Fundamentals of Evaluation of dyestuff by analytical techniques

CHARACTERIZATION OF NATURAL DYES

Studies on the analysis/ identification of natural dyes started as early as nineteen hundred thirties [Pfister, 1935 and Knecht et al., 1893]. A French chemist Pfister used a micro chemical analysis in which he achieved the result by color reactions with different chemicals. Abraham et al., 1964 reported a method using infrared structural analysis. Many workers have used thin layer chromatography to identify natural dyes in textiles [Karbade and Agarwal, 1985

Various methods used in Identification

Chromatographic methods:

- Thin layer chromatography
- Column chromatography
- High pressure liquid chromatography
- Gas chromatography

Spectroscopic methods:

- UV-Vis spectrometry
- FT-IR spectrometry
- NMR spectrometry
- Mass spectrometry

Identification

UV– Visible spectroscopic studies were carried out by Schweppe, 1988. Identification of dyes in historic textiles using chromatographic and spectrophotometric methods as well as by using sensitive colours reaction was done by Schaffe. A non-destructive method was reported of faded dyes on textiles fibers through examination of their emission and excitation spectra. High performance liquid chromatography (HPLC) has been used [Walker et al., 1986] to identify synthetic as well as natural dyes.

Solubility Studies

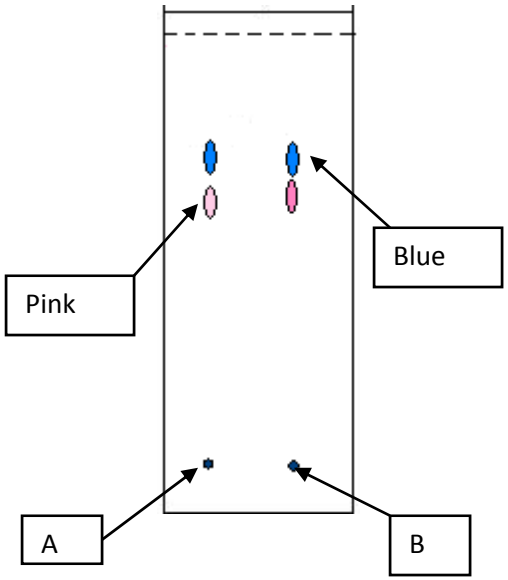
Various techniques that are used for characterization of natural dye are given below:

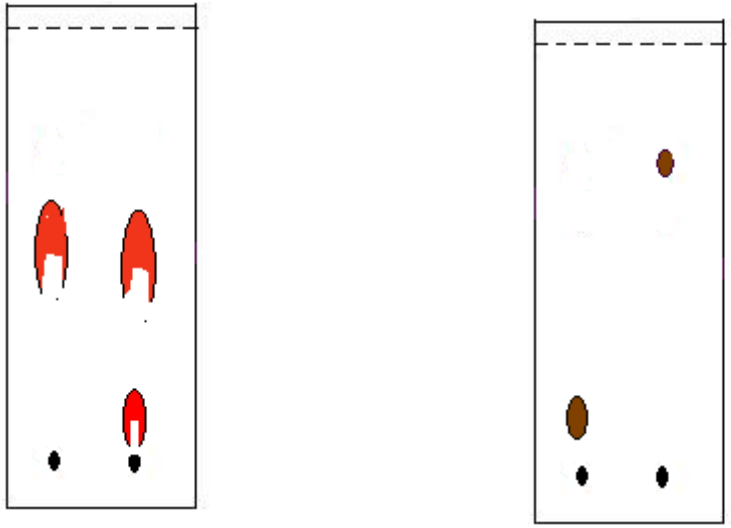
- Solubility of extracted dyes is determined in different solvents, extraction is done on the basis of the polarity e.g. water, ether, methanol, alcohol, acetone, ethyl acetate, dilute acid and alkali. The solubility is determined both at room temperature and higher temperature (50-60°C).

THIN LAYER & COLUMN CHROMATOGRAPHIC STUDIES

Thin layer chromatography is a versatile technique for identification of natural dyes T.L.C. studies carried out on dye extract using suitable eluent system for a specific dye. The spots are visualized in visible light as well as in iodine chamber. The possible constituents of the extracts are identified by comparing the TLC data i.e. color of the spot and R_f values of known compounds. Column chromatography is used to separate the colored components form single dye or mixture of dye after eluting with a suitable solvent. This is also used as clean–up procedure for the subsequent instrumental analysis.

