Experimental methods and characterization techniques

Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FT-IR), UV-Visible (UV-Vis) spectral techniques were used to characterize complexes of polyphenolic acids with Fe(III). The theoretical studies of the complex have been studied by Density Functional Theory (DFT).
2.1.0. Introduction

Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FT-IR), UV-Visible (UV-Vis) spectral technique were used to characterize complexes of polyphenolic acids with Fe(III). The theoretical study of the complex has been studied by Density Functional Theory (DFT).

2.2.0. Experimental

2.2.1 Chemicals and reagents

Syringic acid (3, 4-Dihydroxycinnamic acid) chlorogenic acid, caffeic acid (3,4-Dihydroxybenzeneacrylic acid), were purchased from Molekula Ltd., United Kingdom. 7-Hydroxy-4-methylcoumarin and 4-hydroxycoumarin were purchased from Himedia chemicals, India. Source of Fe(III) salt of ferric chloride was obtained from Fisher Scientific India. All the reagents and solvents were of analytical grade and chemically pure and were used as received.

2.2.2 Preparation of solutions

All experiment was performed in aqueous solution. Stock solution of Fe(III) was prepared in double distilled water (10 ppm) in 100 ml volumetric flask. Stock solution of syringic acid, caffeic acid and chlorogenic acid were prepared in double distilled water (10 ppm) at pH 9.0 and ethanol (10 ml) was added in their stock solutions for complete dissolution. Similarly stock solution of 7-Hydroxy-4-methylcoumarin and 4-hydroxycoumarin were also prepared in double distilled water at pH 11.0. When equimolar concentration of Fe(III) reacts with different polyphenolic acid, they formed coloured complex after the reaction. After 30 to 45 minutes, the precipitates of different polyphenol complex with iron were formed at room temperature. The precipitates of each complex were filtered and dried separately by rota vapour for further analysis.
Table 2.1. Data showing the polyphenolic acid complex colour formation with Fe(III)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Fe(III) + Polyphenols complex</th>
<th>Complex colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fe(III) + Syringic acid</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>2.</td>
<td>Fe(III) + Caffeic acid</td>
<td>Dark brown</td>
</tr>
<tr>
<td>3.</td>
<td>Fe(III) + Chlorogenic acid</td>
<td>Dark green</td>
</tr>
<tr>
<td>4.</td>
<td>Fe(III) + 4-Hydroxycoumarin</td>
<td>Dark green</td>
</tr>
<tr>
<td>5.</td>
<td>Fe(III) + 7-Hydroxy-4-methylcoumarin</td>
<td>Dark brown</td>
</tr>
</tbody>
</table>

2.3.0. Various characterization techniques

Synthesized complex was characterized by various characterization techniques given below.

2.3.1.0. Optical Characterization

2.3.1.1. UV-Visible spectroscopy

UV-Vis spectrophotometer uses visible light and ultraviolet to analyze the chemical structure of the substance. A spectrophotometer is a special type of spectrometer, which is used to measure the intensity of light, and the intensity is proportional to the wavelength. When the ultraviolet light project to various organic compounds, these compounds will absorb it. So, you can use UV-Vis spectrophotometer to measure the absorption of a compound by the result and have its molecular structure, as well as the related information.

2.3.1.2 Principle of UV-Visible spectroscopy

The spectrophotometer is a much more refined version of a colourimeter. In a colourimeter, filters are used which allow a broad range of wavelengths to pass through, whereas in the spectrophotometer a prism (or) grating is used to split the incident beam into different wavelengths. By suitable mechanisms, waves of specific wavelengths can be manipulated to fall on the test solution. The range of the wavelengths of the incident light can
be as low as 1 to 2 nm. The spectrophotometer is useful for measuring the absorption spectrum of a compound, that is, the absorption of light by a solution at each wavelength.

![Spectrophotometer Diagram](image)

Fig.2.1. Absorption of light by a sample in UV-Visible spectrophotometer

### 2.3.1.3 Quantitative relationships for optical spectroscopy

**Beer and Lambert laws**

\[ A = \varepsilon bc \]

\[ A = -\log T = \log \frac{I_0}{I} = \varepsilon bc \]

\[ T = \frac{I}{I_0} \]

(Where \( A \) = Absorbance, \( I_0 \) = intensity of incident light, \( I \) = Intensity of emitted light, \( \varepsilon \) = molar absorptivity coefficient, \( T \) = Transmittance, \( b \) = path length of sample, \( c \) = molar concentration of solute)

From the Beer-Lambert law, it is clear that greater the number of molecules capable of absorbing light of a given wavelength, the greater the extent of light absorption.
Experimental Methods and Characterization Techniques

2.3.1.4. Instrumentation and working of UV-Visible spectroscopy

Instrumentation and working of the UV spectrometers can be studied simultaneously. Most of the modern UV spectrometers consist of the following parts-

**Light source**

Tungsten filament lamps and Hydrogen-Deuterium lamps are most widely used and suitable light source as they cover the whole UV region. Tungsten filament lamps are rich in red radiations; more specifically they emit the radiations of 375 nm, while the intensity of Hydrogen-Deuterium lamps falls below 375 nm.

