KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University Established Under Section 3 of UGC Act 1956)

Coimbatore – 641 021.

LECTURE PLAN

DEPARTMENT OF BIOCHEMISTRY

STAFF NAME: Dr. L. HARIPRASATH

SUBJECT NAME: PRACTICAL- FUNDAMENTALS OF NANO TECHNOLOGY

SUB.CODE: 16BCU612-B

SEMESTER: VI **CLASS: III B.Sc.(BC)**

S.No	Duration of period	Topics covered	Books referred
1	**1**	Nanoparticles synthesis using chemical methods	R1
2	**1**	Synthesis of nanoparticles using plant extracts	R1
3	**1**	Nanoparticles synthesis using fungal species	R1
4	**1**	Nanoparticles synthesis using *Actinomyces*	R1
5	**1**	UV-Visible spectra analysis of nanoparticles (Demo)	R2
6	**1**	FT-IR spectra analysis of nanoparticles (Demo)	R2

REFERENCES

R1 : Edward, L.W., (2006). Nanophysics & Nanotechnology: An Introduction to Modern Concepts in Nanoscience, WILEY-VCH.

R2 : Fahrner, W.R., (2005). Nanotechnology and Nanoelectronics Materials, Devices, Measurement Techniques, Springer.

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1. Nanoparticles synthesis using chemical methods

Synthesis of silver nanoparticles by chemical reduction method

AIM: To synthesize silver nanoparticles using trisodium citrate and ascorbic acid as a surfactant.

Materials required:

1. Silver nitrate (AgNO3) as the metal precursor
2. Trisodium citrate is used a reducing agent and
3. Ascorbic acid as a surfactant
4. Test tubes
5. Water bath

Stock preparation:

1. Silver nitrate (1 mM): Molarity is moles per liter. Since the molar mass of AgNO3 is 169.87g/mol, a 1 M solution of AgNO3 would be 169.87 g (1 mole of AgNO3) in 1 liter. For preparation of 100ml of solution 0.017g of silver nitrate is taken.
2. Trisodium citrate (2mM to 5 mM)
3. Ascorbic acid (2mM and 3 mM)

Procedure:

- 8 ml of AgNO3 was first heated to 60°C
- Then added (with vigorous stirring) to 2 ml of a C6H5O7Na3and C6H8O6 solution that was pre-heated to 60°C.
- The mixture was then stirred for 20 minutes.
- After that, the heating was stopped and the solution was cooled at room temperature with continuous stirring.
- The colour change in different tubes was observed and the synthesis of silver nanoparticles is confirmed.

Result:

Formation of orange colour indicates the formation of silver nanoparticles.

	1mM AgNO3	The AgNo3 solution is heated to 60°C with vigorous stirring for 10 min	**Trisodium citrate**	**Ascorbic acid**	1 mL each of Trisodium citrate and ascorbic acid was mixed in separate tube and heated at 60°C with vigorous stirring for 10 min. Mix the content with AgNO3 and heated again for 60°C
T1 Blank	8 mL		-	-	
T2	8 mL		1 mL (2 mM)	-	
T3	8 mL		1 mL (3 mM)	-	
T4	8 mL		1 mL (4 mM)	-	
T5	8 mL		1 mL (5 mM)	-	
T6	8 mL		1 mL (2 mM)	1 mL (2 mM)	
T7	8 mL		1 mL (3 mM)	1 mL (2 mM)	
T8	8 mL		1 mL (4 mM)	1 mL (2 mM)	
T9	8 mL		1 mL (5 mM)	1 mL (2 mM)	
T10	8 mL		1 mL (2 mM)	1 mL (3 mM)	
T11	8 mL		1 mL (3 mM)	1 mL (3 mM)	
T13	8 mL		1 mL (4 mM)	1 mL (3 mM)	
T14	8 mL		1 mL (5 mM)	1 mL (3 mM)	

2. Synthesis of silver particles nanoparticles using plant extracts

Synthesis and characterization of silver nanoparticles using leaf extract of *Azadirachta indica* (Neem extract)

Aim: To synthesize and characterize silver nanoparticles using Neem leaf extract.

