Chapter 13

Textbook questions

13-13

描述以下選項之間的不同以及寫出其優點 (是另一項所沒有的)

a) Hydrogen and deuterium discharge lamps as sources for UV radiation.
b) Filters and monochromators as wavelength selectors.
c) Photovoltaic cells and phototubes as detectors for electromagnetic radiation.
d) Photodiodes and photomultiplier tubes.
e) Double beam in space and double bean in time spectrophotometers.
f) Spectrophotometers and photometers.
g) Single beam and double beam instruments for absorbance measurements.
h) Conventional and multichannel spectrophotometers.

13-13

Answer:

(f) Spectrophotometers and photometers

(f) *Spectrophotometers* have monochromators or spectrographs for wavelength selection. Photometers generally have filters and may use an LED source for wavelength selection. The spectrophotometer can be used for wavelength scanning or for multiple wavelength selection. The photometer is restricted to one or a few wavelengths.

(g) Single beam and double beam instruments for absorbance measurements.

(g) A *single-beam* spectrophotometer employs one beam of radiation that irradiates one cell. To obtain the absorbance, the reference cell is replaced with the sample cell containing the analyte. With a *double-beam instrument*, the reference cell and sample cell are irradiated simultaneously or nearly so. Double-beam instruments have the advantages that fluctuations in source intensity are cancelled as is drift in electronic components. The double-beam instrument is readily adapted for spectral scanning. Single-beam instruments have the advantages of simplicity and lower cost. Computerized versions are useful for spectral scanning.

13-13

Answer:

(h) Conventional and multichannel spectrophotometers.

(h) *Multichannel spectrophotometers* detect the entire spectral range essentially simultaneously and can produce an entire spectrum in one second or less. They do not use mechanical means to obtain a spectrum. *Conventional spectrophotometers* use mechanical methods (rotation of a grating) to scan the spectrum. An entire spectrum requires several minutes to procure. Multichannel instruments have the advantage of speed and long-term reliability. Conventional spectrophotometers can be of higher resolution and have lower stray light characteristics.

13-16

為什麼氘燈 (deuterium lamp) 在 UV 中是產生連續光譜而不是線譜?

Answer:

在氘燈中，電源的燈能量產生激發態的氘分子，氘分子會解離為基態的兩個原子和輻射的光子。 隨著激發態的氘分子弛豫 (relaxation)，其量化的能量分佈在光子和兩個原子的能量之間。後者可以幾乎從零到激發分子的能量變化。因此輻射的能量，即激發態分子的量子化能量與原子的動能之差，也可以在同一範圍內連續變化。因此，放射出的光譜是連續光譜。

$$D_2 + E_e \rightarrow D_2^* \rightarrow D' + D'' + h\nu$$

$$E_e = E_{D_2^*} = E_{D'} + E_{D''} + h\nu$$

可參考課本 p.315

13-16

為什麼光電倍增管不能用於紅外光輻射?

光電倍增管
一種具有高靈敏度與超快響應時間的光探測元件，在一般典型的光電倍增管中，在其響應範圍最佳的近紅外光區到紫外光區，可以將只有數百個光子的光訊號轉換為有用的脈衝電流，進而利用此脈衝電流來做訊號的分析。

Answer:

因為紅外光輻射所產生的光子沒有足夠的能量可以讓光電倍增管產生光電發射(photoemission)

http://www.phys.ncku.edu.tw/~optlab/modernoptics/lock-in%20amplifier/2.1.htm

Question 13-18

Why is iodine sometimes introduced into tungsten lamp?

Tungsten/halogen lamps often include a small amount of iodine in the evacuated quartz envelope that contains the tungsten filament. The iodine prolongs the life of the lamp and permits it to operate at a higher temperature. The iodine combines with gaseous tungsten that sublimes from the filament and causes the metal to be redeposited, thus adding to the life of the lamp.

有時鎢絲燈 (白熾燈) 的玻璃中會填入碘氣體，稱為鹵素燈，可增加發光效率及使用壽命。鎢原子 (tungsten) 被蒸發後和碘結合形成碘化鎢。碘化鎢在燈泡中循環並回到被氧化的燈絲上，遇熱後又重新分解成碘和鎢，鎢又可在燈絲上沉積下來，延長燈泡壽命。

Chapter 15

Textbook questions

Question 15-1

Explain the difference between a fluorescence emission spectrum and a fluorescence excitation spectrum.

