International Journal of Scientific Research in Modern Science and Technology, ISSN: 2583-7605 (Online)
Vol. 1, Issue 1 [2022] www.ijsrmst.com

Study on Optical Measurements

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Abstract:

This article focuses on the study of the fundamental ideas related to Fourier transform infrared (FTIR) and highlights recent work on the integration of FTIR spectroscopy technology into lab on a chip gadgets & its applications in the area of biological.

Keywords: blood cells, FTIR, spectroscopy, lab-on-a-chip, functional group.

1. Introduction:

The experimental details are explained in this paper. In this work we use Powder form polymers PVA, PMMA and PS. The molecular weight of PVA is about 125,000 g/mol. Properties such as water solubility, good film-forming property and low permeability of PVA have made it a suitable host polymer for investigation. The molecular weight of PMMA is approximately 120,000 g/mol. It is a colorless transparent plastic and optical clarity is the main characteristic of this plastic. It also has good mechanical properties. It is resistant to many chemicals but soluble in organic solvents such as ketones and esters. Polystyrene has low heat deformation temperature, poor weathering property, brittleness and is soluble in many organic solvents. Table 1 lists the sources and compounds that were employed in the current investigation.

Compounds	Abbreviation	Source
Poly(vinyl alcohol)	PVA	S.D.Fine, India
Poly(methyl methacrylate)	PMMA	Scientific Allied products, Bangalore, India
Polystyrene	PS	S.D. Fine, India
Ethidium Bromide	EtBr	Sigma Aldrich, India
5(6) Carboxyfluorescein	5(6)CF	Sigma Aldrich, India
Acridine Orange	AO	Scientific Allied and products Bangalore, India.

Table1. Table of compounds used in experimental work

2. Optical Measurements:

The current study makes use of a variety of optical approaches to comprehend the altered microstructure and optical characteristics of dyes in various mediums.

2.1. FTIR Measurement:

The KBr pelleting technique was used to acquire infrared spectra for this investigation, and the spectra were recorded on a NICOLET FTIR-6700 spectrometer at a resolutions of 4 cm^{-1} across the wave range of 400-4000 cm^{-1}.A glass mortar and glass pestle are used to fully combine and pulverise the polymer sample and dried potassium bromide at a ratio of 1:100. An opaque spherical disc with a 13 mm diameter is produced by pressing the mixture into a specific die under 5 tonnes of pressure using a hydraulic pelleting equipment. In order to conduct a spectroscopic analysis, the disc is then put into the instrument beam [1]. The building of an FTIR device may use a wide variety of materials and parts. The main components of FTIR spectrometer are source, interferometer, tape dispenser, indicator and computer.

