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Review on LC-NMR Hyphenated Technique

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Abstract: *A hyphenated technique refers to the integration of two distinct analytical methodologies to facilitate seamless interfacing. Typically, this involves the combination of chromatographic techniques with spectroscopic methods. LC-NMR, an influential analytical tool, has been instrumental in resolving intricate mixtures. LC-NMR, a hyphenated system that merges liquid chromatography (LC) with nuclear magnetic resonance (NMR), is a meticulously organized analytical approach extensively applied across various domains. These applications encompass the analysis of metabolites, profiling drug impurities, elucidating molecular structures, identifying volatile oils, scrutinizing pesticides, and investigating other natural products.*

Keywords: LC-NMR, NMR, Hyphenated Techniques, instrumentation, Development, Analytical method.

I. INTRODUCTION

HYPHENATED TECHNIQUES:

A Hyphenated technique is the mixture of two different analytical techniques which assist a proper interface, mainly chromatographic techniques are combined with spectroscopic techniques.

In chromatography, the pure or nearly pure tiny layer of chemical components in a mixture was separated & spectroscopy produced selective information for identification using standards or library spectra.

The term Hyphenated technique range from the merger of

Separation - separation

Separation - identification &

Identification - identification Techniques

The spell “Hyphenation” was first adapted by Hirsch feldin (1980).

ADVANTAGES:

- For quick & accurate Analysis.
- A higher degree of automation.
- Higher sample throughput.
- Better reproducibility.
- Separation of quantity at the same time.

DISADVANTAGES:

- By this method only volatile sample can be investigated.

Types of Hyphenated Technique:

- Mainly two types. They are
  1. Double Hyphenated
  2. Triple Hyphenated

1.Double Hyphenated:

A.LC-MS

B.LC-NMR

C.LC-IR

D.GC-IR

E.GC-MS

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2.Triple Hyphenated:

A.LC-API-MS
B.APCI-MS-MS
C.LC-ESI-MS
D.LC-NMR-MS
E.LC-APPI-MS
F.LC-PDA-MS

II. LC-NMR HYPHENATED TECHNIQUE

INTRODUCTION:

- NMR is the one of the most powerful techniques for the structural identification of organic compounds.
- Separation and isolation of the individual components of a sample mixture are the normal step before analysing their structure by NMR.
- Prior to isolate, LC/MS is used routinely to analyse the components of mixture to evaluate the need to isolate the compounds of interest.
- In many cases, however, NMR is suitable for the identification of complicated structures.
- Even though Hyphenated LC-NMR has been known since the late 1970s, the technique was not implemented widely until the past two decades.
- For more complete structural analysis, LC-MS and LC-NMR have been combined (LC-NMR-MS) with its efficacy demonstrated successfully.
- The first real sample analysed by LC-NMR was military jet fuel using normal phase columns and deuterated chloroform and Freon.

PRINCIPLE OF LC (HPLC):

- High performance liquid chromatography (HPLC) is basically a improved form of column liquid chromatography
- The replacement of a solvent being allowed to drip through a column under gravity, it is forced under high pressures of up to 400 atmospheres.
- That makes its much faster.
- All chromatographic separations, including HPLC operate under the same basic principles; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the active phase and the stationary phase used in the separation.

PRINCIPLE OF NMR:

- The principle behind NMR is that many nuclei contain spin and all nuclei electrically charged.
- When the external magnetic field is applied, the energy transfer is possible between the base energy to a higher energy level [generally a single energy gap].
- When the energy transfer takes place at a wavelength that corresponds to radio frequencies and the spin returns to its base level, energy is emitted at the same frequency.
- The signal which matches this transfer is measured in many ways and processed in order to yield an NMR spectrum for the nucleus concerned.

INSTRUMENTATION OF LC-NMR:

It is the combination of three units. They are
1. LC UNIT : ContainAuto sampler, LC pump, Column.
A non NMR detector [eg: UV, DAD,EC, refraction index or radioactivity]
2. LC-NMR INTERFACE:Contains Direct line ,
Indirect [intermediate storage loop].