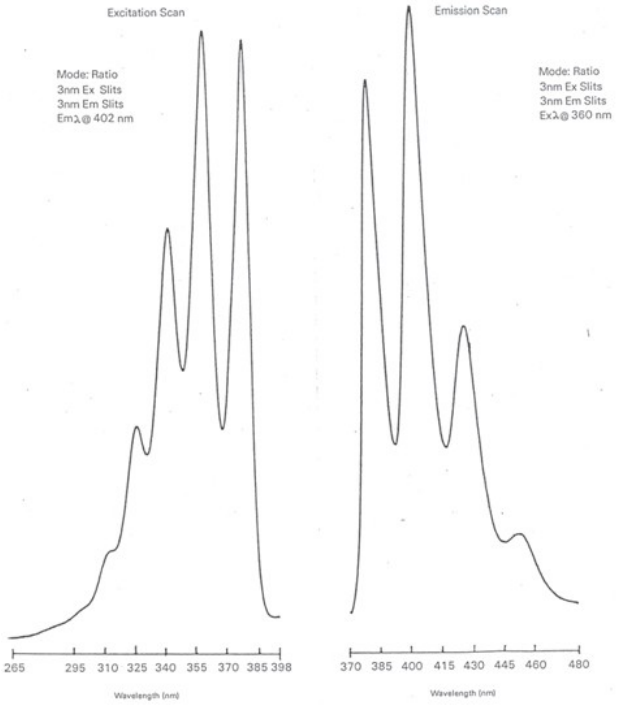
Which is more closely resembles an absorption spectrum?

15-1. In a fluorescence emission spectrum, the excitation wavelength is held constant and the emission intensity is measured as a function of the emission wavelength. In an excitation spectrum, the emission is measured at one wavelength while the excitation wavelengths are scanned. The excitation spectrum yields those wavelengths that are responsible for fluorescence emission which are those that absorb radiation.

Emission spectrum: 固定 excitation wavelength 掃描不同波長 emission light 的強度

Excitation spectrum: 固定 emission wavelength 掃描不同波長 excitation light 的強度

Excitation spectrum 較接近吸收光譜，因為分子吸收了 excitation light 後放出 emission light



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Question 15-2

Define the following terms

(a) Fluorescence

The process in which a molecule, excited by the absorption of radiation, emits a photon while undergoing a transition from an excited singlet electronic state to a lower state of the same spin multiplicity (e.g., a singlet → singlet transition).

(b) **Phosphorescence**

The process in which an excited molecule emits a photon while undergoing a transition from an excited triplet state to a lower state of a different spin multiplicity (e.g., a triplet → singlet transition).

(c) **Resonance fluorescence**

Can be observed when an excited species emits radiation of the same frequency as that used to cause the excitation.

(d) A **singlet state**

One in which the spins of the electrons of an atom or molecule are all paired so there is no net spin angular momentum

(e) A **triplet state**

One in which the spins of the electrons of an atom or molecule are unpaired so that their spin angular moments add to give a net non-zero moment.

(f) **Vibrational relaxation**

The process by which a molecule loses its excess vibrational energy without emitting radiation.

(g) **Internal conversion**.

The intermolecular process in which a molecule crosses to a lower electronic state with emitting radiation.

(h) **External conversion**

A radiationless process in which a molecule loses electronic energy while transferring that energy to the solvent or another solute.

(i) Intersystem crossing

The process in which a molecule in one spin state changes to another spin state with nearly the same total energy (e.g., singlet → triplet).

(j) **Predissociation**

Occurs when a molecule changes from a higher electronic state to an upper vibrational level of a lower electronic state in which the vibrational energy is great enough to rupture the bond.

(k) **Dissociation**

Occurs when radiation promotes a molecule directly to a state with sufficient vibrational energy for a bond to break.

(l) **Quantum yield**

The fraction of excited molecules undergoing the process of interest. For example, the quantum yield of fluorescence is the fraction of molecules absorbing radiation that fluoresce.

(m) **Chemiluminescence**

A process by which luminescent radiation is produced as a result of a chemical reaction

Question 15-3

Why is spectrofluorometry potentially more sensitive than spectrophotometry?

For spectrofluorometry, the analytical signal F is proportional to the source intensity P_0 and the transducer sensitivity.

In spectrophotometry, the absorbance A is proportional to the ratio of P_0 to P. Increasing P_0 or the transducer sensitivity to P_0 produces a corresponding increase in P or the sensitivity to P. Thus the ratio does not change.