Red spot in Natural indigo is darker but fades off after sometime about 10-15 mins (this is due to presence of very small quantities of iso-indigo, which is red in color) in the case of Natural indigo





Column chromatography:

- In case of column chromatography mixture of various components enters a chromatography process, and the different components are flushed through the system at different rates. These differential rates of migration as the mixture moves over adsorptive materials provide separation. Repeated sorption/desorption acts that take place during the movement of the sample over the stationary bed determine the rates. The smaller the affinity a molecule has for the stationary phase, the shorter the time spent in a column.

- In the analysis of natural products column chromatography is especially advantageous as to purify individual chemical compound from mixture of compounds. Two methods are generally used to prepare a column; the dry method, and the wet method. In most applications the stationary phase is either silica (SiO_2) or alumina (Al_2O_3), which is mixed with the solvent being used as the mobile phase to yield thick white slurry. The mobile phase is a liquid that is chosen to maximize the separation of the sample. This can be water or any organic solvent. Different other chromatographic techniques were also used in colorant identification and isolation from the plant species described.

ULTRA VIOLET-VISIBLE SPECTROPHOTOMETRIC STUDIES

The dye is dissolved in a suitable solvent system and scanned through UV-Visible spectrophotometer. Identification of the dye by this method involves as empirical comparison of the details of the spectrum, i.e. maxima and minima point of the unknown with those of pure compounds. A close match is considered to be good evidence of the chemical identity, particularly if the spectrum contains a number of short and well-defined peaks.

Principle of UV

- When a material is illuminated by light, specific wavelengths are absorbed depending on the molecular structure present. Electrons in the ground state molecule absorbing light energy and moving to an excited state cause this. The absorption intensity depends on the wavelength and the absorption spectrum (curve measuring absorption intensity changes accompanying wavelength changes for monochromatic light illuminating a material) is characteristic of a specific material. Analysis of materials based on this principle is called absorptiometry. This analysis can be used for various purposes such as:

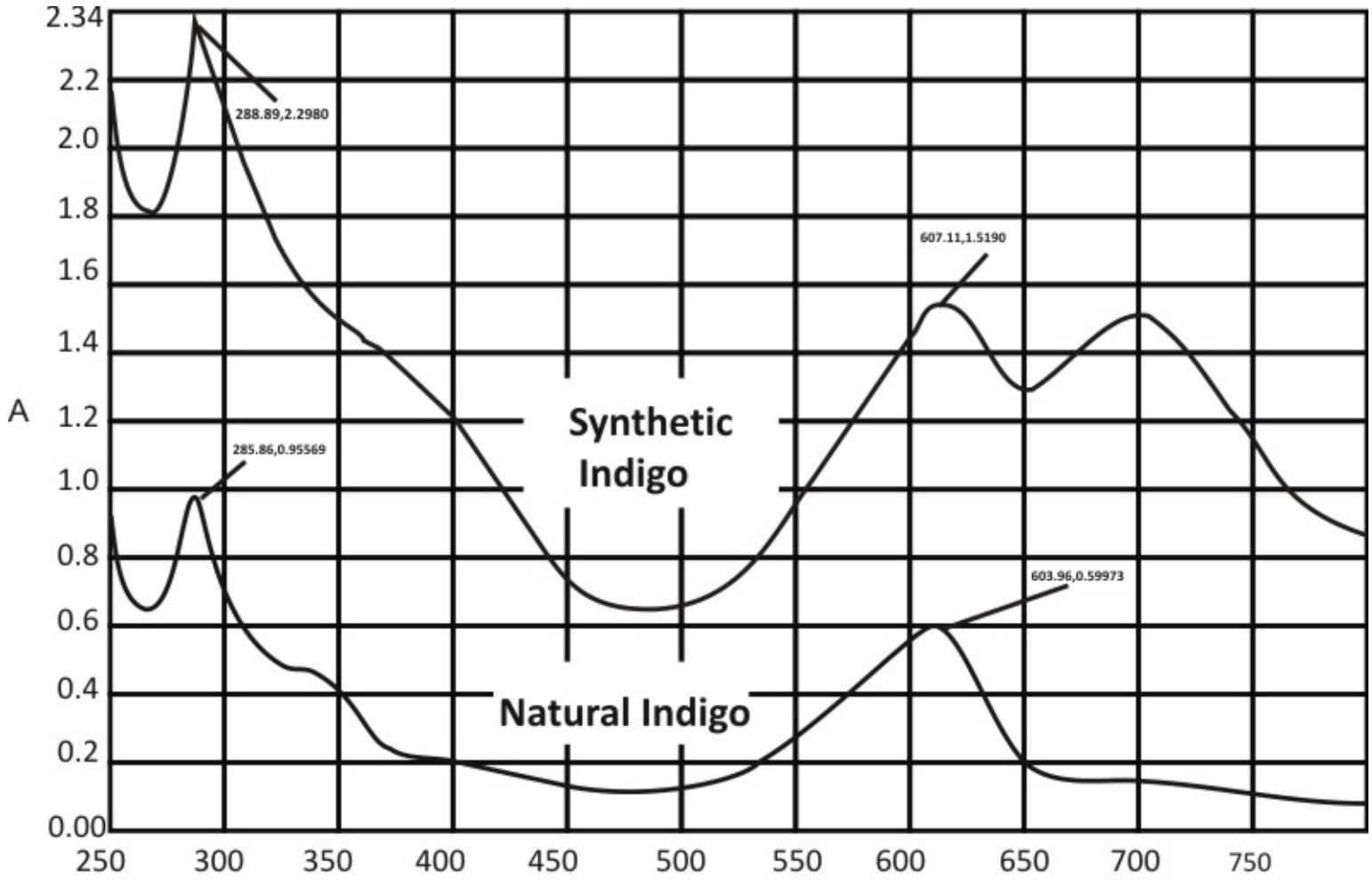
- -Identification

- -Quantitative analysis

Electronic state analysis

In addition, molecules absorbing light and entering the excited state later lose energy, through thermal dissipation, collisions with other molecules or other processes and return to the ground state. These processes are called radiation less transitions and may include the emission of the absorbed light energy in the form of light. Such re-emission processes include florescence and phosphorescence. Analysis using these phenomena is called fluorescence photometry.

A UV-Visible spectrophotometer of make Thermo, HeλIOS α



Comparison of Synthetic Indigo and Natural Indigo

FOURIER TRANSFORM – INFRA-RED STUDIES

Functional groups were identified by the FT-IR of purified dye extract. Major peaks were identified for different types of C-C, C-H, C-O stretching and bending vibrations. Absorption in the infrared region is due to molecular vibration of one kind or another; the spectrum is generally very complicated and contains many absorption peaks.

