



## UV SPECTROPHOTOMETER: PRACTICAL CONSIDERATIONS

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### ABSTRACT:

This article deals with UV Spectroscopy its application (practical) and its mode. There are various modes of UV spectrophotometer namely photometric, spectrum, quantitation, kinetics, time scan, multi-component and bio-method. Photometric measures absorbance or % transmittance of a sample at arbitrary wavelengths. Spectrum Scans a wavelength range to measure the absorbance and % transmittance of a sample as a function of wavelength. Single beam energy measurement can also be performed. Data processing such as peak detection, smoothing, and mathematical calculation may be applied to the measured spectrum. Quantitation creates a calibration curve from a standard sample and quantitates an unknown sample. Kinetics Calculates enzyme activity from the time dependent change in absorbance. Time scan This function is used to measure the change in the rate of absorbance, transmittance, or energy in the fixed wavelength. Multi-component Enables samples with up to 8 constituent components to be measured and quantitated. In the Bio-method Mode, DNA Quantitation and Protein measurement are done. These modes are used for different analysis.

### Introduction

UV Spectroscopy is the measurement of the attenuation of a beam of light after it passes it through a sample or after reflection from a sample surface. Absorption measurements can be at a single wavelength or over an extended spectral range.

The region beyond red is infra-red whereas region beyond violet is ultra-violet. The wavelength range of UV radiation starts at blue end of visible light (4000 Å) and ends at 2000 Å<sup>1</sup>.

There are various modes of UV Spectrophotometer for the analysis of sample. The following are:

1. Photometric
2. Spectrum
3. Quantitation
4. Kinetics
5. Time Scan
6. Multi-component
7. Bio-method

### PHOTOMETRIC

Measures the absorbance or transmittance at a single wavelength or at multiple (up to 8) wavelengths<sup>2</sup>.

### SPECTRUM

The spectrum mode is used to measure spectra. Measure the absorbance and transmittance spectra of the sample by performing the wavelength scan. Changes in the sample can be tracked using repeated scans<sup>2</sup>. This mode also allows you to measure the energy of a light source using its single beam.

#### Application 1

Identification of compounds.

UV spectroscopy can characterize those types of compounds which absorb UV radiation. It is done by comparing the absorption spectrum with the spectra of known compounds<sup>15</sup>.

## QUANTITATION

The quantitation mode is used to quantify an unknown sample by creating a calibration curve from the standard sample. Various combinations of wavelength and calibration curve factor and first-to-third order regression calibration are possible. The following four types of measurement methods are available according to the number of wavelengths used:

- One-wavelength method: The sample is quantified using its absorbance at a single wavelength. The operation procedures in this chapter mainly describe this method.
- Two-wavelength method: The absorbance at a wavelength other than the quantitation wavelength is used to eliminate the interfering components and contaminants.
- Three-wavelength method: The absorbance of two wavelengths is used to eliminate the interfering components.
- Derivative quantitation: The derivative value (1st through 4th orders) for the spectrum at the quantitation wavelength is used.

The calibration curve is created using one of the following three methods<sup>3</sup>:

- K factor method: In the equation, "concentration = K x absorbance + B", K and B are predetermined.
- Single-point calibration curve method: One standard sample is measured to create the calibration curve.
- Multi-point calibration curve method: Multiple standard samples (10 maximum) are measured to create the calibration curve.

If the measurement is repeated, the measurement results may be displayed as a list. A single list can include a maximum of 200 measurement results<sup>1</sup>.

EXAMPLE: The calibration curve of Ezetimibe is plotted through photometric mode<sup>4</sup>.

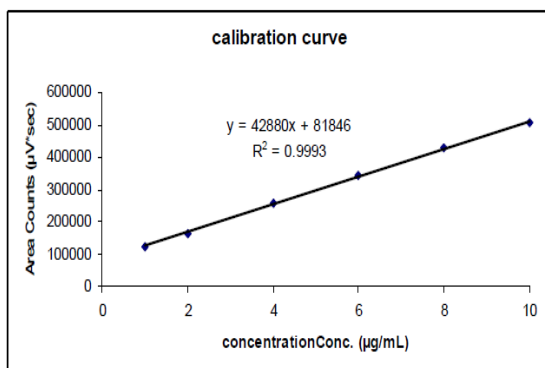


Figure 1: calibration curve of ezetimibe

## KINETICS

The Kinetics mode allows you to measure the changes in the absorbance (Abs), which occur over time from the enzyme reaction, and to obtain the activity value of the enzyme from that measurement result as shown in fig 3<sup>2</sup>.

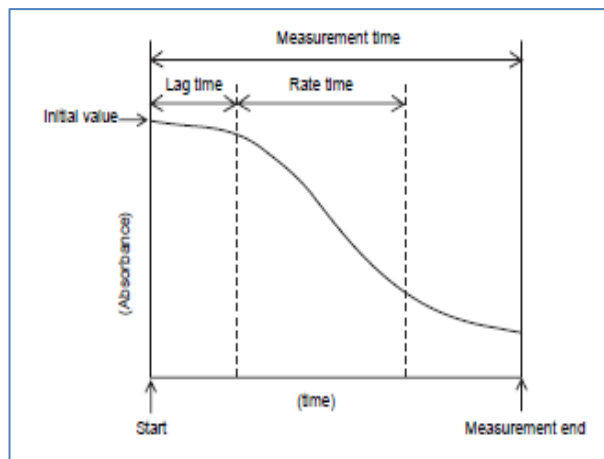


Figure 2: lag time and rate time

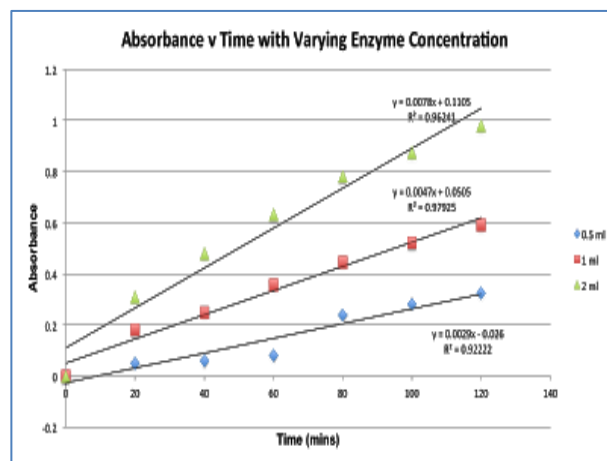


Figure 3: enzyme kinetic reaction<sup>5</sup>

### Kinetic Rate

The kinetics rate measurement mode is used to measure the change in the rate of absorbance for one wavelength and obtain the change in quantity of absorbance per rate interval. A discriminant is used to determine whether the absorbance is being changed linearly and to display the linearity (L) or the non-linearity (N). The discriminant is as follows. Linearity (L) is determined by whether the ratio of the rate of change of absorbance during one cycle and the rate of change of one cycle before are within the proportion (%) set in the criteria<sup>2</sup>.