As a result, the sensitivity of fluorescence can be increased by increasing P_0 or transducer sensitivity, but that of absorbance does not change.

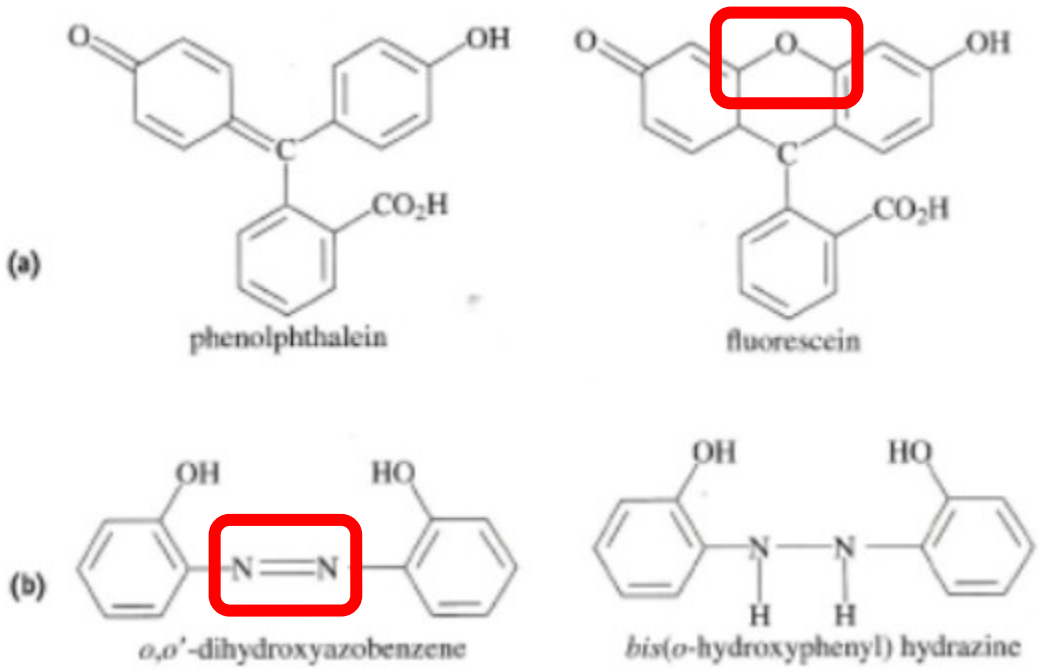
螢光訊號與光源強度成正比，同時也與傳感器 (transducer) 的靈敏度成正比

分光光度法中，吸收度 A 指的是光源強度與通過 sample 的光強度的比例，增加光強度或增加傳感器靈敏度的同時只會增加通過 sample 的光，無助增強吸收度訊號

因此在我們可簡單的藉由增加光源強度與傳感器靈敏度增加螢光光譜法的靈敏度，但分光光度法無法藉由此方法增加

Question 15-4

Which compound in each of the following pairs would you expect to have a greater fluorescence quantum yield? Explain.



Keyword 關鍵字 : rigidity

a) Fluorescein
b) o,o´-Dihydroxyazobenzene

(a) Fluorescein because of its greater structural rigidity due to the bridging –O– groups.

(b) *o,o´*-Dihydroxyazobenzene because the –N=N– group provides rigidity that is absent in the –NH–NH– group.

Question 15-5

Why do some absorbing compound fluoresce but others do not?

Compounds that fluoresce have structures that slow the rate of nonradiative relaxation to the point where there is time for fluorescence to occur.

Compounds that do not fluoresce have structures that permit rapid relaxation by nonradiative processes.

Question 15-6

Discuss the major reasons why molecular phosphorescence spectrometry has not been as widely use as molecular fluorescence spectrometry?

The triplet state has a long lifetime which makes it susceptible to collisional deactivation.
Thus, most phosphorescence measurements are made at low temperature in a rigid matrix or in solutions containing micelles.
Also, electronic methods must be used to discriminate phosphorescence from fluorescence.
Not as many molecules give good phosphorescence signals as fluorescence signals.
As a result, the experimental requirements to measure phosphorescence are more difficult than those to measure fluorescence and the applications are not as large.