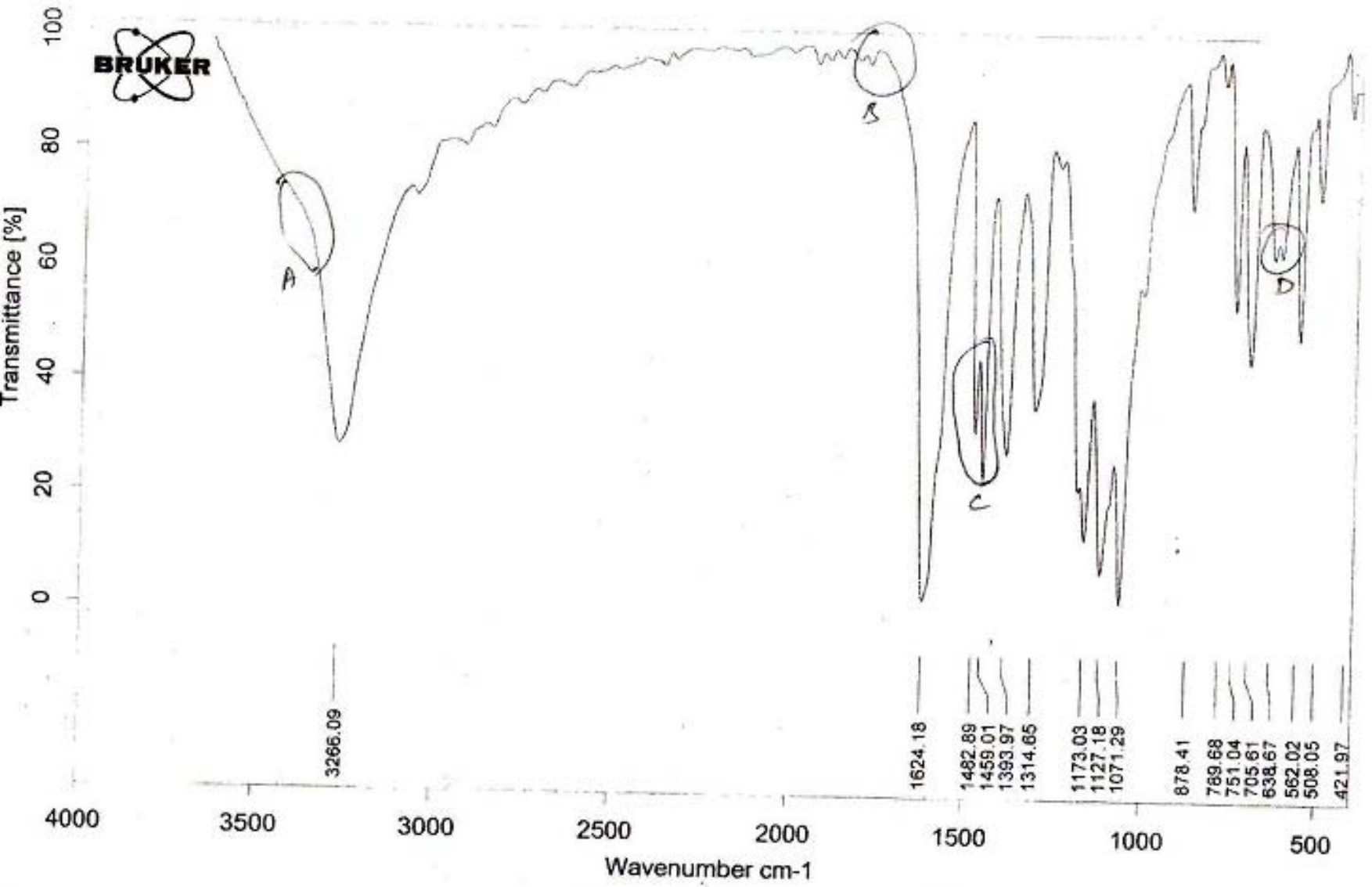
IR absorption spectroscopy

The IR absorption spectroscopy *(Bruker, Vertex 70)* is based on the absorption of infra red radiation by molecules and is most widely used for the identification of the organic compounds. The atoms in molecules vibrate constantly in a variety of stretching and bending motions. The different types of motion are called vibrational modes. Atoms that are connected by covalent bonds can stretch or bend at natural resonance frequencies, which depend on the strength or stiffness of the bonds.