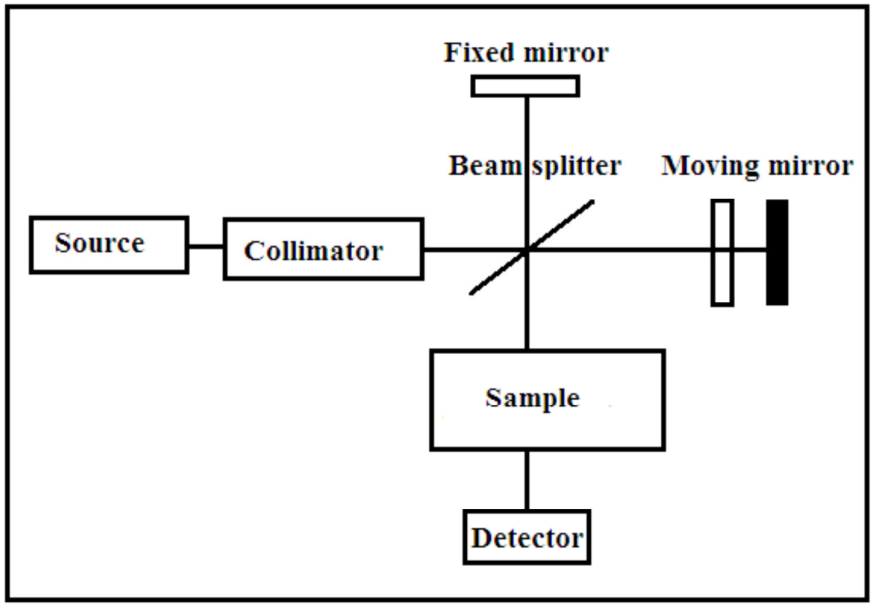

Fig.1.1 Schematic diagram of Fourier Transform Infrared Spectrophotometer

- **Interferometers:**

It calculates the degree of interference between two light beams. Fig. 1.1 depicts light coming from an infrared source entering the interferometer from the left. A single beam of light is divided into two by the interferometer. In Fig. 1.1, a beam of light emerges from the interferometer after the two beams of light have combined after travelling along separate pathways.

- **Sources:**

In the spectrometer function of the source into exclude IR radiation. The Most of the IR sources work on the basis of heat generation due to the source's resistance to electric current. This resistance heats the source (above 800^{o}C) from which it emits radiation.

- **Beam splitter:**

The beam splitter is a semi-reflecting device and is often made by depositing a thin film of germanium on a flat KBr substrate. The radiation as on broadband IR source into collimated & directed to interferometer & applied to the beam splitter. The half of the IR beam to be transmitted into the fixed mirror & the remains half reflected in the moving mirror on the beam splitter. After the split beam to be reflected from the 2-mirrors they are recombined at the beam splitter. An interference pattern arises due to the change in the relative position of the moving mirror to that of the stationary mirror. The resulting beam then passes through the sample and is eventually focused on the detector.

- **Detector:**

The function of the detector is to transduce the light intensity received by it to electrical signals. From the IR source the detector response for a single frequency component is considered first. This stimulates an idealized situation where the source is monochromatic, such as a laser source.

- **Computer:**

The measured signal is digitized and sent to the computer where the Fourier transform takes place. In the last infrared spectrum is presented for an interpretation & further manipulation.

The radiation source beam is split by a mirror to pass through both the sample and the reference chamber. Light is reflected in a monochromator using mirrors that allow light of only one wavelength at a time to reach the detector. The detector receives signals from both the sample beam and the reference. This information goes to the processor which translates the information in the plot.

All IR spectra were scanned in the 1000–2000cm^{-1}range using an FTIR instrument Milko Scan FT2 (FOSS ANALYTICAL, Hilerød, Denmark). The acquisition was performed according to the method described in [4] with an optical resolution of 14 cm^{-1}. Each sample was manually injected through a loop in the cuvette inside the FTIR apparatus using a syringe. The cuvette was maintained at a temperature of 42 °C and had a path length of 50 µm.

Data Handling and Analysis FOSS Integrator (version 1.5.3, FOSS Analytical, Hillerod, Denmark) was used to export the acquired spectral data. Subsequent data analysis was performed using MATLAB (The MathWorks Inc., MA, USA) and the n-way toolbox as previously described [2,3,5].

The FTIR spectroscopy limitation is related to the penetration of infrared light into the tissue depth, which is allowed to the biochemical analysis of tissues down to only some dozens of micrometers [6]. When using liquid samples then operates in transmission mode and their results in no incident angle between the emitter and the sample, there is a difficulty in ensures reproducibility of spacer thickness [9]. The attenuated total reflectance FTIR technique with FTIR spectroscopy to the overcome its limitation as a complementary technique. ATR-FTIR to be specialize FTIR spectroscopy method and from a sample which measures the reflected signal. In this reflection setup the IR radiation passes with a crystal through a high refractive index and through total internal reflection before leaving the crystal [9,10]. Finally, micro-FTIR (micro-FTIR) [11] relates to another specialized FTIR technique that combines an IR spectrometer with a visible lights

microscope to be achieving better sensitivity when detective condensed phases compound [12] and solid or enough to measure samples of liquid thin films. An easy using to a free from reagent method based on a spectroscopy to the quantify protein contents of the extracellular vesicle samples without any sample preparation [13]. This method is present a free from reagent alternative to the modern colorimetric protein determination assay & do not requires any special sample preparation for the investigation of EVs [13]. Therefore, this IR spectroscopy-based on protein quantification method will be successfully adapted for the routine analyze of an extracellular vesicles.

