BITS PILANI, DUBAI CAMPUS
SECOND SEMESTER 2012-2013
COMPREHENSIVE EXAM

Course Title: Instrumental Methods of Analysis	**Course No.: BIOT C244**
Maximum Marks: 40	**Weightage: 20%**
Time: 2 hours	**Date: 8th June 2013**

1a. Explain the generation of plasma in the ICP torch? [4]
b. Justify, why some molecules exhibit fluorescence while all molecules do not? [3]
c. Different columns can be used in gas chromatography. Describe them and justify their right use. [3]

2a. why are molecular spectra are more complex than atomic spectra? [3]
b. What are signal generators? Explain with examples the different signal generators. [3]
c. Give the advantages of an autoanalyzer and mention any two applications of the same. [4]

3a. In a PCR, the starting material of DNA was 3ng/ml. What would be the amount of DNA after 30 rounds of PCR? [3]
b. An aqueous solution containing 4.07 g of an optically pure compound was diluted to 200.0 mL with water and was placed in a polarimeter tube 20.0 cm long. The measured rotation was -3.99 degrees at 25 degrees Celsius. Calculate the specific rotation of the compound. [3]
c. Describe the working of the photomultiplier tube. [4]

4a. In an IR spectra what is the peculiarity of the nitro group? [2]
b. Mention the mode, stationary phase and mobile phase used in a HPLC run for separating inorganic cations. What is meant by an isocratic system? [3+1]
c. Complete the following table for the hydrogen atom by determining the wavelength of light absorbed when an electron jumps between the subsequent energy levels. (Given: c=3 x 10^8 m/s and h= 4.14 x 10^{-15} eV s). [4]

Energy Level	*Energy*	*Wavelength of photon absorbed*
1	-13.6 eV	-
2	-3.4 eV	
3	-1.51 eV	
4	-0.85 eV	
5	-0.54 eV	

********** ALL THE BEST **********

BITS PILANI, DUBAI CAMPUS
SECOND SEMESTER 2012-2013
COMPREHENSIVE EXAM
ANSWERKEY

Course Title: Instrumental Methods of Analysis	**Course No.: BIOT C244**
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1a. Explain the generation of plasma in the ICP torch? [4]

The argon gas is directed through a torch consists of 3 concentric tubes made of quartz. A copper coil called the Load Coil surrounds the top end of the torch and connected to a Radio Frequency Generator (RF)

When RF power (700 – 1500 Watts) is applied to the load coil, an alternating current moves back and forth within the coil or oscillates at a rate corresponding to the frequency of the generator (27 – 40 MHz). This RF oscillation causes the RF electric and magnetic fields to be set up in the area at the top of the torch.

With Argon gas being swirled through the torch, a spark is applied to the gas causing some electrons to strip out from their Argon atoms. These electrons are then caught up in the magnetic field and accelerated by them. Adding energy to the electrons by the use of the coil in this manner is called "Inductively Coupling". These high-energy electrons in turn collide with other Argon atoms, stripping off still more electrons.

This collisional ionization of Argon continues in a chain reaction thus breaking down the gas into a plasma consisting of Argon atoms, electrons, and Argon ions know as "ICP" discharge. This ICP discharge is sustained. This ICP discharge appears as intense, brilliant, white and tear-drop shaped.

1b. Justify, why some molecules exhibit fluorescence while all molecules do not? [3]

In a non-fluorescent molecule when an electron is excited to the electronic excited state, it return back to the ground state by losing the energy it has acquired through conversion of the excess electronic energy into **vibrational energy** .

If a molecule has a rigid structure the loss of electronic energy through its conversion into vibrational energy is relatively slow and there is a chance for the electronic energy to be emitted as **ultraviolet or visible radiation.**

The energy emitted is of **lower energy** than the energy absorbed because the excited electron moves to the lowest energy **vibrational state** in the excited state before returning to the ground state.

Thus fluorescence emission is typically shifted by 50-150 nm (***Stokes shift***) to the longer wavelength in comparison to the wavelength of the radiation used to produce excitation.