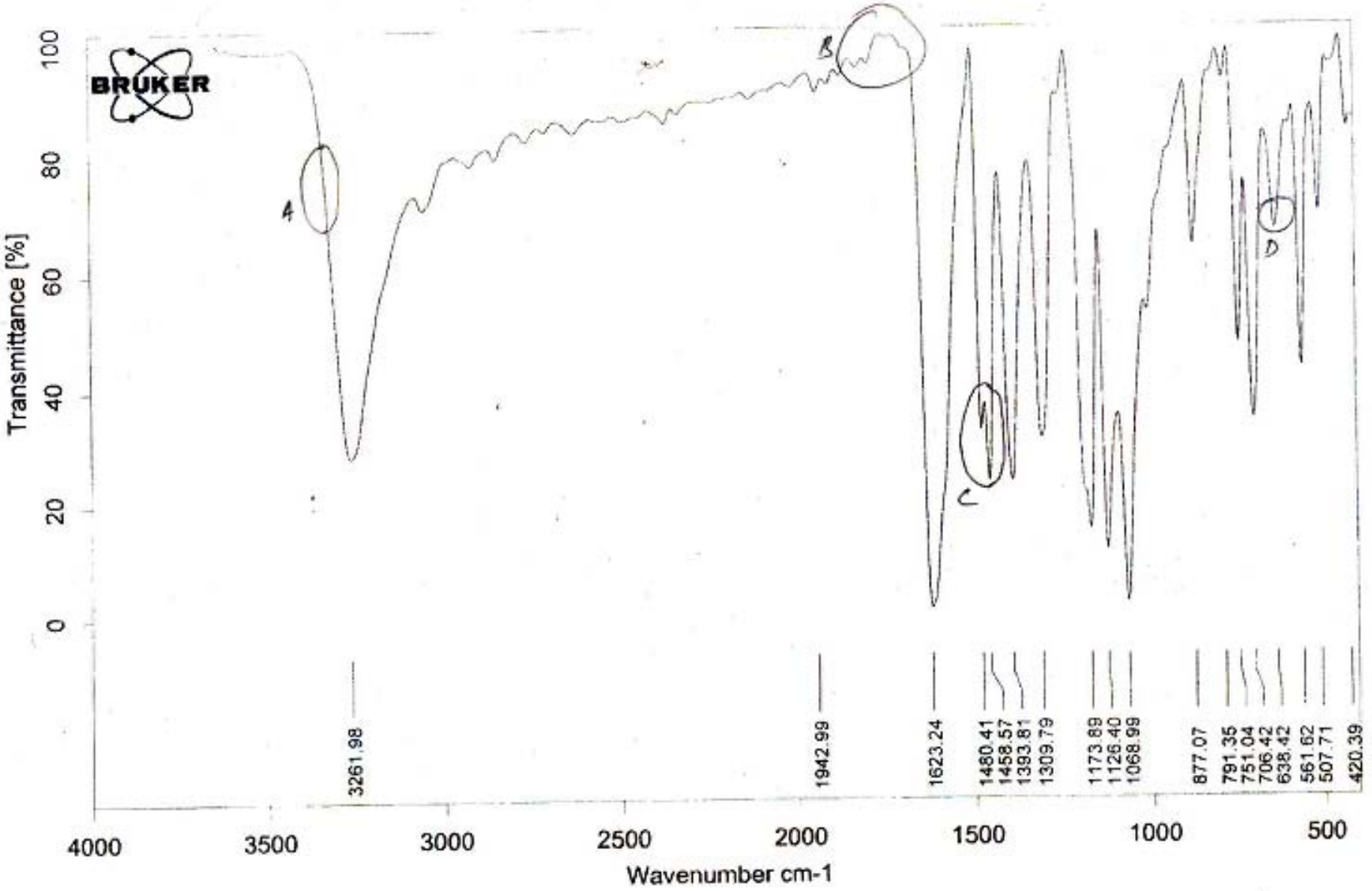
- C - C C = C C º C

  1200cm-1 1650cm-1 2150cm-1

- The double and triple bonds are stronger than a single bond and have correspondingly higher energies of vibration. Similarly, stretching modes have higher energies than bending modes for the same atoms. These vibrational modes can be excited to higher energy states, which cause the atoms to vibrate with greater amplitude that is a greater displacement from their average position. Vibrations can be excited by increasing the temperature or by absorption of photons of the appropriate energy. The energies of the vibrational modes are quantized, can be excited only with discrete amounts of energy. A photon that has the same energy as vibration is said to be in resonance with that vibration and can be absorbed.