Principle:

Materials and methods:

1. AgNO3 – 1mM
2. *Azadirachta indica* (Neem)

Procedure:

Preparation of plant extract: Fresh leaves of *Azadirachta indica*, were collected from KAHE campus, and washed several times with water to remove the dust particles and then sun dried to remove the residual moisture and grinded to form powder. Then plant extract was prepared by mixing 1% of plant extract with deionized water in a 250ml conical flask. Then the solution was incubated for 30 minutes and then subjected to centrifuge for 30 minutes. at room temperature with 5000 rpm. The supernatant was separated and filterd with (mm filter paper pore size) filter paper with the help of vaccume filter. Then the solution was used for the reduction of silver ions Ag^{+}) to silver nanoparticles (Ag^{o}).

Synthesis of silver nanoparticles: Four concentration ratios of plant and metal ions were prepared (30:1, 60:1,120:1 & 240:1) by increasing the concentration of plant extract concentration in the solution. 0.17% of 1mM AgNO3 metal ion was added in the prepared plant extract. Then the bio-reduced aqueous component was used to measuring UV-Vis spectra of the solution

Characterization of silver nanoparticles:

UV-Vis Analysis: The optical property of AgNPs was determined by UV-Vis spectrophotometer (PerkinElmer, Lamda 35,Germany). After the addition of AgNO3 to the plant extract, the spectrum was taken in different time intervals up to 24Hrs. between 350 nm to 500 nm. Then the spectra were taken after 24Hrs. of AgNO3 addition.

FTIR analysis: The chemical composition of the synthesized silver nanoparticles was studied by using FTIR spectrometer (perkin-Elmer LS-55- Luminescence spectrometer). The solutions were dried at 75^{o}C and the dried powders were characterized in the range 4000–400 cm^{-1} using KBr pellet method.

SEM Analysis: The morphological features of synthesized silver nanoparticles from neem plant extract were studied by Scanning Electron Microscope (JSM-6480 LV). After 24Hrs. of the addition of AgNO3 the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 20 KV.

Results:

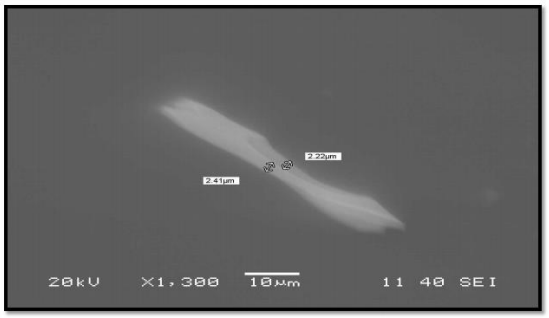
The silver nanoparticles have been synthesized. The characterization was done using UV spectroscopy, FTIR and SEM analysis.

UV VISIBLE SPECTROSCOPY:

Reduction of silver ions into silver nanoparticles during exposure to plant extracts was observed as a result of the color change. The color change is due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of silver nanoparticles were observed around 421 nm.

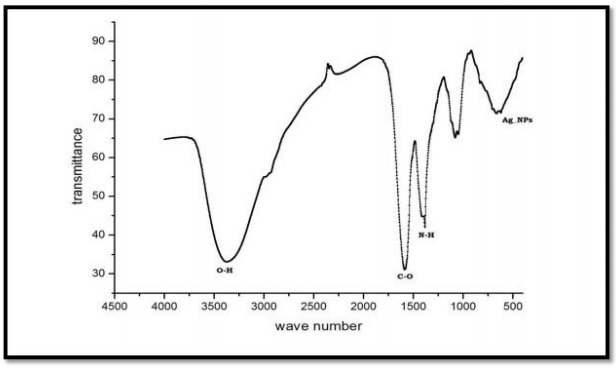
SEM ANALYSIS:

The size of the prepared nanoparticles was more than the size of nanoparticle which should be; i.e.; between 1-100 nm. The size was more than the desired size as a result of the proteins which were bound in the surface of the nanoparticles.