1c. Different columns can be used in gas chromatography. Describe them and justify their right use. [3]

Packed columns: these are filled with a coated inert solid support such as fire brick, alumina, and graphite with a specific mesh size. The coatings are called phases and for best results are chemically bonded to the support. Chemical bonding provides for longer column life and less bleeding (major source of background noise) contributing to lower sensitivity. Column dimensions 1/8" - 1/4" ID x up to about 6' using glass or stainless steel.

Advantages - higher capacity (higher conc), hence used in purification.

Capillary columns: Here the phase (film) is coated on the inside diameter of the capillary wall with film thickness range of 0.1 to 5μ where the ticker film provides for better resolution but also allows for more bleed. Typical dimensions .25mm - .53mm ID x up to 60m made of fused silica coated with polyamide.

Advantages: high resolution and better S/N, hence applied in analytical work.

2a. Why are molecular spectra are more complex than atomic spectra? [3]
In polyatomic molecules, different vibrational and rotational energy levels are also involved. Roughly speaking, a **molecular energy state**, is the sum of the electronic, vibrational, rotational, nuclear, and translational components. The complexcity of molecular spectra increases because of the transitions between electronic states which are accompanied by transitions between rotational and vibrational energy levels that impart fine structure to the electronic absorption bands

2b. What are signal generators? Explain with examples the different signal generators. [3]
Signal generators: The signal results from the direct or indirect interaction of the analyte with some form of energy such as electromagnetic radiation, electricity or thermal heating.

Two general methods are used for signal generation:

1. Application of an external signal to the sample and subsequent modification of this signal by the analyte as in absorption spectroscopy
2. Creation of a sample environment that allows the analyte to produce a signal as in potentiometric measurements.

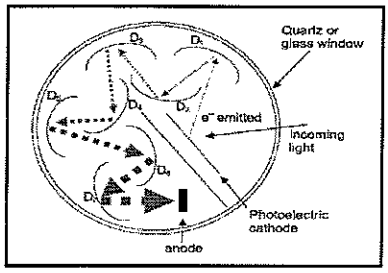
2c. Give the advantages of an autoanalyzer and mention any two applications of the same. [4]
Advantages: i. small volumes are required
ii. cost effective
iii. rapid method
iv. multiple modes like photometry, luminometery and fluorimetry in one instrument
Application: i. ELISA, ii. SGOT/SGPT, iii. Leishmaniasis,

3a. In a PCR, the starting material of DNA was 3ng/ml. What would be the amount of DNA after 30 rounds of PCR? [3]
Starting material of DNA = 3ng/ml
After 30 cycles, the amount of DNA would be = $3(2^{30})$ = 3.221225472 g/ml

3b. An aqueous solution containing 4.07 g of an optically pure compound was diluted to 200.0 mL with water and was placed in a polarimeter tube 20.0 cm long. The measured rotation was -3.99 degrees at 25 degrees Celsius. Calculate the specific rotation of the compound. [3]

α	conc	conc	l	sp rot
-3.99	4.07g/200ml	2.035/100ml	20	98.0344

3c. Describe the working of the photomultiplier tube. [4]
It is very sensitive detectors with very short response times. It contains a photo cathode and a series of dynodes, which are also photosensitive.
A higher successive potential is maintained between each dynodes. Photoelectrons released from the photo cathode are accelerated toward the first dynode by their voltage difference, where they strike to release several electrons. The secondary electrons are then accelerated toward the second dynode where the process repeats. In this way multiplication of the electrons can be achieved. The current from phototubes and photo multiplier tubes never falls to zero. A small residual current called dark current is produced, due to long exposure of the light.



4a. In an IR spectra what is the peculiarity of the nitro group? [2]

The peculiarity of the nitro group is the N=O stretching, which exhibits two peaks at 1550 and 1350 cm^{-1} for the asymmetric and symmetric stretching respectively. Often the 1550 cm^{-1} peak is stronger than the other one.

4b. Mention the mode, stationary phase and mobile phase used in a HPLC run for separating inorganic cations. What is meant by an isocratic system?[3+1]

Mode: ion exchange chromatography

Stationary phase: anionic exchange resins

Mobile phase: aqueous/ buffer with counter ions.

