY, ECOSY.



TOCSY: Total Correlation Spectroscopy (also referred to as the HOHAHA experiment). TOCSY Use a spin-lock for coherence transfer. During the spin-lock, all protons of a coupled system become "strongly coupled," leading to cross peaks. TOCSY have some advantages over COSY i.e., small couplings can be detected (in COSY small couplings give very weak cross-peaks because the peaks are antiphase).[10]

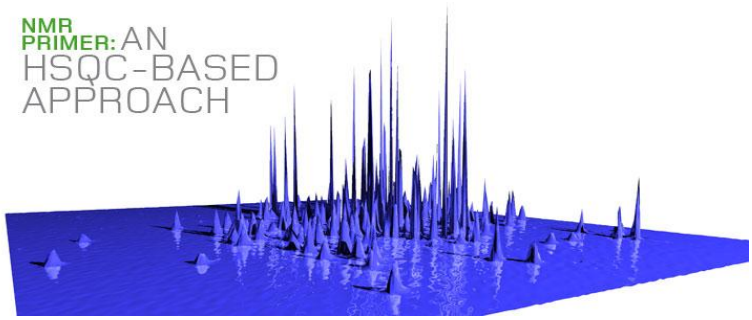


CH-COSY or **HETCOR:** Heteronuclear correlation, usually between 1H and 13C resonances mediated by JC-H. The experiment can be run using either 1 JC-H or longer range couplings. It has poor sensitivity because the observed nucleus is 13C, and has been largely replaced by the inverse detection experiments HMBC and HMQC.



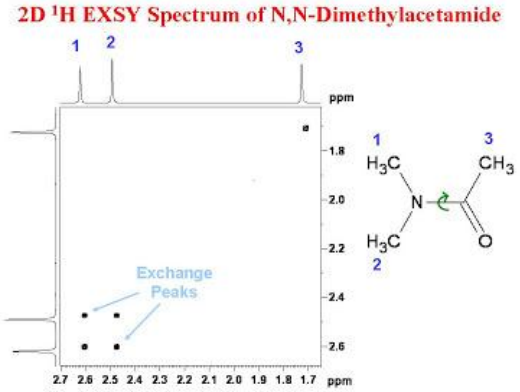
HMQC: Proton detected heteronuclear multiquantum coherence. Similar to CH-COSY or HETCOR experiment, except that the inverse detection using a DEPT sequence provides much better sensitivity. It is used to correlate proton and carbon signals using either one bond or longer range couplings.

HSQC: Heteronuclear Single Quantum Coherence. This is a CH correlation experiment which uses proton detection of the 13C signals using an INEPT sequence. It shows higher resolution in the C-dimension than the related HMQC experiment.



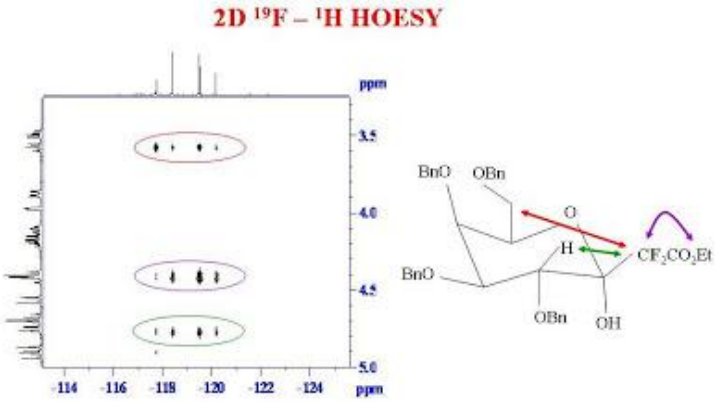
HMBC: Heteronuclear Multi-Bond Connectivity. This experiment is similar to HMQC, but it is optimized to detect proton-carbon correlation over 2 or 3 bonds. The sensitivity is better than direct detection methods, but the magnetization has to be transferred twice, first from proton to carbon and then back to proton via weak long-range interactions, which require relatively long delay times and thus much opportunity for loss of signal intensity by T1 and T2 relaxation.[11]

EXSY: Exchange Spectroscopy - the 2D equivalent of the Forsen saturation transfer experiment. The transfer of magnetization is due to chemical exchange between A and B. EXSY uses an identical pulse sequence to the NOESY experiment, which is subjected to interference by NOE. The magnetization transfer will be the equivalent of a negative NOE (nuclear overhauser effect).

