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Applications of Analytical Instruments in Biotechnology: A Comparative Review

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ABSTRACT

Knowing the appropriate applications of various analytical techniques used in biotechnology, makes the experiment more easier and let the researcher obtain a better result which also reduces the time period of experiment by eliminating the trial and error methods. Along with the knowledge of application of the analytical technique, the researcher also should have the knowledge of the sampling techniques for the analytical instruments as it is one of the deciding factors to choose a specific technique for the analysis. At the same time, the results obtained by using the analytical equipments are relatively authentic and accurate. With the development of biotechnology, the usage of analytical equipments has increased by many folds. The areas of biotechnology such as nanotechnology, cancer biology, genetic engineering and many more are dependent on the analytical equipments for most of their experimental analysis. Analytical equipments find its application not only in the research works but also at the industry level. Various food and pharmaceutical industries rely upon the analytical techniques like FT-IR and Raman for the quality analysis of the products.

Key words: Analytical techniques, biotechnology, nanotechnology, cancer biology, genetic engineering

INTRODUCTION

The growth of research and development is highly dependent on the validity and precision of the results obtained by performing various experiments in laboratory. One of the major issues of biotechnology experiments is the time dependent of many results and there comes a possibility of human error or any other form of handling error due to a large time gap between the performance of experiment and obtaining result.

By the usage of various analytical equipments, we can obtain an accurate and precise result within a small stipulated time gap. This reduces the chance of handling errors as well any misreading obtained due to transformation of chemical compounds during the time period of experimentation. At the same time, the analytical equipments need a very minute amount of sample for the analysis.

The most ancient, popular and highly used analytical equipments are Raman spectroscopy, Infra Red spectroscopy, Gas chromatography and NMR (Table 1).

Raman spectroscopy: The Raman spectroscopy is derived from an inelastic light scattering process in which a monochromatic beam of light usually a Laser photon is directed towards the

Table 1: History of the development in analytical instrumentation

Analytical instrument	Year	Primary function	Properties of analytes
Raman spectroscopy	1928	Molecular identification	Liquid and solid state
Fourier transform-infra red spectroscopy	1950	Molecular identification	Liquid and solid state
Nuclear magnetic resonance	1969	Determine the composition of organic compound	NMR active nuclei
Gas chromatography (GC)	1947	Testing the purity of a particular compound	Gaseous state
Mass spectrometry (MS)	1919	Identification of compound based on mass-charge ratio	Solid state
GC-MS	1950	MS as detector in GC	Gaseous or solid state
Chromatography	1903	Separation and identification of compound based on color	Liquid state

sample molecule and it is being scattered after interacting with the sample molecules. This scattering of laser photon is measured by the change in wavelength of irradiating photon due to the change in energy of the laser photons during the process. This change in wavelength of the monochromatic light after interacting with the sample is called as the Raman Effect and is measured as Raman shift by the electronic detector. The change in energy of the laser photons is characteristic of the bonds in the molecule and hence the measurement of this change in energy in the form of wavelength shift can be used to obtain the information of presence of a particular molecule in the sample. In this sense Raman spectroscopy and FT-IR is similar but Raman spectroscopy has its own advantages over FT-IR, as summarized here. Raman analysis of a sample does not require intensive sampling processes as it is needed in case of FT-IR analysis such as lyophilisation, mixing with KBr/NaCl and other time consuming steps. Raman analysis is unaffected by sample cell materials such as glass which is a matter of concern in case of FT-IR analysis. Thus, having these advantages, Raman spectroscopy can be used as a powerful tool for chemical monitoring and analysis of samples.

The Raman system is typically consisted of four basic components, namely as a monochromatic light source in the form of Laser light to be used as an excitation source, a light collection system, a spectrophotometer for the selection of wavelength and a detector in the form of photodiode array.

A laser beam is focused on the sample and the irradiated beam of light is collected through a lens and sent through the interferometer or spectrophotometer to obtain the Raman spectrum of the required sample.