**Monochromator**

Monochromators generally composed of prisms and slits. The most of the spectrophotometers are double beam spectrophotometers. The radiation emitted from the primary source is dispersed with the help of rotating prisms. The various wavelengths of the light source which are separated by the prism are then selected by the slits such the rotation of the prism results in a series of continuously increasing wavelength to pass through the slits for recording purpose. The beam selected by the slit is monochromatic and further divided into two beams with the help of another prism.

**Sample and reference cells**

One of the two divided beams is passed through the sample solution and second beam is passed through the reference solution. Both sample and reference solution are contained in the cells. These cells are made of either silica or quartz. Glass can't be used for the cells as it also absorbs light in the UV region.

**Detector**

Generally two photocells serve the purpose of detector in UV spectroscopy. One of the photocell receives the beam from sample cell and second detector receives the beam from the reference. The intensity of the radiation from the reference cell is stronger than the beam of sample cell. This results in the generation of pulsating or alternating currents in the photocells.
Amplifier

The alternating current generated in the photocells is transferred to the amplifier. The amplifier is coupled to a small servometer. Generally, current generated in the photocells is of very low intensity, the main purpose of an amplifier is to amplify the signals many times so we can get clear and recordable signals.

Recording devices

Most of the time amplifier is coupled to a pen recorder which is connected to the computer. Computer stores all the data generated and produces the spectrum of the desired compound (Aman Thakur, 2011)

Fig. 2.2. Typical flow diagram of UV-Visible Spectrophotometer working

With the help of double beam spectrophotometer, the colourimetric study of the various polyphenolic acid complex with Fe(III) has been carried out (Perron N.R., 2010) and such representative data has been given in Table.

Fig. 2.3. UV-Visible spectrophotometer at DAC, BBA University, Lucknow, U.P.
### Experimental Methods and Characterization Techniques

#### Table 2.2. Data showing the $\lambda_{\text{max}}$ of different iron-polyphenol complexes

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Fe(III) + Polyphenols complex</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fe(III) + Syringic acid</td>
<td>292.5</td>
</tr>
<tr>
<td>2.</td>
<td>Fe(III) + Caffeic acid</td>
<td>321</td>
</tr>
<tr>
<td>3.</td>
<td>Fe(III) + Chlorogenic acid</td>
<td>323</td>
</tr>
<tr>
<td>4.</td>
<td>Fe(III) + 4-Hydroxy coumarine</td>
<td>280</td>
</tr>
<tr>
<td>5.</td>
<td>Fe(III) + 7-Hydroxy-4-methylcoumarin</td>
<td>318</td>
</tr>
</tbody>
</table>

#### 2.3.1.2 Fourier Transform Infrared (FT-IR) spectroscopy

Fourier transform Infrared Spectroscopy (FT-IR) is an extremely useful technique particularly for identifying to different types of chemical bonds in a molecule of unidentified materials [P. Pandey et. al., 2011]. FT-IR spectroscopy is a very powerful method for the identification of functional groups. In general, the goal of FT-IR spectroscopy is to measure how well a sample absorbs or transmits light at each different wavelength. To use the FT-IR, a continuum source of light is used to produce light over a broad range of infrared wavelengths. The principal experimental method in this work is FT-IR, which allows us to detect infrared (IR) absorption and reflection properties over a broad spectral region. IR spectroscopy also known as vibrational spectroscopy, which concerned with the study of absorption of IR radiation by a molecule, causes the molecular bonds to vibrate including vibrational transition in a molecule.

FT-IR is used with the basic goal of determining changes in the intensity of infrared light as it interacts with a material as a function of wavelength. Therefore, infrared spectroscopy can apply as a very powerful tool for qualitative identification of different functional group and chemical bonds in a different environment [B.C. Smith, 1996].

In FT-IR analysis there are three commonly examined pieces of data known as peak position, the peak width and the peak intensity. The peak position is probably the most commonly used for the identification of materials. These peaks are unique since, at characteristic frequencies, certain functional groups will display their own set of peaks. This is because infrared techniques measure the vibrational energies of the molecules. In order for a molecule or functional group to be IR active, the dipole moment of the molecule must change. When the desired sample for testing is opaque, transmission experiments are not
practical. To overcome this problem, reflection experiments in the FT-IR become more appropriate.