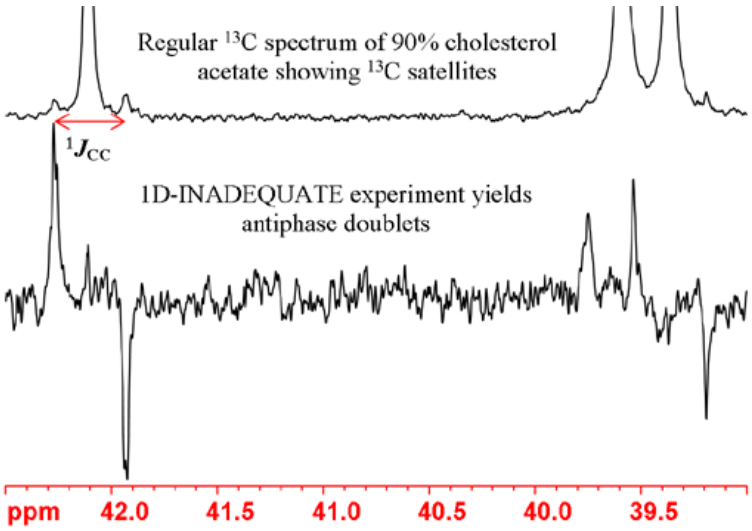


ROESY: Proton-proton correlation mediated by dipolar coupling. Correlation between protons that are close in space. This is the single most powerful NMR technique for determining the 3-dimensional structure of molecules - from conformations of small molecules to the 3-dimensional structure of small proteins.

HOESY: Heteronuclear Overhauser Effect Spectroscopy. There will be Correlation between protons and heteronuclei that are close in space. Heteronucleus is relaxed significantly by dipole-dipole interactions with nearby protons. It is used extensively in the spectroscopy of organolithium reagents, since 6 Li works well in this experiment.



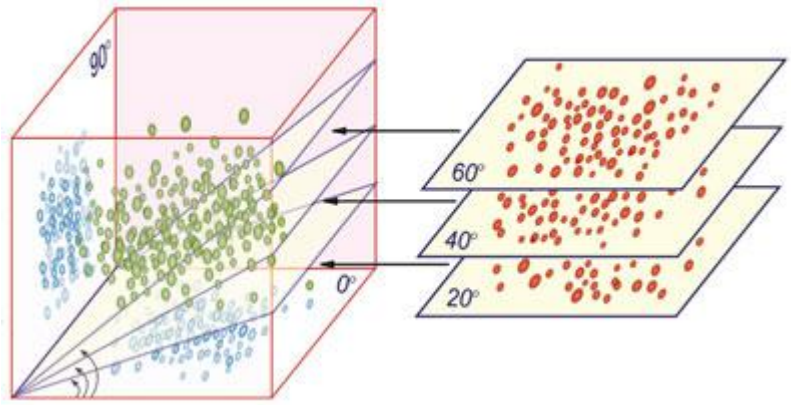
INADEQUATE: Incredible Natural Abundance Double Quantum Transfer Experiment. It has 2-Dimensional JC-C. This double quantum coherence experiment produces a C-C correlation using 1 JCC. It is the most powerful structure-determination tool but has very low sensitivity.



THREE DIMENSIONAL NMR SPECTROSCOPY

A three dimensional NMR experiment can easily be constructed from a two dimensional NMR experiment by inserting an additional indirect evolution time and the direct data acquisition. Each of the different indirect time periods(t1, t2) is incremented separately.[12]

Triple resonance experiments are the method of choice for the sequential assignment of larger proteins (> 150 AA). These experiments are called "triple resonance" because three different nuclei (^{1}H, ^{13}C, ^{15}N) are correlated. The experiments are performed on doubly labelled (^{13}C, ^{15}N) proteins.



RESEARCH METHODOLOGY

Research methodology is a careful, systematic, patient study and investigation in some field of knowledge undertaken to establish principles / policies.

Research can also be defined as

1. Search for knowledge
2. Systematic and scientific search for getting relevant answers on any taken up specific topic.
3. It is the voyage of discovery.

Objectives of research

- Defining and redefining the problems.
- Formulating the hypothesis or suggested solutions.
- Collecting, organizing and evaluating data.
- Making deductions and reading conclusions.
- And at last carefully testing the conclusions to determine whether they fit the formulating hypothesis.[13]

Types of research

- **Descriptive Research:-**Means description of the state of affairs as it exists at present, research only reports only what has happened or what is happening.
- **Applied Research:-**Aims at finding solution for an immediate problem facing a society or an industry/business organizations
- **Quantitative research:-**Based on the measurement of quantity or amount. applicable to phenomena that can be expressed in terms of quantity.