Isocratic system: A system that is operated under similar condition, without altering the parameters during the run.

4c. Complete the following table for the hydrogen atom by determining the wavelength of light absorbed when an electron jumps between the subsequent energy levels. (Given: c=3 x 10^8 m/s and h= 4.14 x 10^{-15} eV s). [4]

Energy Level	*Energy*	*Wavelength of photon absorbed*
1	-13.6 eV	-
2	-3.4 eV	1.21 x 10^{-7} m
3	-1.51 eV	6.571429 x 10^{-7} m
4	-0.85 eV	18.81818 x 10^{-7} m
5	-0.54 eV	40.06452 x 10^{-7} m

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BITS PILANI DUBAI CAMPUS
DUBAI INTERNATIONAL ACADEMIC CITY
SECOND SEMESTER 2012-2013
Test 1 (OPEN BOOK)

Course NO: BIOT F244 **Maximum Marks: 20**
CourseTitle: INSTRU METHODS OF ANAL **Weightage: 10%**
Date: 13.05.2013

Answer all the questions in the given sequence

1a. An analytical laboratory receives a sample from a cosmetic industry for the qualitative and quantitative determination of mercury. As an analyst suggest an appropriate technique along with the description of sample preparation. [4]

b. Justify, 'in a HPLC, wider the base of a peak suggests a low efficiency column'. [3]

c. State the role of a nebulizer in ICP-OES. [2]

2a. Why is it important to develop a good temperature program for Gas chromatography? [3]

b. Differentiate between AAS and ICP-OES. [2]

c. We have a sample from the pharmaceutical company. Suggest a method for analyzing and purifying the formulation. Justify your answer. [3]

d. Describe a detector system that makes use of a heated tungsten filament. [3]

**********ALL THE BEST**********

Answer solution

BITS PILANI DUBAI CAMPUS
DUBAI INTERNATIONAL ACADEMIC CITY
SECOND SEMESTER 2012-2013
Test 1 (OPEN BOOK)

Course NO: BIOT F244 **Maximum Marks: 20**
CourseTitle: INSTRU METHODS OF ANAL **Weightage: 10%**
Date: 12.05.2013

Answer all the questions in the given sequence

1a. An analytical laboratory receives a sample from a cosmetic industry for the qualitative and quantitative determination of mercury. As an analyst suggest an appropriate technique along with the description of sample preparation. [4]

ICP. **Wet digestion:** Wet digestion is the method used for sample preparation, since the metal to be analyzed is mercury, which is a volatile element. This method requires the use of strong oxidizing acids, such as nitric; sulphuric and perchloric acids, or, in certain cases, hydrofluoric, phosphoric and hydrochloric acids, either alone or in various combinations, depending on the nature of the sample and the metals to be analyzed. Nitric and perchloric acids are very strong oxidizing agents. Generally, sulfuric acid is used with an additional substance such as hydrogen peroxide to yield a cleaner decomposition mixture. Combinations of the above acids are normally recommended for food analyses. Wet digestions of biological samples commonly were carried out in open vessels, such as Kjehdahl flasks.

b. Justify, 'in a gas chromatogram, wider the base of a peak suggests a low efficiency column'. [3]

Theoretical plates (N) measure **how efficiently a column can separate a mixture into its components**. This efficiency is based on the retention time of the components and the width of the peaks. Wider the base of the peak, lower is the number of theoretical plates and hence lower is the efficiency of the column to separate the solutes. $N = 16 \left(\frac{t_R}{W_b}\right)^2$

c. State the role of a nebulizer in ICP-OES. [2]

Nebulizer plays a duel role of aerosole generation and droplet selection. The nebulizer helps in splitting the sample liquid into tiny aerosols with the help of a peristaltic pump and a constant gas flow.

2a. Why is it important to develop a good temperature program for Gas chromatography? [3]

Temperature programming means change of temperature of the column at a rate predetermined rate during the analytical run. This has the same influence on elution time of separated components as gradient programming in HPLC analysis. Temperature programming helps reduce analysis time by permitting early elution of less volatile components.