3. UV-Visible Absorption Measurement:

Optical studies of pure and doped PVA, PMMA, PS and PMMA/PVA composite films were performed using SHIMADZU (wavelength range UV spectrometer 195 in the beam path without sample as reference) and then polymerized film the samples are placed in the sample holder and placed in the beam path. Fig. (1.2) Representative Diagram of UV- Visible Spectrophotometer

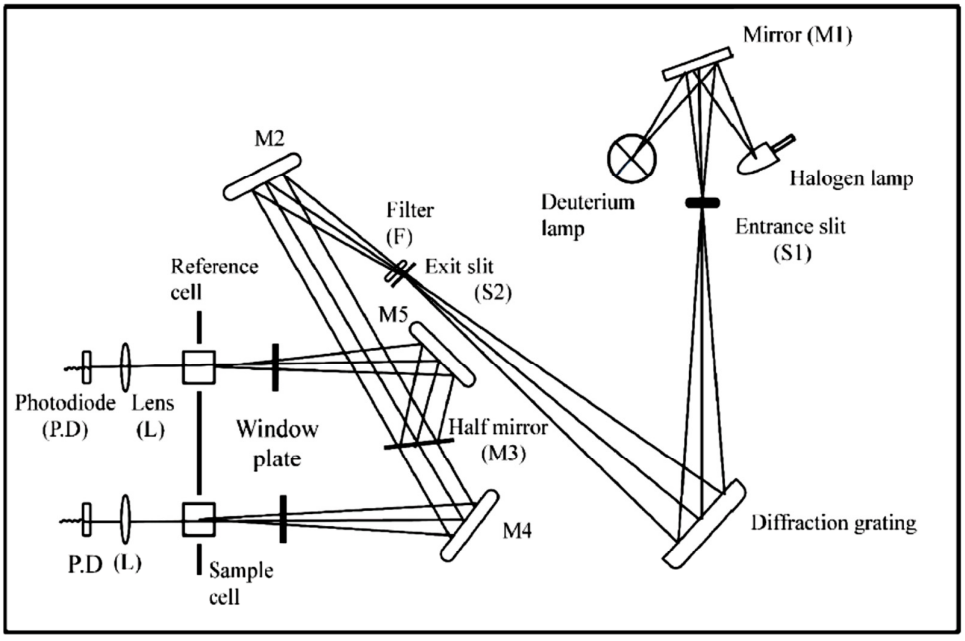

Fig.1.2. Representative Diagram of UV-Visible Spectrophotometer

- **Source:**

Deuterium and tungsten filament lamps usually serve as a source of visible light. This type of lamp is using in the range of wavelengths 350-1100 nm. The energy emitted by a tungsten filament lamp is relative to the four powers of the operative voltage. This means that for the energy output to be stable, the voltage to the lamp has to be very constant indeed. An electronic voltage regulator or constant-voltage transformer is used to ensure this stability.

- **Monochromator:**

The monochromator slit aperture is fixed at 2 nm and the diffraction grating includes a 900 line/mm aberration - a concave holographic grating. The light coming from the source to be reflected by the mirror M1 and then enters the monochromator. Light source switching is fully automatic, with the device selecting

the next light source according to the wavelength. The light as the monochromator passes through a filter F and strikes the mirror M2 and to be split with the quasi-mirror M3 into the sample side beam and the reference side beam, then passes through their respective cells and the detector (photodiodes). The image of the exhaust slit S2 is visible near position of cell in the sample compartment. When the sample side beam and reference side beam are picked up by the detectors and converted into voltage by the pre amplifier. The signal is then fed into the A/D converter and finally read by the CPU.

Conclusion:

This paper highlights the details of the various techniques used in the present study, including the preparation of pure and doped polymer films, characterization of polymer blends and doped films. Optical and structural studies of pure and dye doped polymers have been carried out using various experimental techniques such as FTIR, absorption spectroscopy (UV-visible and infra-red). FTIR measurements uniquely allow the simultaneous measurement of spectral changes resulting from product formation and substrate consumption. In order to capture these changes and make maximum use of the obtained information (including the detection of specific structural changes resulting from a particular enzymatic attack preference), as well as to handle the complexity of the data, multivariate regression analysis has to be used.

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