### TIME-SCAN

The time scan mode is used to measure the change in the rate of absorbance (Abs), transmittance (%T), or energy (E) in the specified arbitrary wavelength<sup>6</sup>.

Application 1: Time scan graph of hydrogen peroxide.

In this study hydrogen peroxide was kept at 50°C for 10 minutes after that it runs for 150 seconds. As time passes, there is decrease in absorbance which tells that at high temperature the absorbance of hydrogen peroxide increase<sup>7</sup>.

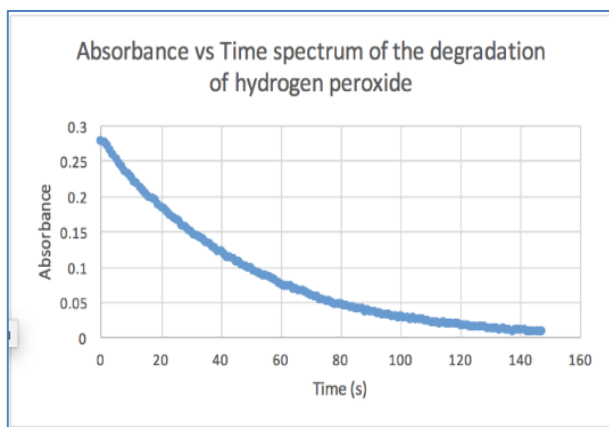


Figure 4: time scan graph of hydrogen peroxide

### MULTI-COMPONENT

The multi-component quantitation mode is the mode in which the concentration of each constituent component is determined by using the absorption spectrum of the mixed sample with pure standards or standards made up of multiple constituent components<sup>8</sup>.

- 1) Mixed samples with up to 8 constituent components can be quantitated.
- 2) In addition to using pure samples of each constituent component as the standard samples, a mixed sample in which the concentration of each constituent component is known may also be used. The effects of interference among the various constituent components can be minimized by using a mixed sample as the standard sample.
- 3) Parameter files for this mode store measurement parameters and standard sample data, making their file size relatively large. Therefore, they can only be saved by using a USB memory device.

In addition, a spectrum saved in memory can be loaded and used as the standard sample or unknown sample data. However, unlike other parameter files, these can only be saved one at a time.

- 4) The measurement wavelengths can be set at uniform intervals or randomly set<sup>9</sup>.

### BIO-METHOD

The Bio-method mode allows you to obtain the DNA and protein concentrations with various quantitation methods<sup>2</sup>.

### APPLICATIONS

1. Concentration of NaCl

Different concentration of NaCl were prepared and the spectrum was run between 400-200nm<sup>10</sup>.

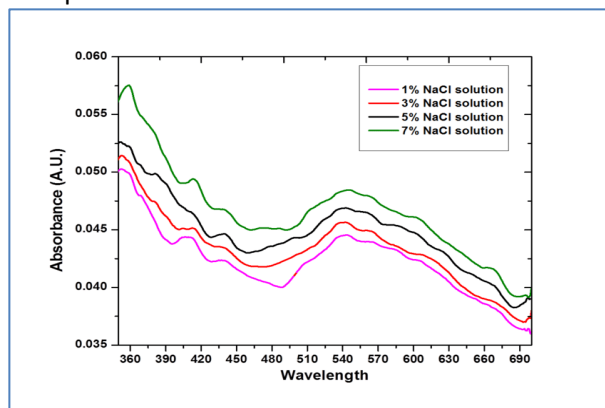


Figure 5: Different concentration of NaCl

2. Identification of compounds.

UV spectroscopy can characterize those types of compounds which absorb UV radiation. It is done by comparing the absorption spectrum with the spectra of known compounds. 10ppm Ibuprofen solution was prepared in hexane, ethyl acetate and methanol and spectra were taken<sup>11</sup>.

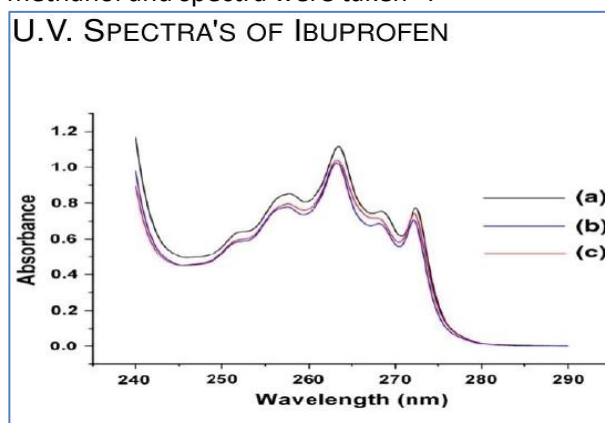


Figure 6: Identification of Ibuprofen

3. Determination of total organic carbon  
 The sample is oxidised to produce carbon dioxide which is then measured by a detection system. Aquatic humic water divided into two portions and kept at ph ≤ 2 and other at ph between 5-7and sit

capped for 30 minutes in cold place and 40µL HCl, H<sub>2</sub>SO<sub>4</sub> were added and spectrum was run between 400-200nm<sup>12</sup>.

**17.2.1 TOC Instrument 1: UV/persulfate wet oxidation with permeation/conductivity detection**

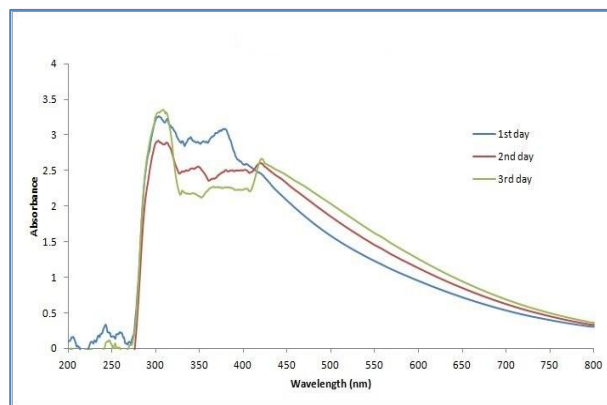
Dissolved Organic Carbon, mg/L <sup>a</sup>				
Source Water	Unfortified Sample Conc.		Fortified Sample Conc.	
	Mean	%RSD	Mean	%REC
Boulder Creek	1.63	1.62	12.2	105
Shingobee R.	2.98	0.19	13.5	105
Bolten Well	1.27	0.00	12.0	107
Ohio R. (Fernbank)	2.79	0.36	13.6	108
Muddy Creek	3.81	0.15	14.6	108
Great Miami R.	3.18	0.00	13.7	104
Saint Leon Well	0.53	0.97	11.0	104
Total Organic Carbon, mg/L <sup>a</sup>				
Source Water	Unfortified Sample Conc.		Fortified Sample Conc.	
	Mean	%RSD	Mean	%REC
Boulder Creek	1.73	0.33	12.1	103
Shingobee R.	3.16	0.18	13.0	98
Bolten Well	1.32	0.44	11.4	100
Ohio R. (Fernbank)	3.02	0.57	13.2	102
Muddy Creek	4.24	0.00	14.6	103
Great Miami R.	3.51	0.33	13.8	102
Saint Leon Well	0.66	0.52	11.1	104