2b. Differentiate between AAS and ICP-OES. [2]

AAS	ICP
Atomic absorption depends upon the number of ground state atoms	Atomic emission depends upon the number of excited atoms
Presence of a light source	Absence of the light source
The temperature in the atomizer is adjusted to atomize the analyte atoms in the ground state only (1700 – 3150C)	The temperature in the atomizer is big enough to atomize the analyte atoms and excite them to a higher energy level (6000 – 8000C)

2c. The IMA lab of BITS Pilani Dubai Campus has received a sample from the pharmaceutical company. Suggest a method for analyzing and purifying the formulation. Justify your answer. [3]

HPLC would be the method of choice for analyzing and purifying the sample. HPLC can separate and purify the sample, taking care of the analysis. However, since the detector is a non destructive detector, it is possible to recover the purified sample from the column.

2d. Describe a detector system that makes use of a heated tungsten filament. [3]

Thermal Conductivity (TCD) :It uses a heated filament placed in the emerging gas stream. The amount of heat lost from the filament by conduction to the detector walls depends on the thermal conductivity of the gas phase. A tightly coiled tungsten filament is place in the cavity of the metal block. The filament is heated to a constant temperature by dc current. Heat lost from the filament ot the metal block is constant when only the carrier gas is flowing throught eh detector. The thermal conductivities of hydrogen and helium are 6-10 times greater than those for most organic compounds, hence the presence of even small amounts of organic materials cause a relatively large decrease in the thermal conductivity of the column effluent.

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BITS Pilani Dubai Campus
Dubai International academic City
Second Semester 2012-2013
Quiz 2

Course: INSTRUMENTAL METHODS OF ANALYSIS		Course No. : BIOT F244
Date: 11-04-2013	Total marks: 10	Weightage: 10%
NAME:		ID NO:

1. Define Emission spectroscopy. [1]

2. Explain the phenomenon of fluorescence with an example. [2]

3. Explain the consequence on fluorescence of a molecule when the temperature of the solution is increased from 28°C to 45°C. [2]

4. Define the term Plane Polarized Light. [1]

5. List the factors which influence the optical rotation of a chiral compound. [2]

6. The optical rotation of pure α-glucose is +112.2. A new purification plant recovered the first batch of glucose and found it was only 25% pure. The manufacturers worked on the process controls to increase the purity, which now exhibited an optical rotation of +52.5. Were the manufacturers capable of improving the purification process in the new plant. What do you infer from the new optical rotation value that is obtained? [2]

**********ALL THE BEST**********

BITS Pilani Dubai Campus
Dubai International academic City
Second Semester 2012-2013
Quiz 2 ANSWERKEY

Course: INSTRUMENTAL METHODS OF ANALYSIS		Course No. : BIOT F244
Date: 11-04-2013	Total marks: 10	Weightage: 10%
NAME:		ID NO:

1. Define Emission spectroscopy. [1]

Emission spectroscopy is a spectroscopic technique which examines the wavelengths of photons emitted by atoms or molecules during their transition from an excited state to a lower energy state.

2. Explain the phenomenon of fluorescence with an example. [2]

The molecule absorbs energy, gets excited and returns back to the ground state by losing the energy it has acquired through conversion of the excess electronic energy into **vibrational energy**. The energy emitted is of **lower energy** than the energy absorbed because the excited electron moves to the lowest energy **vibrational state** in the excited state before returning to the ground state. Thus fluorescence emission is typically shifted by 50-150 nm (***Stokes shift***) to the longer wavelength in comparison to the wavelength of the radiation used to produce excitation.

Eg: NADH absorbs at 340nm and emits at 465nm, Quinine absorbs at 350nm and emits at 450nm

3. Explain the consequence on fluorescence of a molecule when the temperature of the solution is increased from 28°C to 45°C. [2]

Fluorescent property decreases with increasing temperature because the increased frequency of collision at elevated temperatures increases the probability of collisional relaxation.