Raman spectroscopy can be applied in forensic sciences by comparing the body fluid samples obtained from the crime scene to that of the database created of all the body fluids using Raman signals. The body fluid obtained from the crime scene is dried and small amount of the sample is analyzed by Raman spectroscopy to obtain the Raman spectrum. This spectrum is compared with the library and the identity of the sample can be revealed. The advantage of using Raman spectroscopy is the need of a very minute amount of sample for the analysis and just a sample from the crime scene to be exploited for DNA extraction can provide with the result.

The Raman spectroscopy was not much applied in biological sciences till the invention of laser. After the laser development by scientists, Raman spectroscopy has been exploited vigorously by the researchers. The various technical advancements lead to a better role of Raman spectroscopy in biological science experiments. The most recent advancement in technology for Raman is SERS which resulted in spectacular outcomes in biology by enhancing the relatively weaker Raman signals for a comparatively better result analysis. Raman spectroscopy is also a notable technique and highly favored by researchers because of the requirement of minimal amount of sample which can be ranging in few milligrams.

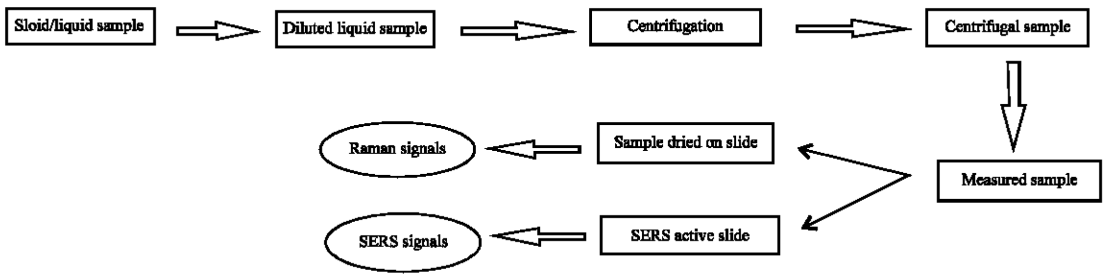

Fig. 1: Schematic diagram of sampling process for raman and SERS analysis

Surface Enhanced Raman Scattering (SERS) can be used for label free sensing of bacteria because of its spectacular enhancement in Raman signals (Liu *et al.*, 2011). The difference between the process of Raman and SERS is due to the SERS active substrate. A typical SERS active substrate contains an arrangement of nano-metallic substances such as silver-nanoparticles and the etch pits on silver surfaces, sustain surface Plasmon Polariton resonance and enhances the molecular Raman signal near and on the substrate (Nie and Emory, 1997; Fang *et al.*, 2008; Li *et al.*, 2010). The high reproducibility and sensitivity of SERS active substrate lead to the efficient application of SERS for biological experiments (Tripp *et al.*, 2008; Jarvis and Goodacre, 2008; Graham *et al.*, 2008; Efrima and Zeiri, 2009; Naja *et al.*, 2007; Yan and Reinhard, 2010; Han *et al.*, 2009). Raman and SERS can is also being used in the case of study of antibacterial activity of various drugs, to detect the lethal changes in the bacterial cell wall and structure on the interaction of antibiotics and bacterial bio-molecules (Liu *et al.*, 2009; Prakash *et al.*, 2013; Alok *et al.*, 2013) (Fig. 1).

Surface enhanced Raman scattering spectroscopy can also be used to check the purity of a substance. Recently, there has been a case of milk products adulteration by melamine which caused various numbers of infant deaths in China (Zhang *et al.*, 2010). In various fields, Raman spectroscopy is being considered as a non-invasive and rapid analytical technique for the detection as well as quantification of adulterants.