<sup>a</sup> N = 3, samples fortified at 10mg/L OC using KHP

**Figure 7: Total Organic Carbon content**

4. Change in absorbance with time

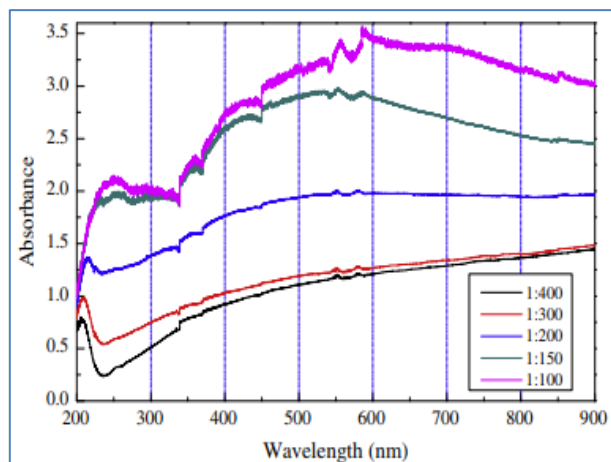
Silver nanoparticle were kept for three days and change in absorbance were observed. culture of endophytes in 250 mL flask using LB media for 48 hrs at 37° C, centrifuge at 10,000 rpm for 10 min, AgNO<sub>3</sub> solution (1 mM) added into 100 mL filtrate (1:1, v/v), addition of PVP (5 mL, 1% w/v),Incubation at room temperature in an incubator shaker (180 rpm). UV-Vis reading (200-800 nm) at 24 hrs interval<sup>13</sup>.



**Figure 8: change in absorbance with time**

5. Absorption performance on emulsified asphalt with UV-Vis spectrophotometer

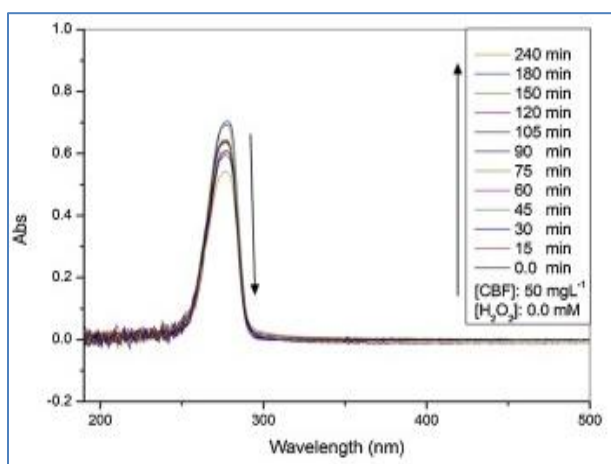
Emulsified asphalt mixed with cement and different concentrations were prepared with cement and emulsified asphalt. Water is used as solvent as it acts as binder for them<sup>14</sup>.



**Figure 9: UV-Vis scanning spectra of emulsified asphalt**

6. carbofuran in aqueous solution by using UV-irradiation/hydrogen peroxide

50 mgL<sup>-1</sup> aqueous solution of carbofuran were exposed to UV light with different irradiation time (15–240 min) without hydrogen peroxide. The experiments were performed with addition of hydrogen peroxide 4.8 ± 0.1 mM<sup>15</sup>.

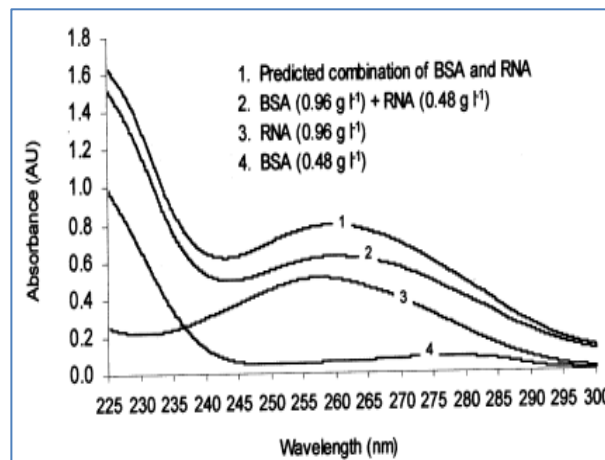


**Figure 10: Absorption spectra of Carbofuran solutions as a function of UV irradiation time, [CBF]: 50 mgL<sup>-1</sup>, [H<sub>2</sub>O<sub>2</sub>]: 4.8 ± 0.1 mM.**

7. Monitoring of Bio-processes

Protein standards were made up using BSA and phosphate buffer, in the range 0.0 to 1.0 g l<sup>-1</sup> at

0.1 g l<sup>-1</sup> intervals. A 0.1 ml sample (i.e. calibration standards and actual samples) was pipetted into a 4.0 ml plastic cuvette, and then 3.0 ml dye reagent added. The solution was left for 10 to 15 min and then the absorbance measured at 595 nm<sup>16</sup>.



8. DNA extracted from human saliva

0.4 ml of DNA extraction buffer was added to tubes containing 0.5 ml of saliva sample. e. 0.02 ml Protease K was added to all the tubes to digest the cellular material and proteins. Centrifuge tubes were placed at 50–60 C in an incubator for 3 h for fresh samples and for dry samples kept at 50–60 C in an incubator overnight. To collect the DNA the tube was centrifuged at 14,000-g for 10 min. 0.2 ml of DNA wash was added to the pellet and centrifuged at 10,000-g for 10 min. 50 μl of TE buffer was added to the pellet, incubated at room temperature for 10–15 min and then stored in a refrigerator at 20 C. Quantity of DNA was calculated by taking the absorbance of samples at 260 nm. Concentration of DNA calculated from absorbances for fresh samples ranged from 125 to 795 μg/ml and for dry samples ranged from 15 to 85 μg/ml<sup>17</sup>.

