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Kaplan: Clinical Chemistry, 5th Edition

Chapter 2: Spectral Techniques

Test Bank

MULTIPLE CHOICE

1. The wavelength of electromagnetic radiation is:
1. directly proportional to the energy of a photon
2. inversely proportional to the energy of a photon
3. directly proportional to the frequency of a photon
4. inversely proportional to the frequency of a photon
a. 1, 3
b. 2, 4
c. 1, 4
d. 2, 3

ANS: B
The equation $\upsilon = c/\lambda$ shows the inverse relationship between wavelength and frequency, and the equation $E = hc/\lambda$ shows the inverse relationship between energy and wavelength.

DIF: 2 REF: 46

2. Once a molecule absorbs electromagnetic radiation, it becomes unstable and spontaneously returns to its unexcited, or ground, state by which of the following processes?
a. release of kinetic energy
b. release of vibrational energy
c. release of light energy
d. all of the above

ANS: D
As a molecule in solution absorbs light and becomes excited, molecular motion and collisions with solvent molecules increase, resulting in a loss of energy in the form of kinetic energy. There will also be vibrational energy loss associated with adjustments in bond angles and lengths. In addition, some molecules may release a portion of excess energy in the form of electromagnetic (or light) energy, which is known as *fluorescence*.

DIF: 1 REF: 47

3. Absorbance is __________ related to percent transmittance.
a. directly
b. inversely
c. directly and logarithmically
d. inversely and logarithmically

ANS: D

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Referring to Figure 2-3, absorbance and percent transmittance are inversely and logarithmically related.

DIF: 1 REF: 48-49

4. What are the units for ***a*** (absorptivity) in Beer's law when ***c*** (concentration) is expressed in g/dL, and ***b*** (path length) is expressed in cm?
   a. cm/g•dL
   b. g/dL•cm
   c. dL/g•cm
   d. absorptivity is a unitless term

ANS: C
In Beer's law, **A = abc**, ***A*** (Absorbance) is a unitless term. Therefore, the units for absorptivity ***(a)*** must be the reciprocal of the units for ***b*** (path length) and ***c*** (concentration). Given that ***c*** is expressed as g/dL, and ***b*** is expressed as cm, the combination of these units gives g•cm/dL. The reciprocal is dL/g•cm.

DIF: 2 REF: 49

5. The percent transmittance of a solution is 50%. Calculate the absorbance.
   a. 3.7
   b. 1.7
   c. 0.7
   d. 0.3

ANS: D
Use the equation A = 2-log%T to determine the absorbance. Solving, A = 2-log 50; A = 2-1.7; A = 0.3

DIF: 2 REF: 49

6. If a glucose standard has an absorbance of 0.480, and an unknown has an absorbance of 0.120, which of the following best describes the unknown's glucose concentration?
   a. one fourth the standard concentration
   b. equal to the standard concentration
   c. four times the standard concentration
   d. not enough data to calculate

ANS: A
Use the equation $C_u = A_u/A_s \times C_s$ to answer the question. Inserting data from the question, $C_u = .120/.480 \times C_s$. This is equivalent to $C_u = 1/4 \times C_s$. Thus C_u equals one fourth the standard concentration.

DIF: 2 REF: 49

7. A solution of chloramphenicol (MW 323.1) in water gave an absorbance of 0.610 at 278 nm in a 1-cm cuvette; the concentration was 0.02 mg/mL. What is the molar absorptivity of chloramphenicol?

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a. 30.5 L/mol•cm
b. 3050 L/mol•cm
c. 9855 L/mol•cm
d. 11241 L/mol•cm

ANS: C
Use the equation $A = abc$, with ***a*** defined as molar absorptivity. To give ***A*** (Absorbance) as a unitless term, when solving for molar absorptivity, ***c*** (concentration) must be expressed in terms of mol/L and ***b*** (path length) must be expressed in terms of cm. In the question, ***b*** is given in terms of cm, but ***c*** is given in terms of mg/mL. Therefore, the first step is to convert mg/mL to mol/L, using the molecular weight:
$0.02 \text{ mg/mL} = 0.02 \text{ g/L} \times 1 \text{ mol}/323.1 \text{ g} = 6.19 \times 10^{-5} \text{ mol/L}$
Once all the correct units are obtained, plug into Beer's law: $0.610 = a(1 \text{ cm})(6.19 \times 10^{-5} \text{ mol/L})$.
a = 9855 L/mol•cm

DIF: 2 REF: 49

8. Any radiant energy that is measured by the detector but outside the spectral region isolated by the monochromator of the instrument is referred to as ______. As this increases, Beer's law linearity will ______.
a. Rayleigh scatter, increase
b. stray light, decrease
c. transmitted light, increase
d. polychromatic light, decrease

ANS: B
Stray light is radiant energy that reaches the detector at wavelengths other than those isolated by the monochromator and passed through the sample. Since stray light reaches the detector without passing through the sample, there will be deviation from Beer's law, and linearity will decrease. See Figure 2-5.