IR Spectrum of Natural Indigo

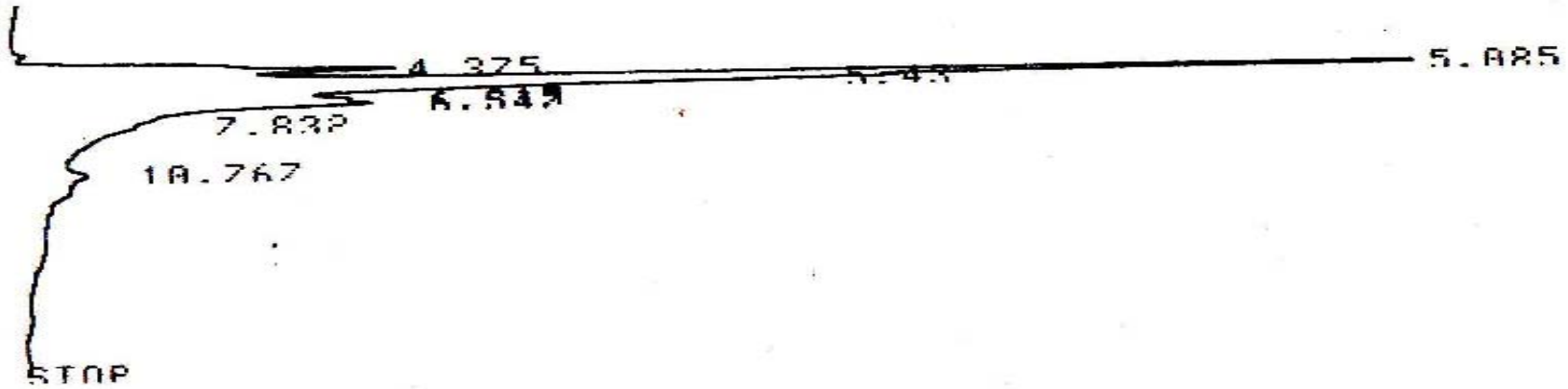


IR Spectrum of Synthetic Indigo

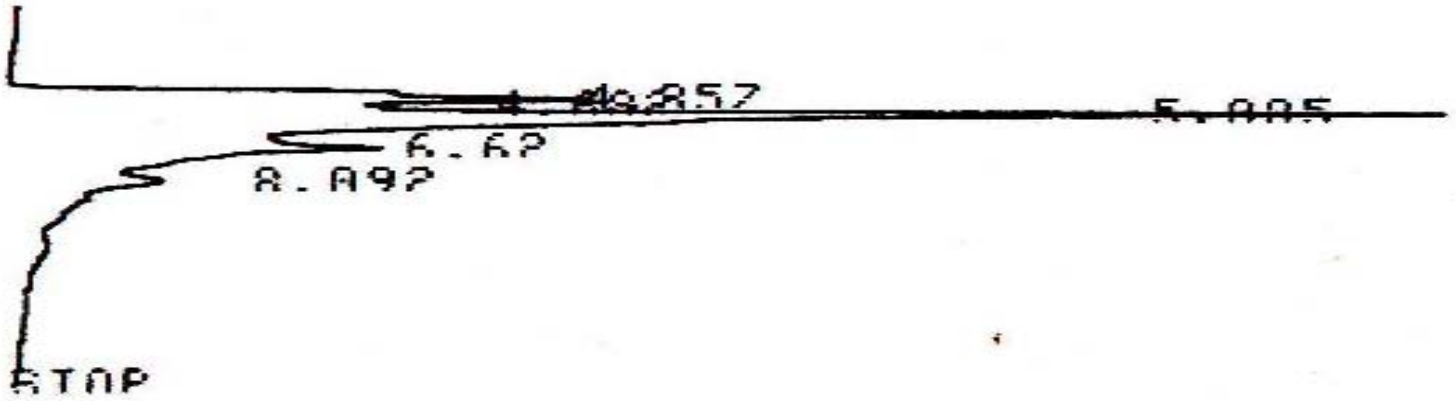
HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC STUDIES

- The applicability of high performance liquid chromatography (HPLC) to analyze ancient textile dyes was first successfully demonstrated [Wouters, 1985]. HPLC linear gradient elution method was first described for the analysis of Indigoid dyes [Wouters and Verhockem, 1991]. Identification of blue and purple indigoid dyes was also described using HPLC techniques [Wouters, 1991]. Analysis of manjistha, alizarin, turmeric sandalwood etc. were carried using HPLC techniques [Bhattacharya, 1999].