9. Para-Nitrophenol on Raney Alloy

Stock concentration of para nitrophenol (PNP) 272 ppm was prepared in aqueous solution. For the sonication process, the reactor cell volume was kept at 5 ml. 50%Ni–50%Al raney alloy (100 mg) which was suspended in 3.62 mL of deionized water and sonicated for 5 minutes. 40ppm, 50ppm and 75ppm PNP solution was prepared and stirred for 15-30-45 minutes at 30 °C and centrifuged for 3 minutes at 14500 rpm. The spectra was run between 4500-350 cm<sup>-1</sup><sup>18</sup>.

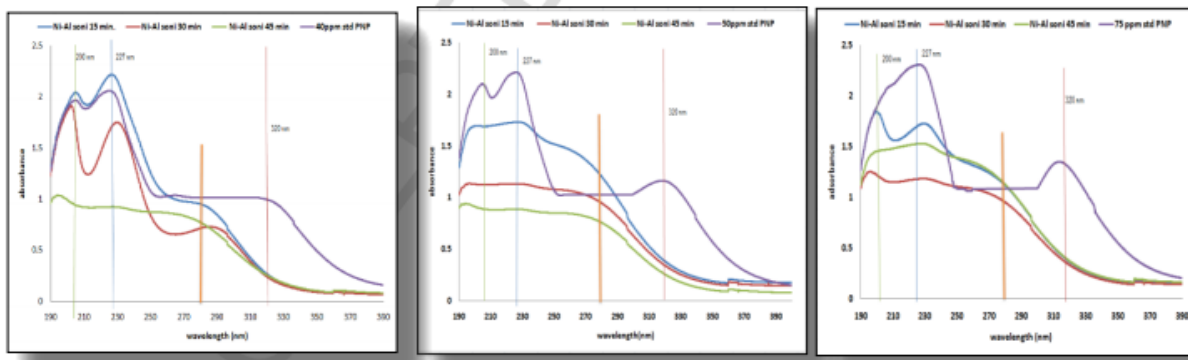


Figure 11: UV-visible spectra of PNP and 50%Ni-50%Al rane alloy/ PNP (sample alloy)

10. Carboxyterfenadine antacid interaction

10 ml of 1 mMole fexofenadine to the dissolution medium at zero time while after 15 min before collecting the sample, 2 gm of aluminium hydroxide (antacid) was added<sup>19</sup>.

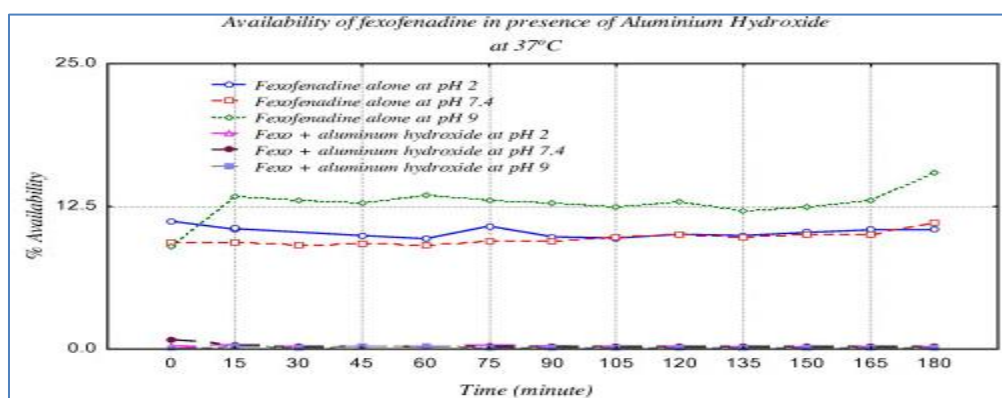


Figure 12: Availability of fexofenadine in presence of aluminium hydroxide at 37 °C.

11. Effect of Chromium on two Lewatit-anion exchange resins

Contact time: Initial concentration of Cr (VI),  $1 \times 10^{-3}$  M; amount of resin, 0.04 g; volume of adsorption medium, 25 ml; temperature,  $25 \pm 1$  °C; stirring rate 180 rpm; initial pH, 5.0.

pH: Initial concentration of Cr (VI),  $1 \times 10^{-3}$  M; amount of resin, 0.04 g; volume of adsorption medium, 25 ml; temperature,  $25 \pm 1$  °C; stirring rate 180 rpm; stirring time, 50 min<sup>20</sup>.

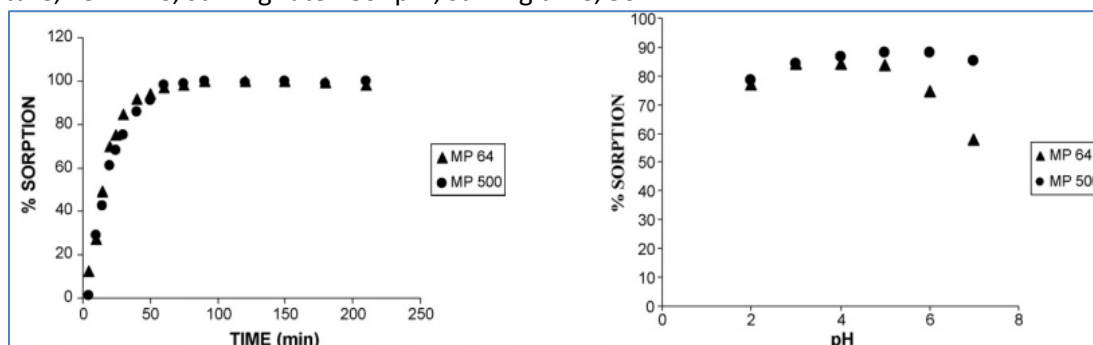


Figure 13: Effect of contact time and pH on the sorption of Chromium by Lewatit MP 64 and Lewatit MP 500

12. UV spectra of Aldehydes

10ppm solution of trans-2-pentenal, trans-2-hexenal and 2-methyl-2-pentenal were prepared and spectra was run between 400-240nm<sup>21</sup>.