訊號弱、反應時間慢、易將能量傳給 solvent ，所以不好測

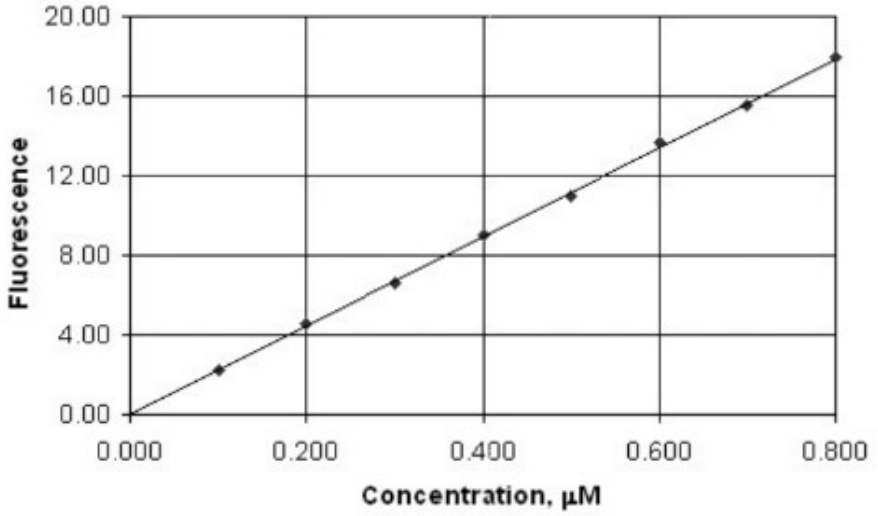
15-7

The reduced form of NADH is an important and highly fluorescent coenzyme. It has an absorption maximum of 340 nm and an emission maximum at 465 nm. Standard solutions of NADH gave the following fluorescence intensities

	Intensity / a.u.
0.100	2.24
0.200	4.52
0.300	6.63
0.400	9.01
0.500	10.94
0.600	13.71
0.700	15.49
0.800	**17.91**

A) Construct a spreadsheet and use it to draw a calibration curve for NADH
B) Find the least squares slope and intercept for the plot
C) Calculate the standard deviation of the slope and the standard deviation about regression for the curve
D) An unknown exhibit a relative fluorescence intensity of 12.16. Use the spreadsheet to calculate the concentration of NADH
E) Calculate the relative standard deviation for the result in (D)
F) Calculate the relative standard deviation for the result in (D) if the result of 7.95 was the mean of three measurements

	A	B	C	D	E	F	G	H	I
1	**Determination of NADH**								
2	Part (a)								
3	Concentration in μM	Fluorescence							
4	0.100	2.24							
5	0.200	4.52							
6	0.300	6.63							
7	0.400	9.01							
8	0.500	10.94							
9	0.600	13.71							
10	0.700	15.49							
11	0.800	17.91							
12	unknown	12.16							
13	Part (b)								
14	**Regression equation**								
15	Slope	22.3464							
16	Intercept	3.571E-04							
17	Concentration of unknown	0.544							
18	Parts (c), (d), (e), and (f)								
19	**Error Analysis**								
20	s_r (standard error in y)	0.175							
21	N	8							
22	S_{xx}	0.42							
23	s_m	0.27							
24	y bar (average fluoresence)	10.056							
25	M for part (e)	1							
26	M for part (f)	3							
27	Standard deviation in c for part (e)	0.008							
28	RSD in c for part (e)	0.015							
29	Standard deviation in c for part (f)	0.005							
30	RSD in c for part (f)	0.010							
31	**Spreadsheet Documentation**								
32	Cell B15==SLOPE(B4:B11,A4:A11)		Cell B24 =AVERAGE(B4:B11)						
33	Cell B16=INTERCEPT(B4:B11,A4:A11)		Cell B25= Replicates part (e) (entry)						
34	Cell B17=(B12-B16)/B15		Cell B26=Replicates part (f)						
35	Cell B20=STEYX(B4:B11,A4:A11)		Cell B27 =B20/B15*SQRT(1/B25+1/B21+((B12-B24)^2)/((B15^2)*B22))						
36	Cell B21=COUNT(B4:B11)		Cell B28=B27/B17						
37	Cell B22=B21*VARP(A4:A11)		Cell B29=B20/B15*SQRT(1/B26+1/B21+((B12-B24)^2)/((B15^2)*B22))						
38	Cell B23=SQRT(B20^2/B22)		Cell B30=B29/B17						



(b) $F = 22.35c + 3.57 \times 10^{-4}$ (c) 0.27, 0.175

(d) 0.544 µM (e) 0.015% (f) 0.010%