Chromatogram of Synthetic Indigo



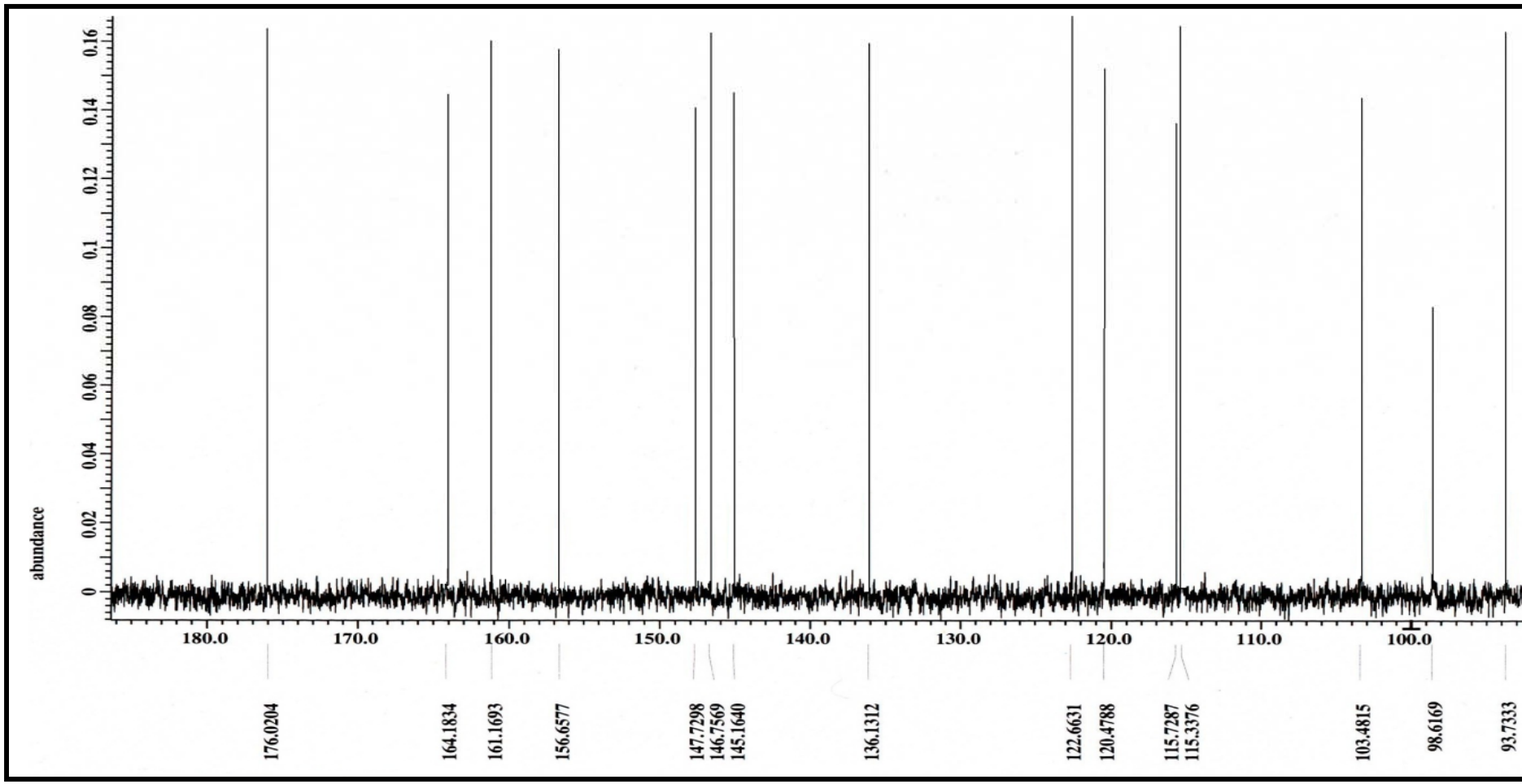
Chromatogram of Natural Indigo



GAS CHROMATOGRAPHY-- MASS SPECTROPHOTOMETRIC STUDIES

Gas chromatography with mass spectrophotometer (GC-MS) is an important detection method for natural products providing chemical fingerprints from the peaks. Electron impact source (EI) and automated library searching makes chemical identification easy. The gas chromatograph serves a method to separate a mixture so that they enter the mass detector one at a time for identification.