**Fig. 2.4. IR beam interaction in diffuse reflection mode of FT-IR**

When the infra-red beams enter in the sample as shown in Fig. 2.3(a), it can be reflected, transmitted or absorbed. The infra-red energy reflecting off the surface is typically lost. The infra-red beam that passes through a particle can either reflect off the next particle or to be transmitted through the next particle. Scattered infra-red energy is collected by a spherical mirror that is focused onto a detector. These are the basics of how the diffuse reflectance mode of the FT-IR works [Ronald A. Holser, 2012].

Diffuse reflectance mode of the FT-IR (DR-FT-IR) can be successfully used for studying the surface chemistry variation of materials. There have been few studies that have shown that the variation in infra-red absorption of materials can be correlated to their variation in free carrier densities on the material surface.

Absorption by free carriers on the surface in the space charge region may differ that in the bulk and need to be studied when dealing with adsorption of humidity species onto the surface of the material. Beer’s law shows that the absorbance can be directly related to the absorption coefficient using the relation given below:

\[ I = I_0 \exp (-kx) \]
In Eqn. 2.6, $x$ is the thickness of measured sample and $K$ represents the absorption coefficient of the sample. This is typically referred to as the broad background of the sample.

![Fig. 2.5. FTIR at USIC, BBA University, Lucknow, U.P., India](image)

Spectra of the sample prepared during the research period were recorded on KBr pellet using a Thermo Scientific (Nicole 6700) FT-IR spectrometer Fig. 2.3(b), in the wave number region of 400-4000 cm$^{-1}$. For recording the spectra, the sample pellets were prepared by mixing a small amount (2 mg) of the sample with 200 mg of KBr powder and was grinding until a homogenous mixture is formed. The mixtures were then taken in a hydraulic pressure die and pressed under high pressure to form pellets before recording the spectra.

### 2.3.2 Surface morphological characterization

#### 2.3.2.1 Scanning Electron Microscopy (SEM)

A scanning electron microscope (SEM) is a special type of electron microscope which produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that can be detected and give information about the sample’s surface topology and composition. The electrons beam is generally scanned in a raster scan pattern and the beam’s position is pooled with the detected signal to produce an image. SEM can achieve resolution better than 1 nm. The most common SEM mode for the detection of secondary electrons is emitted by atoms excited by the electron beam. The numbering of secondary electrons depends on the angle at which
beam meets the surface of the specimen, i.e. on specimen topography. By scanning the sample and collection the secondary electrons with the special detector, an image displaying the topography of the surface is created [Antonio da Costa, 2014]. The surface morphological studies of the different polyphenol complex were obtained in mesoporous in nature.

### 2.3.3.1.1. Principle and working of SEM

Different types of signals produced by an SEM include secondary electrons, back-scattered electrons (BSE), characteristic X-rays, cathode luminescence (light), specimen current and transmitted electrons. In all SEM, secondary electron detectors are standard equipment but it is rare that a single machine would have detectors for all possible signals, result from exchanges of the electron beam with atoms at or near the outer part (surface) of the sample. In the standard detection mode, SEM can produce very high-resolution images of a sample surface, revealing details less than 1 nm in size. SEM microscopes have a large depth of field yielding a characteristics three-dimensional appearance useful for understanding the surface structure of a sample. A wide range of magnification is possible, from about 10 times (about equivalent to that of a powerful hand-lens) to more than 5,00,000 times, about 250 times the magnification limit of the best light microscopes [C.W. Oatley et al., 1965].

BSE is the beam electrons that are reflected from the sample by elastic scattering. It is often used in analytical SEM along with the spectra made from the characteristics X-rays because the intensity of the BSE signal is strongly related to the atomic number (Z) of the specimen. BSE images can provide information about the distribution of different elements in the sample. For the same reason, BSE imaging can image colloidal gold immune-labels of 5 or 10 nm diameters, which would otherwise be difficult or impossible to detect in secondary electron images in biological specimens [K.C.A. Smith and C.W. Oatley, 1955]. Characteristics X-rays are emitted when the electron beam removes an inner shell electron from the sample, causing a higher-energy electron to fill the shell and release energy and used to identify the composition and quantify the abundance of elements in the sample. The ray diagram of a typical SEM with photograph has been represented by Fig. 2.4(a) and (b).
Fig. 2.6. (a) Schematic diagram of SEM (b) Photograph of SEM at USIC, BBAU, Lucknow, U.P., India

References


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