- **Conceptual Research:-**Related to some abstract ideas or theory, Used by philosophers and thinkers to develop a new concept or re-interpret existing ones.
- **Empirical Research:-**Relies on experience or observation alone, often without due regards for system and theory.
- **Qualitative research:-**Concerned with qualitative phenomenon, i.e., phenomenon relating to or involving quality or kind.
- **Fundamental research:-**Mainly concerned with generalization and with the formulation of a theory.
- **Analytical research:-**Researcher has to use facts on information already available and analyze these to make a critical evaluation of the material.[14]

Research hypothesis

A research hypothesis is the statement created by researchers when they speculate upon the outcome of a research or experiment. Every true experimental design must have this statement at the core of its structure, as the ultimate aim of any experiment. The hypothesis is generated via a number of means, but is usually the result of a process of inductive reasoning where observations lead to the formation of a theory. Scientists then use a large battery of deductive methods to arrive at a hypothesis that is testable, falsifiable and realistic.

Types of hypothesis

The null hypothesis, H_0, is an essential part of any research design, and is always tested, even indirectly**.** The simplistic definition of the null is as the opposite of the alternative hypothesis, H_1, although the principle is a little more complex than that.

The null hypothesis (H_0) is a hypothesis which the researcher tries to disprove, reject or nullify. The 'null' often refers to the common view of something, while the alternative hypothesis is what the researcher really thinks is the cause of a phenomenon.

Null Hypothesis

A **hypothesis** is a speculation or theory based on insufficient evidence that lends itself to further testing and experimentation. With further testing, a hypothesis can usually be proven true or false. Let's look at an example. Little Susie speculates, or hypothesizes, that the flowers she waters with club soda will grow faster than flowers she waters with plain water. She waters each plant daily for a month (experiment) and proves her hypothesis true!

A **null hypothesis** is a hypothesis that says there is no statistical significance between the two variables in the hypothesis. It is the hypothesis that the researcher is trying to disprove. In the example, Susie's null hypothesis would be something like this: There is no statistically significant relationship between the type of water I feed the flowers and growth of the flowers. A researcher is challenged by null hypothesis and usually wants to disprove it, to demonstrate that there is a statistically-significant relationship between the two variables in the hypothesis.[15]

Alternative Hypothesis

An **alternative hypothesis** simply is the inverse, or opposite, of the null hypothesis. So, if we continue with the above example, the alternative hypothesis would be that there IS indeed a statistically-significant relationship between what type of water the flower plant is fed and growth. More specifically, here would be the null and alternative hypotheses for Susie's study:

Null: If one plant is fed club soda for one month and another plant is fed plain water, there will be no difference in growth between the two plants.

Alternative: If one plant is fed club soda for one month and another plant is fed plain water, the plant that is fed club soda will grow better than the plant that is fed plain water.

Research process: Series of various actions, which are necessary to effective research work. Research process consists of a number of closely related activities. Various steps involved in a research process are neither mutually exclusive nor separate & distinct.

Steps involved in research process

1ST STEP-Establishing the needs for research
2ND STEP-Defining the problem
3RD STEP-Formation and Development Working Hypothesis
4TH STEP- Determining research design
5TH STEP-Identifying information types and source
6TH STEP-Determining methods of assessing data
7TH STEP-Designing data collection form
8TH STEP-Determining sample plan and size
9TH STEP-Data collection
10TH STEP-Analyzing data
11TH STEP-Preparing and presenting final research report

STEP 1-Establishing the needs for market research….

This step involves identification of a few problems and selection of one out of them, after evaluating the alternatives against certain selection criteria.

Market research is not needed when

Required information is already available
Decision need to be made now
Organization can't afford the research

STEP 2-Defining the problem..

The most important step in the research process is defining the problem.

Process involved in defining the problem

- Statement of the problem in a general way.
- Undertaking the nature of problem
- Surveying the available literature

- Developing ideas through discussion Rephrasing the research problem

Modes of problem identification….
There are 3 modes of problem identification:
Extraction from a manager's practical problem in a dialogue.
Cognitive identification of an experienced researcher in the area of his expertise.
A two step research process by a novice (scholar): literature search & pilot study.

STEP 3- Formation and Development of working hypothesis
- Assumptions are drawn to test its logical sequence.
- Hypothesis is guiding force of researcher.
- Outcome of researcher is deep thinking of research

Preparation of Research design
- Outline or a conceptual structure
- Collecting relevant data.
- Methods for preparation of research design

STEP 4-Determining research design….
Explanatory research: Collecting information in an unstructured & informal manner.
Descriptive research: Refers to a set of methods & procedures describing research variables.
Casual research: (Experiments & other approches): allows isolation of causes & effects.

STEP 5-Identifying information types & source….
Secondary data: Information that has been collected for some purpose other than the research at hand.
Primary data: Information that has been gathered especially for the research objectives at hand.

STEP 6-Determine methods of accessing data…
Secondary data: Accessing data through source such as the internet & library.
Primary data: Collecting data from participants through methods such as telephone,mail,online & face to face(quantitative)& observation studies & focus groups(qualitative).