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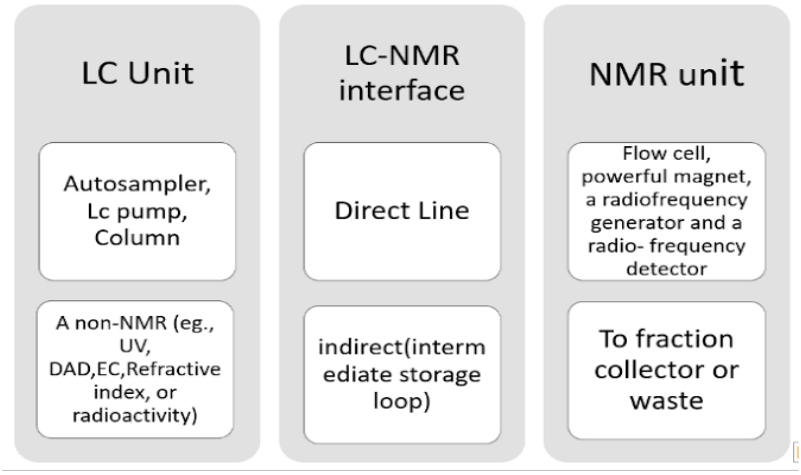
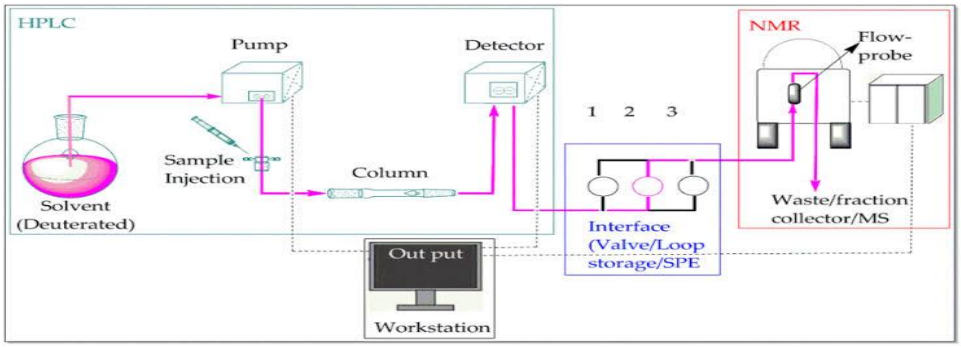
3.NMR UNIT: it Contains flow cell, powerful magnet, a radiofrequency generator and a radio-frequency detector. Fraction collector or waste.



LC UNIT:

- Solvent Reservoir: active phase contents are available in a glass reservoir. This is usually a combination of polar and non- polar liquid components whose corresponding concentrations are varied, depending on the composition of the sample.
- PUMP: A pump removes the active phase from the solvent Reservoir and forces it through the system's column and detector. Depending on the number of factors including column dimensions, particle size of the stationary phase, the flow rate and composition of the active phase, operating pressures maintenance should be up to 42000 kpa (about 6000 psi) could be generated.
- Sample Injector: The Injector should be a single injection or an automated injection system. The Injector for HPLC system should supply injection of the liquid sample in the range of 0.1 - 100 ml of volume with high reproducibility and under high pressure (up to 4000 psi).
- Columns: Columns are generally made up of polished stainless steel, are between 50 and 300 mm long and have an ID (2 and 5 mm). They are commonly filled with a stationary or stable phase with a particle size of 3-10 µm. Columns with an internal diameter of less than 2 mm are often referred to as microbore columns. Ideally the temperature of the active phase and column should be kept stable during an analysis.
- Detector: The HPLC detector, located at the end of the column, detects the analyses that elute from the chromatographic column.
- Generally used detectors are UV (ultraviolet) spectroscopy, fluorescence, mass spectrometric and electrochemical detectors.
- Data collection Devices: Signals projected from the detector may be collected on chart recorders or electronic integrators. The computer integrates the response of the detector to each of the components and places it into a chromatograph that is easy to read and translate.



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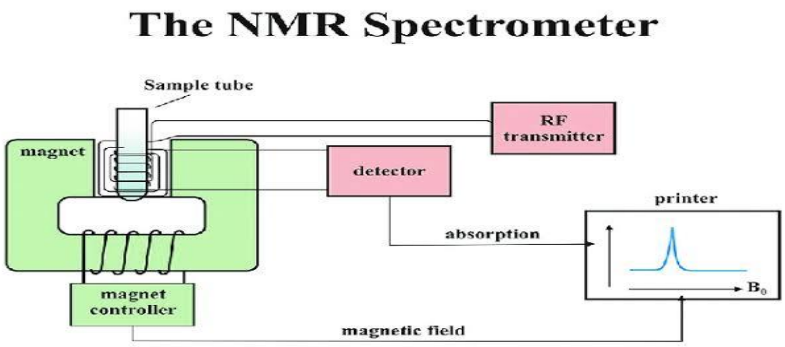
LC-NMR INTERFACE:

Direct coupling: It include direct flow of LC effluent into NMR flow cell continuous recording of spectra
Post - column splitter
Valve - switching interface i.e BNMI (Bruker NMR - Mass spectrometry interface).
Indirect coupling:
Intermediate storage loop which transfers outlet of LC to NMR flow cell at specified time interval
SPE UNIT (Solid phase extraction).