DIF: 2 REF: 50

9. The light source most frequently used for spectrophotometric analysis in the UV region is a ______ lamp and in the visible region, a _____ lamp.
a. tungsten, mercury vapor
b. deuterium, tungsten
c. mercury vapor, deuterium
d. tungsten, deuterium

ANS: B
Deuterium lamps have a maximal output in the UV region, with light output dropping off as the visible region begins. Tungsten lamps have suitable output in the visible region but not in the UV region.

DIF: 1 REF: 50

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10. Suppose the absorbance for a known solution was calculated to be 0.333. The solution was measured on a spectrophotometer, and the measured absorbance was 0.321. The solution was prepared again and gave the same absorbance reading of 0.321. Which of the following explanations would most accurately explain the difference between the calculated absorbance and the measured absorbance?
    a. Calculated absorbance is only an estimate of the true absorbance measured by spectrophotometry.
    b. The molar absorptivity of the compound changes over the bandpass of the spectrophotometer.
    c. There may have been an error in the preparation of the solution measured by spectrophotometry.
    d. Measured absorbance is only an estimate of the true absorbance determined by mathematical calculations.

    ANS: B
    The bandpass is a range of wavelengths that pass through the sample and are measured by the detector. If the molar absorptivity changes over the bandpass of the instrument, which is common, there is less absorbance observed than expected for the concentration of absorber present. This condition results in lower than expected absorbance because the observed absorbance is the integral of absorbance over the band of wavelengths actually passing through the cuvette.

    DIF: 3 REF: 52

11. What would happen to the percent transmittance of a sample that was read in a cuvette with fingerprints on the optical surface?
    a. %T would increase
    b. %T would decrease
    c. %T of quartz cuvettes would not be affected by fingerprints
    d. %T of glass cuvettes would not be affected by fingerprints

    ANS: B
    Fingerprints on the optical surface of cuvettes will block some degree of light from passing through the cuvette. Thus %T would decrease.

    DIF: 2 REF: 52

12. As part of the development of a new spectrophotometric assay, an absorption spectrum was recorded. Which of the following describes the purpose for this absorption spectrum?
    a. to determine the appropriate wavelength of analysis for the assay
    b. to determine the maximal concentration that can be accurately measured for the assay
    c. to check for stray light
    d. to check for linearity

    ANS: A

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An absorption spectrum is a record of the absorbance of the compound of interest at each wavelength of the spectrum. From this spectrum, the wavelength of maximum absorption can be determined. The wavelength of maximum absorption is the desired wavelength for the assay, because this is where the assay will be the most sensitive.

DIF: 2 REF: 54

13. Holmium oxide and didymium filters are used to perform which of the following quality-control checks on spectrophotometers?
    a. wavelength accuracy
    b. linearity of detector response
    c. stray light
    d. photometric accuracy

ANS: A
Holmium oxide and didymium are rare-earth glass filters that have specific wavelengths of absorption. Measuring these known wavelengths of absorption with a spectrophotometer allows a check on the accuracy of the wavelength of the spectrophotometer.

DIF: 2 REF: 55

14. In UV/Visible spectrophotometry, absorption spectra consist of relatively broad bands of absorption, whereas in atomic absorption spectrophotometry, line spectra consist of lines of absorption that are on the order of 0.001 to 0.01 nm in width. Which of the following provides the correct explanation for this difference in width of absorption bands?
    a. The methods of detection in atomic absorption spectrophotometry are much more sensitive than those utilized in UV/Visible spectrophotometry.
    b. Atomic absorption methods measure absorption characteristics of vaporized atoms, whereas UV/Visible techniques measure absorption characteristics of molecules with many bonds and chromophoric groups.
    c. The use of the flame in atomic absorption methods removes the matrix which causes broad absorption bands in UV/Visible techniques.
    d. The use of the flame in atomic absorption methods removes chemical interferences that would otherwise broaden absorption bands.