STEP 7-Design data collection forms...
The design of data collection form that is used to ask or observe projects is critical to the success of the project. It is easy to write a set of questions but very difficult to construct a questionnaire.

STEP 8-Determine sample plan & size…
sample plan and its characteristics
Refers to the process used to select units from the population to be included in the sample.
1) Representativeness.
2) Adequate.
3) Independence.
4) Homogenous.
5) Lack of bias.
6) Accurate and complete.

Sample size and its determination:- Refers to determining how many elements(units)of the population should be include in the sample.
1. Nature of Universe.
2. Number of classes proposed.
3. Nature of study.
4. Type of sampling.
5. Standard of accuracy.
6. Availability of finance.

STEP 9-Data collection...
Sound data collection is very important because regardless of the data analysis methods used, data analysis cannot "fix" bad data. Non sampling errors may occur during data collection. They are related to poor design &/or execution of the data gathering. Sampling error may occur based purely on chance.

STEP 10-Analyze data...
Data analysis: Involves entering data into computer files, inspecting data for errors (data cleaning),running tabulations (frequencies), & conducting various statistical tests.

STEP 11-Prepare & present the final research report…
Findings are presented, often by research objective in a clear & concise way. The need for a good report cannot be over lased. It is the report & for its presentation, that properly communicates the results to the clients. Report should consist of:-

1. Preliminary Pages
- Title page
- Acknowledgement
- Foreword.
- Table of Contents.

2. Main Text
- Introduction.
- Summary of Findings.
- Main Report.
- Conclusion.

3. At the end of the report Appendices & Bibliography should be mentioned.
- Avoid vague language while writing report like "it seems", "there may be", etc.
- Charts and illustrations should be mentioned only if they present clear information.[16]

TECHNIQUES
Z-TEST: The test conditions include
- Population normal and infinite.
- Sample size large or small.
- Population variance is known.
- H_A may be one –sided or two sided.

Test Statistics:-

$$Z = \frac{\bar{X} - \mu H_O}{\frac{P}{\sigma\sqrt{n}}}$$

t-TEST

Test Condition

- Population is infinite and normal.
- Sample size is small.
- Population variance is unkown
- H_A may be one-sided or two sided.

Test Statistics

$$t = \frac{\overline{X - \mu H_O}}{\sigma_{\frac{S}{\sqrt{n}} \times [\sqrt{\frac{N-n}{N-1}}]}}$$

CHI-SQUARE TEST

Chi-squared tests are often constructed from a sum of squared errors, or through the sample variance. Test statistics that follow a chi-squared distribution arise from an assumption of independent normally distributed data, which is valid in many cases due to the central limit theorem. A chi-squared test can be used to attempt rejection of the null hypothesis that the data are independent.

$$\chi_c^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

ANOVA (ANALYSIS OF VARIANCE)

Analysis of variance (ANOVA) is a collection of statistical models used to analyze the differences among group means and their associated procedures (such as "variation" among and between groups), developed by statistician and evolutionary biologist Ronald Fisher. In the ANOVA setting, the observed variance in a particular variable is partitioned into components attributable to different sources of variation. In its simplest form, ANOVA provides a statistical test of whether or not the means of several groups are equal, and therefore generalizes the *t*-test to more than two groups. ANOVAs are useful for comparing (testing) three or more means (groups or variables) for statistical. It is conceptually similar to multiple two-sample t-tests, but is more conservative (results in less type I error) and is therefore suited to a wide range of practical problems.[17]

CONCLUSION

The use of proper restraint and handling techniques reduces stress to animals and also to the researcher. Handling stress represents an experimental variable and should be minimized. Animals can inflict serious injuries to humans and to themselves as a result of improper handling. Most animals, even rodents will respond positively to handling and will learn to recognize individuals. While handling animals, Do not make loud noises or sudden movements that may startle them. Use an assistant whenever possible. Chemical restrain should be considered for any prolonged or potentially painful procedure. The methods described below will assist with performing basic manipulations. Alternate techniques may be needed for special procedures. The hypothesis of the lab was done if the visible spectrum of an element observed, then the element can be identified. There were a couple of steps that needed to be done to collect data for the lab. A spectroscope was used to figure out what colors the spectra light emitted. There were two spectral tubes and each one was observed and data was collected and then it is compared with the visible spectrum of eleven elements. The data then need to be put in charts and from there the charts and light from the elements was analyzed to see whether the hypothesis was refuted or supported. The hypothesis was actually supported because the charts and elements corresponded correctly. With the availability of multisensor, multitemporal, multiresolution and multifrequency, data from operational observation has become a valuable tool in evaluation. This type is a relatively new research field at the leading edge of available technology. It forms a rapidly developing area of research.

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