Infra red spectroscopy: The spectroscopy which operates in the infra-red region of the electromagnetic spectrum is called as infra red spectroscopy. The infra red region of light has a lower frequency and longer wavelength than visible light. This technique can be used to identify and study chemicals. Fourier transform infrared spectrometer is one of the common instruments using this technique.

FTIR: Fourier transform infra red spectroscopy: It is used to identify the functional groups in a molecule and can be applied in quality control of various pharmaceutical and food products to compare the purity of a product with the database collection of pure compounds. Infra red technique can be applied for the detection of protein by a process which can be fast and non-destructive. This proves useful in the case of detection of protein in milk as protein being the important component of milk (Wu *et al.*, 2008; Ramalingam *et al.*, 2013). Infra-red spectroscopy has also been proved useful in the study and detection of fat and total solids in milk, cheese and butter (Laporte and Paquin, 1999; Rodriguez-Otero *et al.*, 1995; Hermida *et al.*, 2001; Sorensen and Jepsen, 1998). Infra red spectroscopy also finds its application in the identification and differentiation of various biological molecules namely milk powder, coffee varieties and Chinese herbs and also in the measurement of chemical components (Downey *et al.*, 1997; Li *et al.*, 2001; He *et al.*, 2006; 2007).

Nuclear magnetic resonance: Nuclear Magnetic Resonance proves to be unique among the various analytical techniques because of its ability to provide non-invasive information of in vivo systems. The metabolic pathways of cellular system can be analyzed by NMR spectroscopy and this finds its need in the genetic engineering field of biotechnology (Hesse *et al.*, 2000; Hugenholtz *et al.*, 2000). Various isotopes have been used for the metabolic pathway study using NMR spectroscopy, such as ^{13}P and ^{13}C (De Graaf *et al.*, 2000; Bibollet *et al.*, 2000; Portais *et al.*, 2000).

The scientific assumption that genetically engineered plants holds an importance in environmental, agricultural and food industries gives rise to a new application of NMR for pathway analysis. It is being applied towards the various metabolic pathways in wild strains and genetically engineered strains (Van Voorthuysen *et al.*, 2000; Roscher *et al.*, 2000). Also, the transgenic plants are being screened for human and animal consumption (Noteborn *et al.*, 2000).

DNA and Protein sequencing techniques are rapid and sophisticated but it is not the same for polysaccharides analysis. NMR along with Mass Spectrometry can be made applicable for sequencing at oligo and polysaccharide level (Leeflang *et al.*, 2000; Mulloy *et al.*, 2000). NMR-imaging technique when combined with metabolic NMR can lead to a better scope for optimization of bioreactors, in which the metabolic pathways of the immobilized cells as well as the transport and diffusion of nutrients, products, oxygen and water are collectively important (Vogt *et al.*, 2000).

Gas chromatography (GC): It is the chromatography technique used in analytical chemistry to separate and analyze volatile compounds which can vaporize without decomposition. Gas Chromatography is mainly being used for testing the purity of a particular substance or separating the different components of mixture. In few of the cases, gas Chromatography may help in compound identification (Niemann *et al.*, 2005).

The instrument used for Gas Chromatography is known as gas chromatograph or aerograph or gas separator. In gas chromatography, the mobile phase is a carrier gas usually an inert gas such as helium or a non-reactive gas such as nitrogen. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside the column which is made up of a piece of glass or metal tubing.

Gas chromatography operates on organic as well as Inorganic analytes, provided it should be volatile. The analytes interact with the wall of the column, coated with a stationary phase. This interaction leads to elution of analytes at different time, known as the retention time of the compound. This comparison of retention time gives Gas Chromatography its analytical importance (Kumar *et al.*, 2011).

Mass spectrometry (MS): MS is the analytical technique based on the principle of measurement of mass-to-charge ration of the charged biological samples such as peptides. The mass spectrum contains various peaks which correspond to the m/z value of various types of peptides in the original sample (McPherson *et al.*, 2001; Dougherty and Braga-Neto, 2006; Suneetha *et al.*, 2013).

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