4. Define the term Plane Polarized Light. [1]

The vibrations may all be restricted to one direction only, in the perpendicular plane .....**PLANE POLARIZED LIGHT.**

5. List the factors which influence the optical rotation of a chiral compound. [2]

Nature of the substance, Length of liquid column, Conc. Of the solution, Nature of the solvent, Temperature of the solution, WL of the light used.

6. The optical rotation of pure α-glucose is +112.2. A new purification plant recovered the first batch of glucose and found it was only 25% pure. The manufacturers worked on the process controls to increase the purity, which now exhibited an optical rotation of +52.5. Were the manufacturers capable of improving the purification process in the new plant. What do you infer from the new optical rotation value that is obtained?. [2]

At 25% purity the OR is +28.05.

The process is now better and high purity sample has probably been recovered, but due to mutarotation the OR value has reached an equilibrium of +52.5, which signifies conversion of some α form to β form of glucose.

BITS Pilani Dubai Campus
Dubai International academic City
First Semester 2012-2013
Quiz 1

Course: INSTRUMENTAL METHODS OF ANALYSIS		Course No. : BIOT F244
Date: 19-03-2013	Total marks: 10	Weightage: 10%
NAME:		ID NO:

1. Define the following with an example [2]

a. Analytical Method:

b. Hypsochromic Shift:

2. What are signal transformation modules? Give an example of the same. [2]

3. Explain with an example the effect of compound geometry on UV-Visible spectroscopy. [2]

4. Calculate the energy associated with a light of wavelength 345nm. [2]

5. What is Rotational Spectroscopy? Which range of the Electromagnetic radiations is studied using Rotational Spectroscopy? [2]

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BITS Pilani Dubai Campus
Dubai International academic City
First Semester 2012-2013
Quiz 1 ANSWERKEY

Course: INSTRUMENTAL METHODS OF ANALYSIS		Course No. : BIOT F244
Date: 19-03-2013	Total marks: 10	Weightage: 10%
NAME:		ID NO:

1. Define the following with an example [2]

a. Analytical Method:

it is a specific application of a technique to solve an Analytical problem.

Eg: the infrared analysis of styrene-acrylonitrile copolymers is an example of an instrumental method.

b. Hypsochromic Shift:

Shifting of λmax to lower value or left hand side due to substitution, solvent, pH etc is called as Hypsochromic shift. e.g. λmax of Phenol in basic media=297nm, λmax of Phenol in acidic media=277nm.

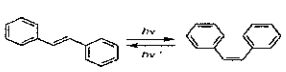
2. What are signal transformation modules? Give an example of the same. [2]

These are electronic components that receive information from the detector, perform necessary and desirable operations, such as amplification and filtering of the signal from the input transducer. These present the information to the output transducer.

Eg: Amplification, Analog -to-digital conversion, Current-to-voltage conversion, Digital-to-analog conversion, Voltage-to-current conversion,

3. Explain with an example the effect of compound geometry on UV-Visible spectroscopy. [2]

e.g. stilbene. Trans-stilbene absorbs at a longer wavelength than cis-stilbene due to stearic effects. Co-planarity is needed for the most effective overlap of the π-orbitals. The cis-isomer is forced in to a non planar conformation due to steric effects. The cis isomer are twisted slightly out of plane by steric interactions so that the degree of conjugation in the π system is slightly less than the trans isomers, resulting in greater energy for the transitions.



4. Calculate the energy associated with a light of wavelength 345nm. [2]

$E = h\nu = \frac{hc}{\lambda}$

therefore, E = (6.62 x 10^{-34} J sec * 3 x 10^{8} m/s)/ 345 or E= (4.14 x 10^{-15} eV s * 3 x 10^{8} m/s)/ 345

E = 5.75 x 10^{-28} j or E = 3.6 x 10^{9} eVs

5. What is Rotational Spectroscopy? Which range of the Electromagnetic radiations is studied using Rotational Spectroscopy? [2]

Rotational spectroscopy measures the absorption or emission of light by molecules in order to understand changes in their rotational energy. The energy difference between the rotational energy levels is small relative to the energy difference between vibrational levels. These transitions are brought about by radiation in the 0.01 to 1cm wavelength range, which include microwaves and longer wavelength infrared radiation.

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