ANS: B
In UV/Visible spectrophotometry, the absorption pattern of a complex organic molecule containing multiple bonds is the sum of the absorption of all of the individual bonds, resulting in broad bands of absorption. In atomic absorption spectrophotometry, individual vaporized atoms are measured and absorb light at very specific wavelengths, causing absorption bands to be extremely narrow.

DIF: 2 REF: 57

15. The flame in atomic absorption spectrophotometry is used to:
    a. provide excitation energy for the atom being measured
    b. dissociate the ionic form of the element from its chemical bonds

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c. place the ionic form of the element in the atomic ground state
d. both b and c

ANS: D
In atomic absorption spectrophotometry, the ionic form of the element is not excited by the flame but is dissociated from its chemical bonds. By attracting free electrons produced by the flame, it is placed in the atomic ground state. Energy provided by the hollow cathode lamp is absorbed by the ground state atom.

DIF: 2 REF: 57

16. In the clinical laboratory, the primary problem with the use of fluorescent techniques is that:
a. this technique is not a very sensitive technique as compared to other spectrophotometric techniques.
b. the flame may not be hot enough to totally dissociate some of the complexes.
c. many compounds do not fluoresce in their natural state.
d. all of the stray light produced in the instrument cannot be filtered out.

ANS: C
For fluorescence to occur, there must be a high probability that the energy of the excited state can be converted to ground state by the ejection of a photon. Not all compounds fluoresce; indeed, only a very few fluoresce.

DIF: 2 REF: 58

17. A linear relationship between concentration and fluorescence intensity is seen as long as solutions being quantified have an absorbance of:
a. less than 0.100
b. less than 0.500
c. greater than 0.500
d. greater than 1.000

ANS: A
Linearity between concentration and fluorescence intensity is maintained as long as the concentration of the solution being measured is maintained at a sufficiently dilute concentration so as to have an absorbance of less than 0.100. Solutions with concentrations above 0.100 are concentrated to the point where all portions of the solution do not absorb light equally. As a result, all portions of the solution do not fluoresce equally. This will cause the relationship between concentration and fluorescence intensity to become nonlinear. This phenomenon is known as the *inner filter effect*.

DIF: 2 REF: 59

18. ______ techniques measure light scattered by a particulate solution, and ______ techniques measure a decrease in light transmission through a particulate solution. _____ measurements can be performed on a basic UV/Visible spectrophotometer.
a. turbidimetric, nephelometric, nephelometric

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b. turbidimetric, nephelometric, turbidimetric
c. nephelometric, turbidimetric, nephelometric
d. nephelometric, turbidimetric, turbidimetric

ANS: D
Nephelometric techniques measure the portion of light that is scattered at an angle away from the incident beam of light. Turbidimetric techniques measure a reduction in light transmission through a solution. Nephelometric measurements require a specialized instrument with the detector at a fixed angle away from the incident beam of light in order to detect scatter. Turbidimetric measurements can be made on a traditional UV/Visible spectrophotometer, since this technique measures a decease in the amount of light transmitted.

DIF: 1 REF: 63

19. In comparing nephelometry and turbidity, nephelometric measurements should demonstrate _______ sensitivity, and turbidimetric measurements should demonstrate _______ sensitivity. In practice, turbidimetric measurements have been found to demonstrate _______ sensitivity.
a. high, limited, high
b. high, limited, limited
c. limited, high, high
d. limited, high, limited

ANS: A
The principle of nephelometry should lend itself to high sensitivity. However, available instrumentation has not been able to achieve this sensitivity. On the other hand, based on the theory of turbidity, this technique should have limited sensitivity. However, currently available instrumentation has excellent discrimination and can quantify small changes in signal, allowing turbidimetric measurements to achieve high sensitivity.

DIF: 3 REF: 64

20. A system in which the light intensity of the wavelength produced by each atom is directly proportional to the number of atoms emitting energy, which in turn is directly related to the concentration of the compound being measured, describes:
1. atomic absorption spectrophotometry
2. atomic emission flame photometry
3. UV/Visible spectrophotometry
4. fluorometry
a. 1, 2, 3, 4
b. 1, 2, 3
c. 1, 3
d. 2, 4

ANS: D

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Atomic emission flame photometry and fluorometry measure the intensity of light energy emitted. Atomic absorption and UV/Visible spectrophotometry measure the amount of light absorbed.

DIF: 2 REF: 49, 57, 58

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