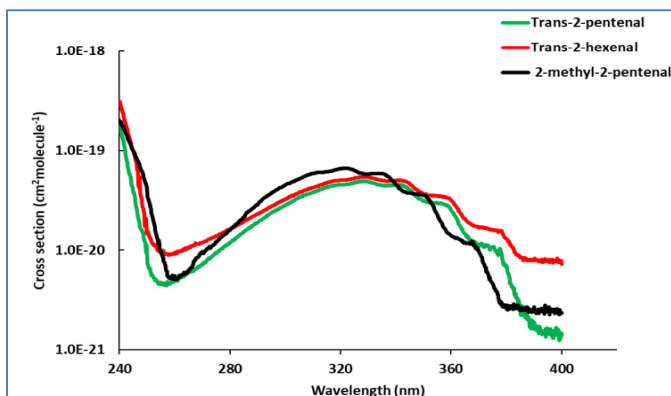


Figure 14: UV absorption spectra of t-2P, t-2H and 2M2P in the domain 240-400 nm

13. Caffeine in coffee beans

The solution was stirred for one hour using magnetic stirrer and heated gently to remove caffeine and filtered by a glass filter<sup>22</sup>.

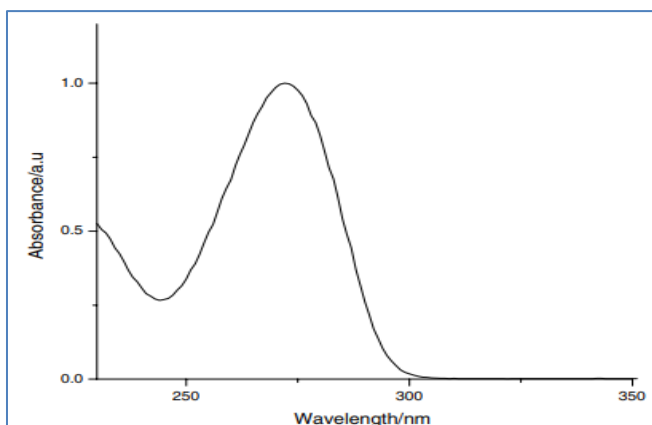


Figure 15: UV-vis spectra of caffeine in water

14. Quantitative estimation of Metronidazole and furazolidone

Metronidazole and Furazolidone were scanned in the spectrum mode over the UV range 200–400 and a mixture of 2 M sodium acetate and 8 M urea (50:50% V/V) solution<sup>23</sup>.

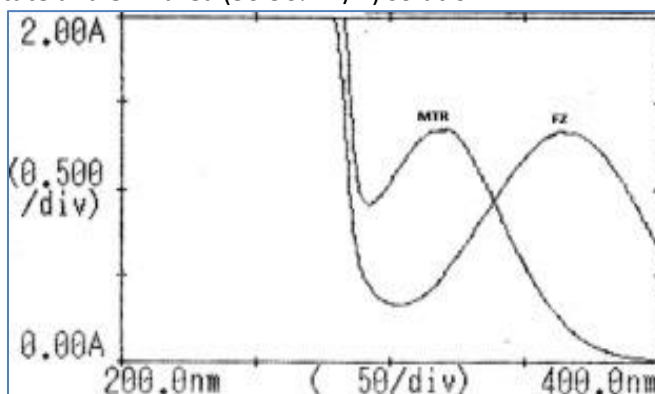


Figure 16: Overlay Spectra of MTR 50 µg/ml and FZ 25 µg/ml.

15. Scattering coefficient of nanostructured glass-ceramics.

Three samples of glass ceramic were prepared with thicknesses  $h_1 = 0.27$  mm,  $h_2 = 0.77$  mm and  $h_3 = 3.02$ mm and optical density  $D_m$  as a function of wavelength  $\lambda$  was measured<sup>24</sup>.

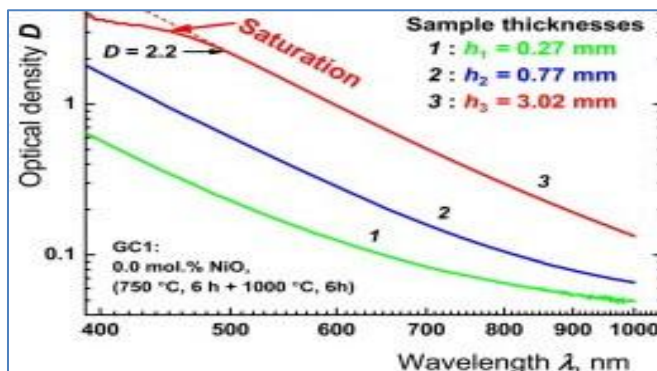


Figure 17: Log-log plots of the measured optical density  $D_m$  spectra for three samples of the GC

16. Neutron fluence with CR-39 using a UV spectrophotometer

The CR-39 (C<sub>12</sub>H<sub>18</sub>O<sub>7</sub>) detectors of (2 × 2) cm<sup>2</sup> size were cut from a sheet of 0.1 cm thickness. These detectors were then exposed to neutrons from two different sources. Each of the detectors was etched in successive time intervals in an aqueous solution of 6 M NaOH at (70 ± 1) °C. The detectors were studied with the help of the spectrophotometer and etched up to 570 min in 14 different time intervals starting from 10 min<sup>25</sup>.

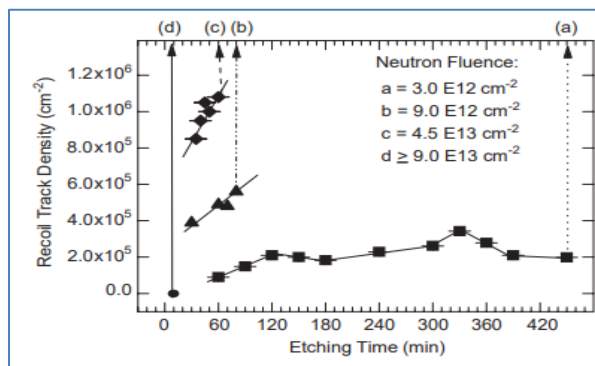


Figure 18: Recoil track density observed with the help of UV spectrophotometer

17. Analysis of anti-oxidant

Anti-oxidants were extracted from the plant *Myrmecodia pendans* by HPLC and analysed by UV Spectrophotometer at 700-200nm. These anti-oxidant were named as 'a' and 'b'<sup>26</sup>.