FTIR ANALYSIS:

The peak found around 1500-1550 cm^{-1} showed a stretch for C-H bond, peak around 1450-1500 cm^{-1} showed the bond stretch for N-H. Where as the stretch for AgNPs were found around 500-550 cm^{-1}. Therefore the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional groups.



3. Synthesis and characterization of silver nanoparticles using mushroom *Pleurotus ostreatus*

Aim: To synthesize and characterize silver nanoparticles using Neem leaf extract.

Principle:

Materials and methods:

1. AgNO3 – 1Mm
2. mushroom *Pleurotus ostreatus*

Procedure:

Mushroom sample and strains:

Pleurotus ostreatus fresh basidiocarps were collected from Coimbatore market. The basidiocarps were sliced, oven-dried at 50 ± 2 °C for 24 hours and ground to fine powder. Ten grams of the P. ostreatus powder was soaked in distilled water in a ratio of 1:10 (w/v) and boiled for 30 min at 60 ± 2°C. The boiled mushroom powder was left covered in room temperature for 30 min and filtered. Suspended residues were removed by centrifuging the filtrate (10,000 x g for 30 min at 4°C) and the supernatant collected was filtered through Whatman No.1 filter paper. The filtrate was freeze-dried at -53 ± 2°C for 48 h. The freeze-dried powder was used as the aqueous extract and stored at 4°C prior to use.

Biosynthesis of silver nanoparticles (AgNPs):

Different concentrations (1-6 mg/mL) of the aqueous extract of P. ostreatus was added to 5 mL of 1 mM aqueous silver nitrate solution and kept at 28 ± 2o C in dark incubation for the bioreduction of Ag+ ions to Ag0 . The mixed solution was continuously stirred and incubated for 6, 12, 18, 24, 30, 36 and 40 hours. The color change of AgNO3 solution was monitored. The fully reduced solution was centrifuged at 20,000 x g for 30 4 min. The residue was retained after discarding the supernatant. The residue was washed in sterile distilled water and dried.

Characterization of AgNPs:

UV-Vis Analysis: The optical property of AgNPs was determined by UV-Vis spectrophotometer (PerkinElmer,Lamda 35,Germany). After the addition of AgNO3 to mushroom, the spectrum

was taken in different time intervals up to 24Hrs. between 350 nm to 500 nm. Then the spectra were taken after 24Hrs. of AgNO3 addition.

FTIR analysis: The chemical composition of the synthesized silver nanoparticles was studied by using FTIR spectrometer (perkin-Elmer LS-55- Luminescence spectrometer). The solutions were dried at 75^{o}C and the dried powders were characterized in the range 4000–400 cm -1 using KBr pellet method.