NMR UNIT:

Basically, NMR instrumentation involves the following units.

- A magnet is used to separate the nuclear spin energy state.
- Two RF channels, one is for the field/ frequency stabilisation and another one to supply RF irradiating energy.
- A sample probe containing coils for coupling the sample with RF filed.
- and contains sample holder, RF oscillator, sweep generator and RF receiver.
- A detector to process the NMR signals.
- A recorder to display the spectrum.



Advantages of LC-NMR :

- The information between the two (three) techniques is so orthogonal; HPLC methods resolve "COMPLEXITY OF A MIXTURE" by separation, whereas NMR resolves virtually any structure questions (especially with different experiments)
- The NMR can identify if the LC peak is impure.
- LC - NMR/MS is "THE" ultimate instrument.
- NMR data can be taken without complete separation of mixture.
- It is non-destructive technique.
- Sample should be maintained for analysis by another method.

Disadvantage of LC-NMR:

- High costs
- Capital equipment costs high; long experiments take times.
- partial use of 2H solvents.
- Operator training requirements.
- Difficulty in solvent selection.
- Doing LC - NMR/MS requires a unique set of skills.

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APPLICATION OF LC- NMR:

- Natural Product Analysis: LC-NMR is extensively used in the analysis of complex natural products such as plant extracts, marine organisms, and metabolites. It helps identify and characterise the various compounds present in these mixtures, which is crucial in drug discovery and phytochemical research.
- Drug Discovery: In pharmaceutical research, LC-NMR is employed to study and identify compounds in drug development. Researchers can verify the purity of synthesised compounds and investigate their structures, ensuring that they meet the desired quality standards.
- Metabolomics: LC-NMR is a valuable tool in metabolomics, the study of small molecules involved in metabolism. It can help identify and quantify metabolites in biological samples, aiding in the understanding of biochemical pathways and disease mechanisms.
- Food and Beverage Analysis: LC-NMR is used in food chemistry to determine the composition of complex mixtures. It's employed to analyse the constituents of food and beverages, detect adulterants or contaminants, and ensure product quality and safety.
- Environmental Analysis: LC-NMR can be applied to analyse environmental samples, such as water, soil, and air, for the presence of pollutants and contaminants. It helps identify and quantify these substances, contributing to environmental monitoring and remediation efforts.
- Polymer Characterization: In polymer science, LC-NMR is used to characterise polymer structures and investigate their composition, molecular weight distribution, and branching. This information is essential for quality control and research in the polymer industry.
- Petroleum and Petrochemical Analysis: LC-NMR plays a role in the analysis of crude oil, petroleum products, and petrochemicals. It helps in identifying and quantifying various components and impurities, aiding in refining processes and quality control.
- Pharmaceutical Quality Control: Pharmaceutical companies use LC-NMR for quality control of drug formulations. It allows for the verification of the identity and purity of active pharmaceutical ingredients (APIs) and helps detect impurities or degradation products.
- Natural and Synthetic Product Comparison: LC-NMR is valuable for comparing natural and synthetic products. It can be used to confirm whether a synthesised compound matches the structure of a natural product, which is essential in drug discovery and understanding biological pathways.
- Structural Elucidation: Perhaps the most crucial application is the determination of the structure of unknown compounds. LC-NMR provides detailed structural information, including connectivity and stereochemistry, making it indispensable in structural elucidation.

IV. CONCLUSION

Mainly single analytics techniques are used to detect unwanted materials present in the organic compound like impurities, structural change etc. But not accurately so , HYPHENATED techniques were introduced. In these techniques there is the merger of two analytical techniques together to produce accurate information of the organic compound by spectroscopic and chromatographic techniques. LC- NMR is a specialised technique which works on nuclear magnetic resonance and liquid chromatography. LC-NMR was an influential analytical tool used for the separation of complicated mixtures. Single analytical techniques are applicable for single application. But Hyphenated techniques are applicable for both chromatographic (identification)and spectrometric (separation& determination).

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