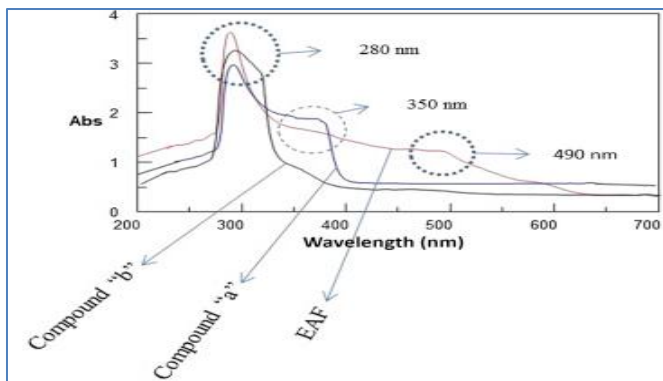
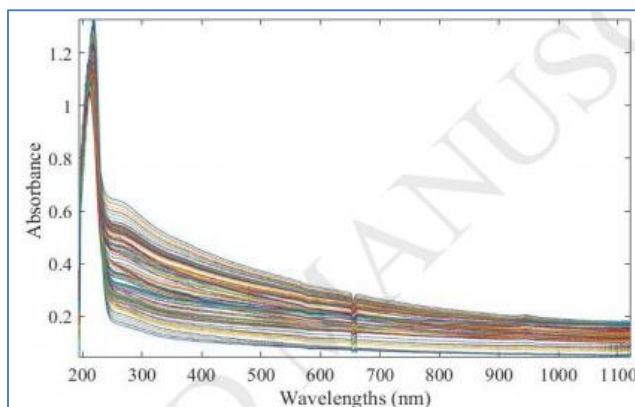


Figure 19: UV-Vis spectra of compound a, compound b, and EAF scanned from 200 nm to 700 nm. EAF = ethyl acetate fraction; Vis = visible.

18. Turbidity compensation method for COD measurement

120 samples were collected from different parts of Qian lake, China and these samples were analysed at 100-200 nm<sup>27</sup>.

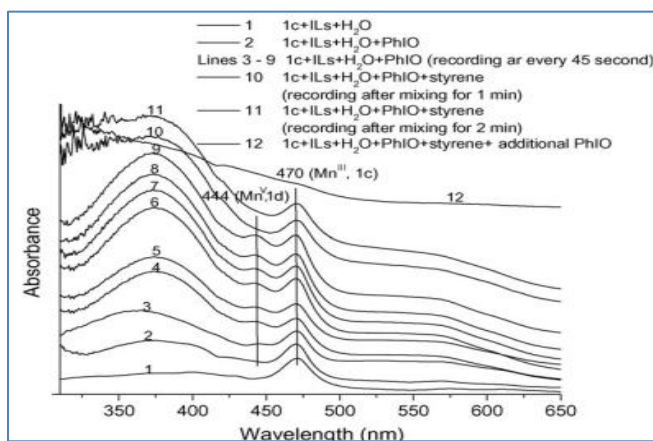




**Figure 20: UV-vis spectra of 120 samples from Qian Lake**

19. Epoxidations catalyzed by an ionic manganese(III) porphyrin and characterization of manganese(V, IV)-oxo porphyrin complexes.

Tetrakis (4-N-dimethylaminophenyl) porphyrin (H2TDMAPP, 1) and manganese tetrakis(4-N-dimethylaminophenyl) porphyrin (MnIIITDMAPP, 1a) were prepared according to the reported work [21]. In N<sub>2</sub> atmosphere, into a three-neck round-bottom bottle were added DMF (50 mL), H2TDMAPP (1, 1.0 g), and CH<sub>3</sub>I (3.0 g, excess). The resulting mixture was stirred at 120 °C for 1 h. After cooled to room temperature, the mixture was added with diethyl ether to precipitate a solid, which was thoroughly washed with acetone and diethyl ether. The collected red-brown solid was then dried in vacuo to yield the product of tetrakis (N-methyl-4-pyridinium)- porphyrin iodide ([H2T(N-Me-4-Py)P][I]<sub>4</sub>, 1b) (0.6 g, Yield: 35%). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>):  $\delta$  = -2.97 (2H, s, 2NH), 3.92 (36H, br, 12N+CH<sub>3</sub>), 8.43 (8H, d, benzyl (CH)<sub>2</sub>),  $\tau$  = 8.49 (8H, s, pyrrole-H), 8.85 (8H, benzyl N=C(CH)<sub>2</sub>) ppm. UV-vis (in CH<sub>3</sub>CN): max (nm) = 418 (s, Soret band), 520 (w, Q band), 580 (w, Q band), 650 (w, Q band). FT-IR (KBr disc, cm<sup>-1</sup>): 3339 (s, stretching vibration of N-H of pyrrolyl), 1623 (s), 1400 (s). TG-DTA analysis: thermal decomposition temperature 210 °C (in N<sub>2</sub> flow). Elemental analysis found (calculated, MW 1354.2) for C<sub>56</sub>H<sub>62</sub>N<sub>8</sub>I<sub>4</sub> (1b): C 49.83 (49.63), H 4.39 (4.58), N 8.14 (8.27). The mixture of 1.36 g [H2TTMAPP][I]<sub>4</sub> (1b, 1.0 mmol) and 1.97 g Mn(OAc)<sub>2</sub>·4H<sub>2</sub>O (8.0 mmol) in 100 mL acetic acid was heated at 80 °C for 6 h. After removal of the solvent in vacuo, the left residue was recrystallized with methanol/diethyl ether to yield a dark-purple solid as [MnIIIT(N-Me-4-Py)P][I]<sub>5</sub>-x[OAc]<sub>x</sub> (x = 0–5). The obtained solid was then dissolved in deionized water, and then treated with excess NH<sub>4</sub>PF<sub>6</sub> aqueous solution. The purple solids precipitated readily. By washing with deionized water and methanol, the purple solids of manganese tetrakis(4-N-trimethylaminophenyl) porphyrin hexafluorophosphate ([MnIIITTMAPP][PF<sub>6</sub>]<sub>5</sub>, 1c) were obtained<sup>28</sup>.