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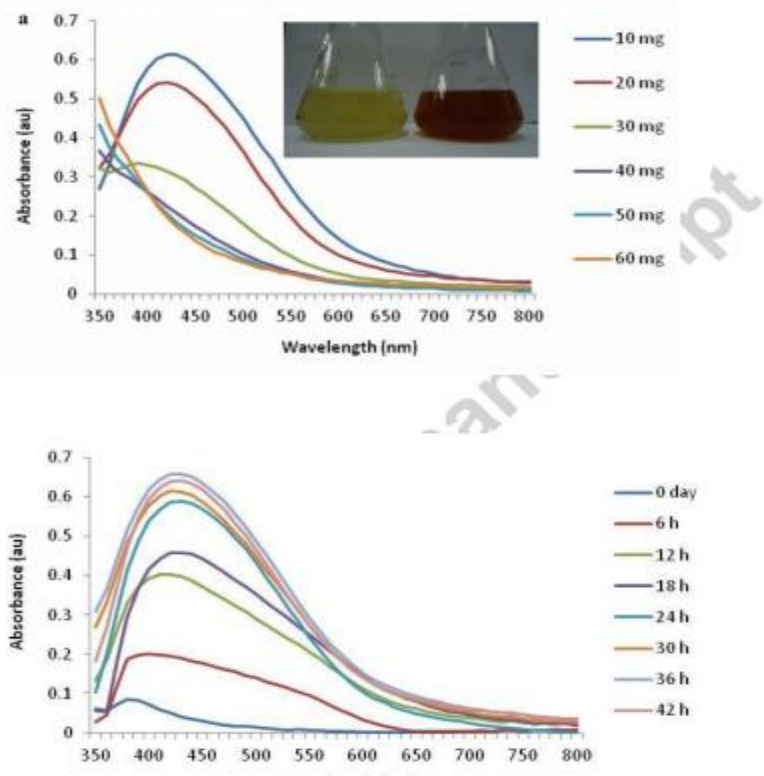
Results:

The silver nanoparticles have been synthesized. The characterization was done using UV spectroscopy, FTIR and SEM analysis.

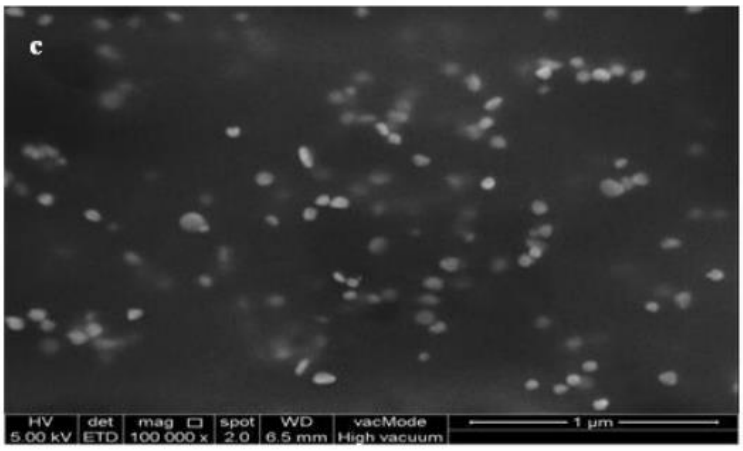
UV-Vis Analysis:

The formation of AgNPs was confirmed by intense absorption peaks at wavelengths in the range of 400-470 nm, which are typical absorption bands of spherical AgNPs due to their surface plasmon resonance. The AgNPs synthesised using low concentrations (10 and 20 mg/mL) of P. ostreatus aqueous extract showed broad absorbance, however the peak turned narrower with further increase in the concentration. At concentrations above 30 mg/mL, the absorbance was not recorded in the range 400-470 nm, this might be because of the high quantity of different types of bioconstituents present in the solution, which results in coupling and clumping of AgNPs.



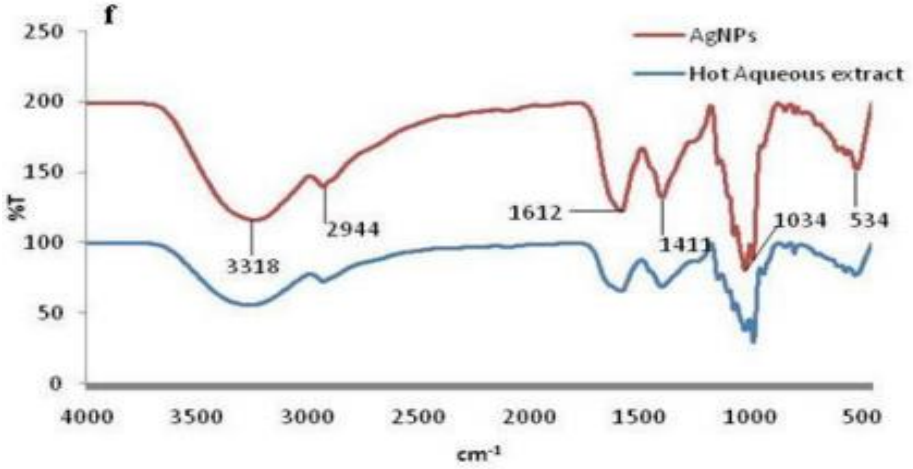
FESEM analysis:

FESEM images provide further insight into the structure and morphology of the synthesised AgNPs. The images depict that the AgNPs are spherical in shape and well dispersed without any aggregation, with an average size ranging from 10 to 40 nm. The size distribution analysis showed the average size of the AgNPs as 28 nm. The presence of different size distribution of AgNPs as evidenced in size distribution study, which is due to the involvement of various biomolecules in capping and bioreduction of AgNO3 solution.



FTIR analysis:

FT-IR spectrum revealed the functional groups involved in the reduction of AgNO3 . The spectrum showed strong absorption peaks from 3318 cm-1 to 534 cm-1. The absorbance peak at 3318 cm-1 corresponds to N-H stretching vibrations , band at 2944 cm-1 corresponds to C-H stretch alkenes and O-H stretch carboxylic acid . Peak at 1612 cm-1 is characteristic of a C=O vibration at the α- and β-unsaturated aldehydes. The presence of a carbohydrate group is evident from the peak at 1411 cm-1.



5. UV-Visible spectra analysis of nanoparticles (Demo)

Aim:

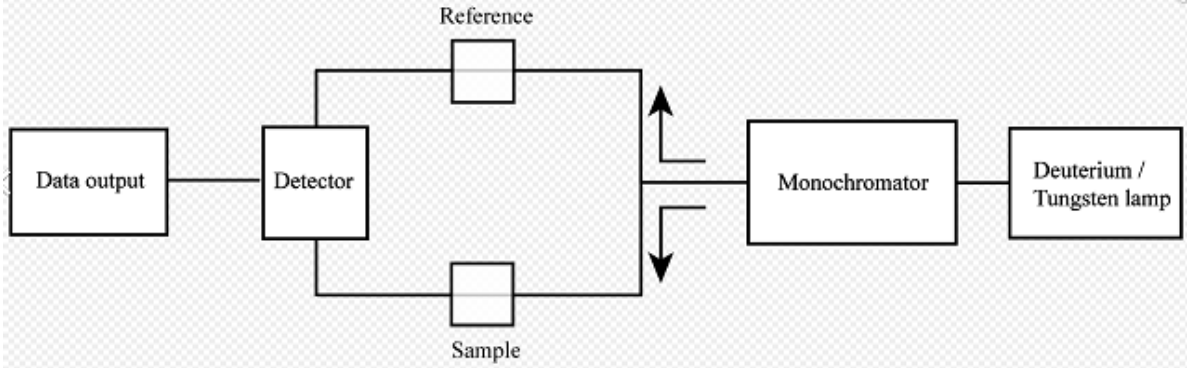
To give the brief understanding about UV-visible spectra analysis

Principle:

Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis or UV/Vis) refers to absorption spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared (NIR)) ranges. UV/Vis spectrophotometer is used in the quantitative determination of concentrations of the absorber in the solutions of transition metal ions and highly conjugated organic compounds.

The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length (Figure 2). Thus, for a fixed path length, UV/Vis spectroscopy can be used to determine the concentration of the absorber in a solution. The absorbance changes with concentration. This can be taken from references (tables of molar extinction coefficients), or more accurately, determined from a calibration curve.

Working diagram



The light source

- A light source which enables to cover the entire visible spectrum plus the near ultra-violet ranging from about 200–800 nm is used.

- A single lamp cannot achieve this, and so a combination of two lights are used – a deuterium lamp for the UV part of the spectrum, and a tungsten/halogen lamp for the visible part.
- The combination of these two bulbs are focused on to a diffraction grating.