Quercetin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0.04
0.06
0.08
0.1
0.12
0.14
0.16
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
176.0204
164.1834
161.1693
156.6577
147.7298
146.7569
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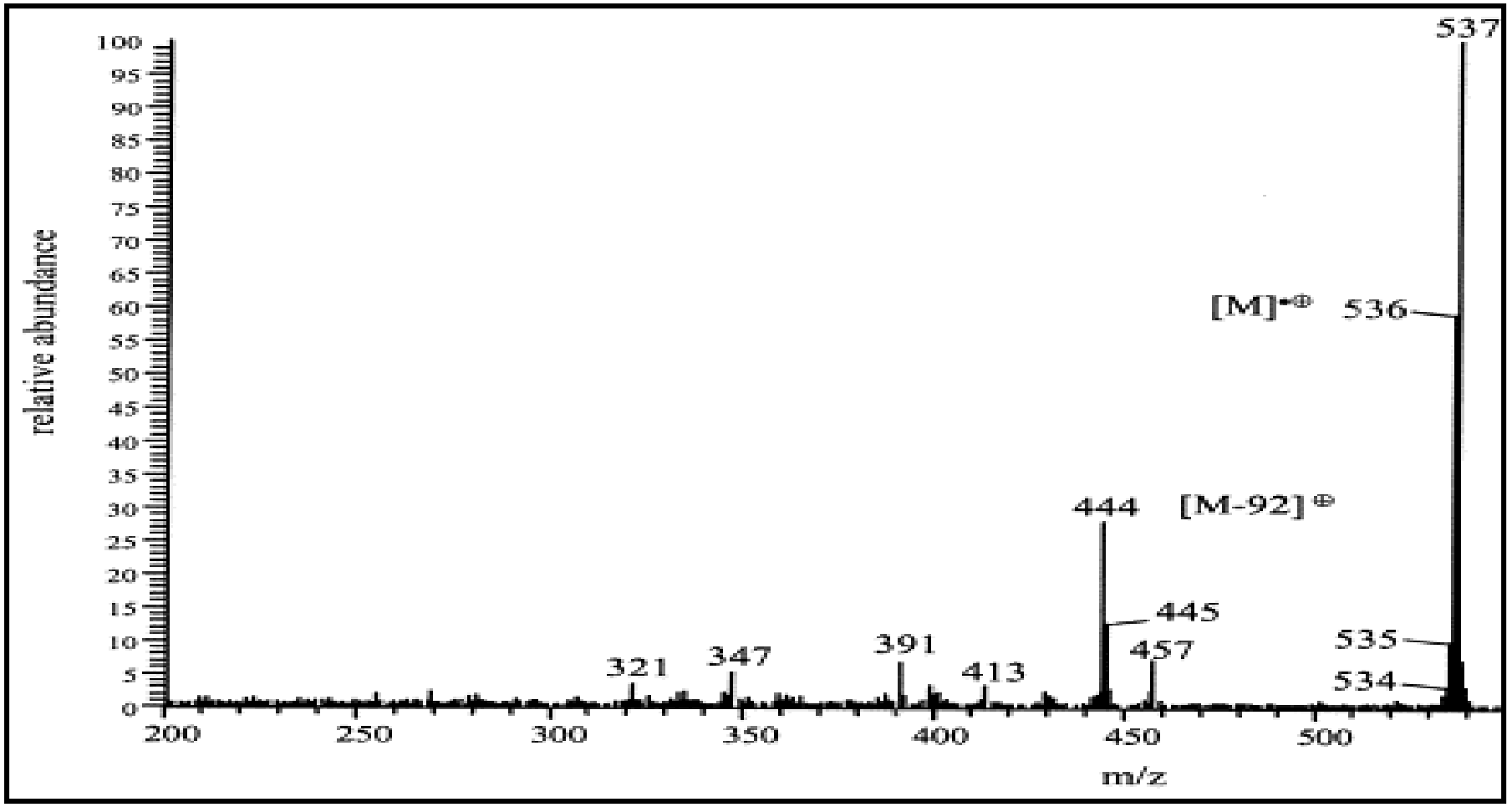
120.4788
115.7287
115.3376
103.4815
98.6169
93.7333

Lycopene


relative abundance
m/z
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[M]•⊕
536
444
[M-92]⊕
445
457
535
534
321
347
391
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100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
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15
10
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200
250
300
350
400
450
500

Nuclear Magnetic Resonance Spectrometry

NMR is the preeminent technique for determining the structure of organic compounds. It is the only one method for which a complete analysis and interpretation of the entire spectrum is normally expected, of all the spectroscopic methods. This technique relies on the ability of atomic nuclei to behave like a small magnet and align themselves with an external magnetic field. When irradiated with a radio frequency signal the nuclei in a molecule can change from being aligned with the magnetic field to identify and/or elucidate detailed structural information about chemical compounds. NMR can differentiate between structural isomers, and provide information about connectivity between atoms within a molecule.

Mass Spectrometry

In order to measure the characteristics of individual molecules, a mass spectrometer converts them to ions so that they can be moved about and manipulated by external electric and magnetic fields. Mass spectrometry is used to identify unknown compounds and quantify known compounds. It is sensitive and selective and is commonly used in combination with a separation technique, such as gas or liquid chromatography, to analyze complex mixtures. A mass spectrometer determines the mass of a molecule by measuring the mass-to-charge ratio (m/z) of its ion. Ions are generated by inducing either the loss or gain of a charge from a neutral species. Once formed, ions are electrostatically directed into a mass analyzer where they are separated according to m/z and finally detected. The result of molecular ionization, ion separation, and ion detection is a spectrum that can provide molecular mass and even structural information.