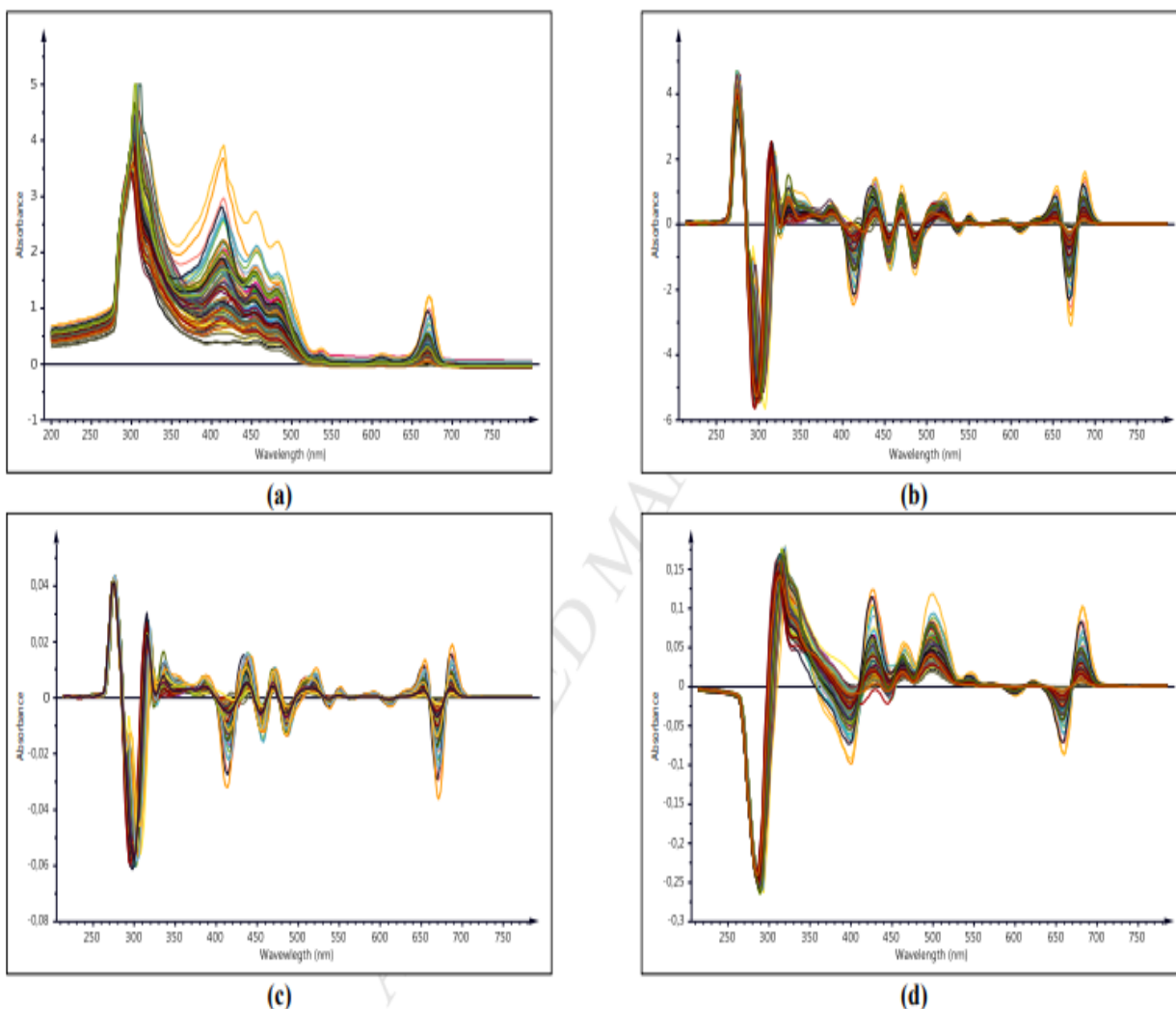


**Figure 21: UV-vis absorption spectra recorded: (1) line 1, for the mixture of 1c-ILs-H<sub>2</sub>O; (2) line 2, after adding PhiO into 1c-ILs-H<sub>2</sub>O immediately; (3) lines 3–9, for the mixture of 1c-ILs-H<sub>2</sub>O-PhiO recorded continuously at every 45 s; (4) line 10, after adding styrene into the mixture corresponding to spectrum of**

line 9 and standing by for 1 min; (5) line 11, after adding styrene into the mixture corresponding to spectrum of line 9 and standing by for 2 min; (6) line 12, after adding PhIO (60 mol) additionally into the mixture corresponding to the spectrum of line 11 (conditions: 1c  $1.3 \times 10^{-5}$  M, ILs 3 mL ([BMIM]BF<sub>4</sub> 1.5 mL + [BzMIM]BF<sub>4</sub> 1.5 g), H<sub>2</sub>O 5 mmol, PhIO 60 mol, styrene 30 mol).

## 20. Determination of chemical characteristics of olive oils

89 samples of olive oil from two harvest years (2015,2016) were used for analysis. The first derivative was obtained after performing the FTIR in presence of silica gel in n-hexane. Second derivative was obtained after performing FTIR in presence of silica gel and n-hexane at 80°C. third derivative was obtained after performing FTIR in presence of second derivative with MSc and SNV<sup>29</sup>.



**Figure 22:** (a) Raw, (b) first derivative, (c) second derivative and (d) second derivative + MSC + SNV of uv-spectra of olive oil sample.

## 21. Writing age of black roller and gel inks

Sample is extracted in a vial with 1 mL methanol containing ethyl benzoate (ethyl benzoate is used as external comparative matter) for 20 min to maximize the amount of all available volatile components of ink. After extraction, 2 mL of every sample is removed and analyzed by GC. The residual solution of extraction is extracted secondarily with 0.9 mL DMF for 20 min to maximize the amount of all available dye components of ink and analysed in UV spectrophotometer<sup>30</sup>.