The diffraction grating and the slit

- A diffraction grating efficiently splits the lights into its component colors.
- The slit allows only a narrow range of wavelengths of light to pass into the spectrometer.
- Light from a whole spectrum is allowed through, by gradually rotating the diffraction grating into the rest of the instrument

The sample and reference cells

- They are made of small rectangular glass or quartz containers, and are designed in such a way that the light beam travels a distance of 1 cm through the solution.
- The sample cell usually contains a very dilute solution of the sample to be tested. The solvent should not absorb any significant amount of light in the wavelength range (200–800 nm).
- The reference cell consists of the pure solvent

The detector and computer

- The light intensity passing through the reference and the sample cells are measured for every wavelength of light passing through the spectrometer.
- The light intensity measured is denoted as I – for the sample cell and Io – for the reference cell
- If the value of I is lesser than Io, this explains that the sample has absorbed some of the light. This is calculated using a formula for absorbance, A

Applications

- Detection of impurities.
- Structure elucidation of organic compounds.
- DNA quantification and Qualitative analysis.
- Identification of functional groups based on the wavelengths

6. FT-IR spectra analysis of nanoparticles (Demo)

Aim:

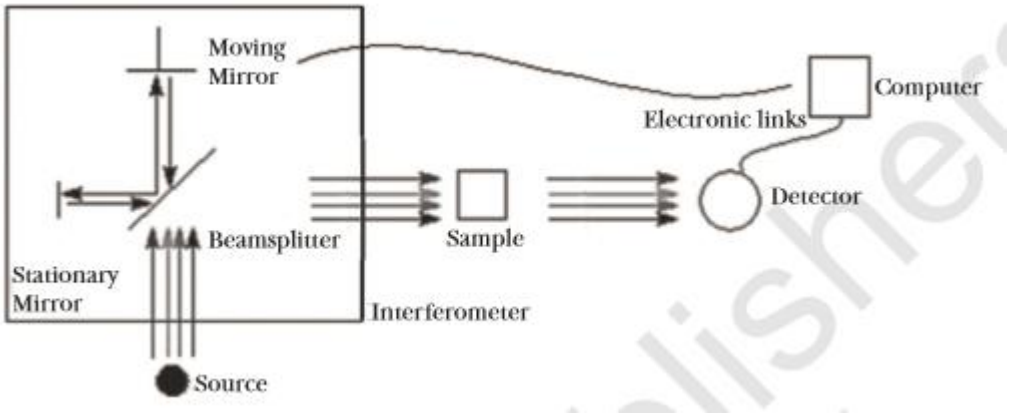
To give the brief understanding about FT-IR spectra analysis of nanoparticles

Principle

- When a sample is made to pass through IR radiation, it absorbs few radiation and transmits the others.
- This results in a spectrum representing the molecular absorption and transmission, leaving a molecular fingerprint of the sample. Even the substances that have unique molecular structures does not exhibit the same IR spectrum.

Working

- Source: A glowing black body source emits the IR energy, which is passed through an aperture, controlling the amount of energy presented to the sample.
- Interferometer: This beam of energy enters the interferometer, where the "spectral encoding" takes place.
- Sample: The beam entering the sample compartment is transmitted through or reflected off the surface of the sample. This depends upon the type of analysis performed.
- Detector: The beam is finally passed into the detector for final measurement. A pyroelectric detector is used.
- The output obtained reaches a maximum, when all the spectral components are in phase and the path difference between them is zero.



Applications

- Helps in determining the composition.
- Identifies unknown materials.
- Determines the amount of components in a mixture (concentration).
- Qualityor consistency of the sample characterized.
- Used to analyse opaque or cloudy samples.
- Micro-samplesin forensic analysis.
- Helps in analyzing raw materials or finished products.

Advantages

- This is a non-destructive technique.
- It requires no external calibration.
- High sensitivity, greater optical throughput.
- Mechanically simple method with only one moving part