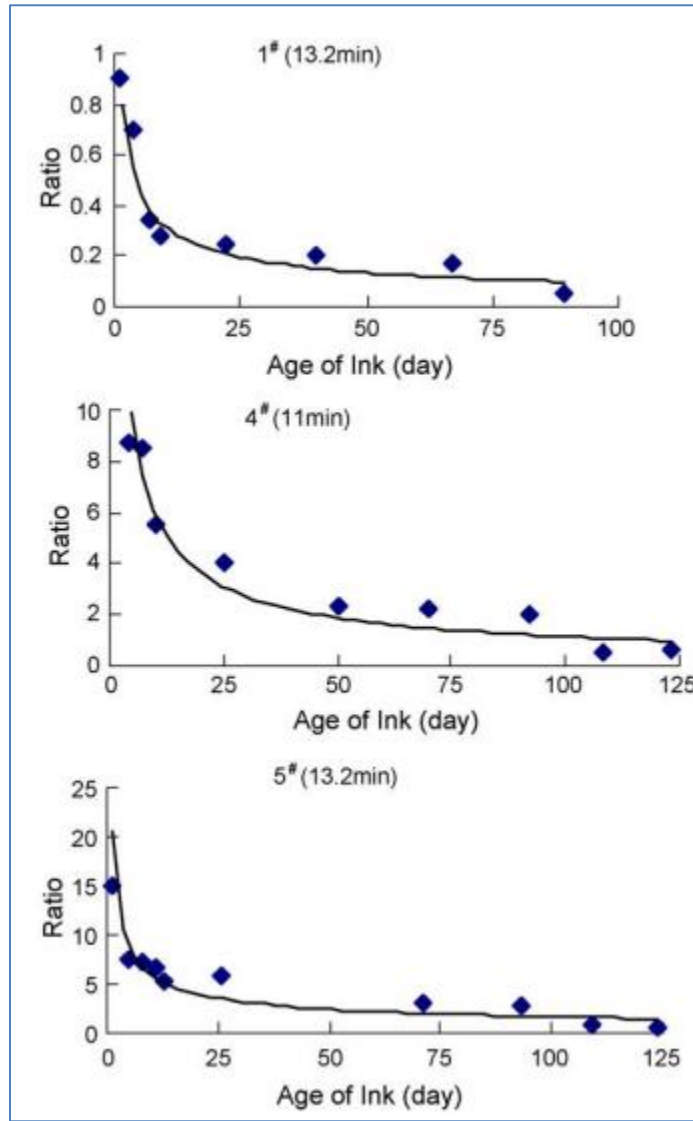


Figure 23: Natural aging curve of the Ratio of known dated samples decreasing

## 22. Nitrates monitoring

Samples were collected from the Moselle River drainage basin. After filtration (cellulose acetate, 0.45mm), nitrates and nitrites were analyzed using ion chromatography and analysed at 300-200nm<sup>31</sup>.

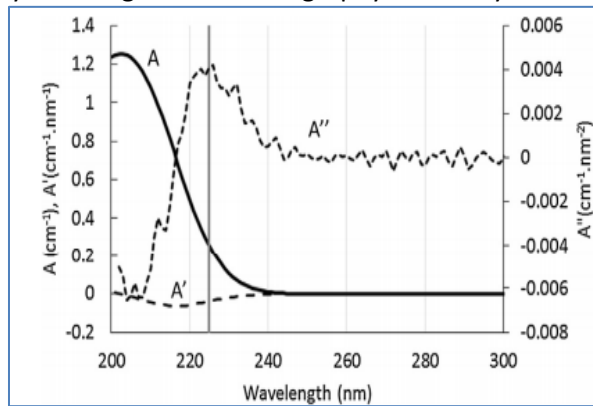


Figure 24: Absorbance (A), first derivative (A') and second derivative (A'') spectra for a 2.0 mg N L1 nitrate standard.

23. Behaviour of DNA under hydrothermal conditions with MgCl<sub>2</sub>.

A sample solution containing 5.0 × 10<sup>-3</sup> M DNA, 5.0 × 10<sup>-4</sup> M EB, 0.2 M MgCl<sub>2</sub> and 0.01 M Tris buffer (pH 7.9) and that containing 5.0 × 10<sup>-4</sup> M EB, 0.2 M MgCl<sub>2</sub> and 0.01 M Tris buffer (pH 7.9) were prepared. Besides, a sample solution containing 5.0 × 10<sup>-3</sup> M DNA and 0.01 M Tris buffer (pH 7.9) and that containing 5.0 × 10<sup>-3</sup> M DNA, 0.2 M MgCl<sub>2</sub> and 0.01 M Tris buffer (pH 7.9) were prepared for direct measurements of UV absorption of DNA<sup>32</sup>.

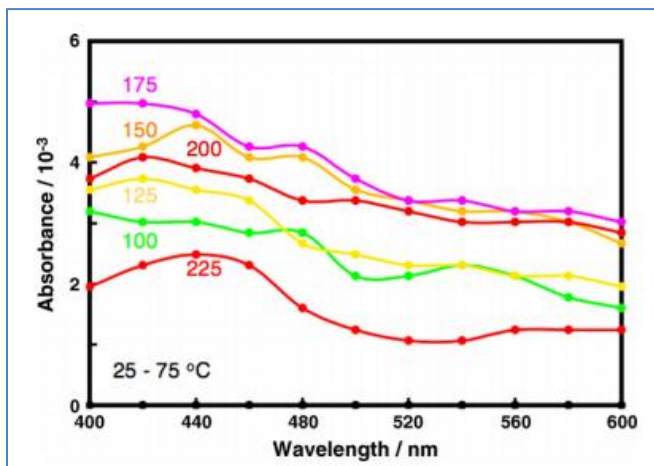


Figure 25: Absorption spectra vs. temperature for a solution containing DNA. Conditions: 5.0 × 10<sup>-3</sup> M DNA, 0.2 M MgCl<sub>2</sub> and 0.01 M Tris buffer (pH 7.9) Heating time: 0.64 s.

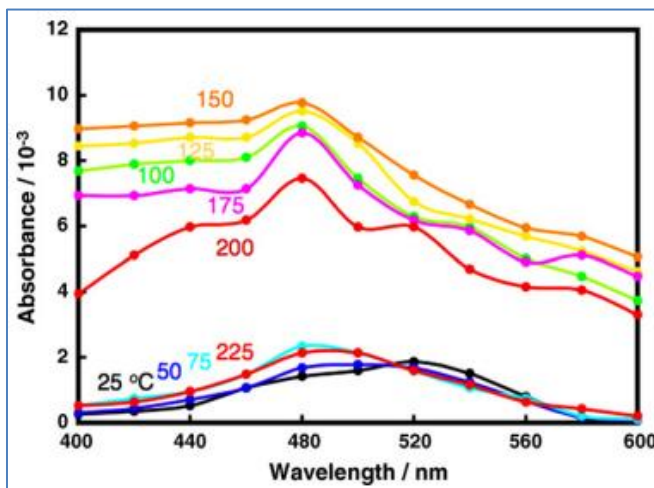


Figure 26: Absorption spectra vs. temperature for a solution containing EB and DNA. Conditions: 5.0 × 10<sup>-3</sup> M DNA, 5.0 × 10<sup>-4</sup> M EB, 0.2 M MgCl<sub>2</sub> and 0.01 M Tris buffer (pH 7.9). Heating time: 0.64 s.

**CONCLUSION**

It can be concluded that UV spectrophotometer can be used for many purposes. It contains various modes like Photometric, Kinetic, Kinetic rate, Spectrum, Time-Scan, Bio-method, Quantitation and Multi-component. Most commonly mode used are Photometric and Spectrum. Photometric is used for calculating the absorbance at a particular wavelength which is the λ<sub>max</sub> of that particular molecule. It gives the exact absorbance of molecule at that particular wavelength. In case of Spectrum, the sample is run in a range (generally

400-200nm is used) a graph is shown which gives the absorbance of sample. Spectrum and Photometric is used for the known concentration. Quantitation is used for plotting the calibration curve of unknown concentration. Kinetics is used to measure the change in absorbance as a function of time, and obtains its enzymatic values. Time scan is used to measure change over time in photometric value at a specific wavelength. Bio-method is used to quantify DNA and protein by using different method.

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