實驗九: 以螢光分光光譜儀測定Quinine的 含量

注意事項:

器材:1.實驗器材放置於窗戶邊櫃,用完需洗淨放回原櫃內。

 定量瓶與測光管置於共用器材籃中,用完洗淨歸位排 列整齊。

3. 藥品用完需蓋上蓋子,取藥用的吸量管亦須清洗乾淨。 步驟: 1. 要先稀釋0.5M H₂SO₄作為Blank與稀釋溶液。

2. 溶液1不需配置,直接取1ppm Quinine溶液

3. 溶液2~7才須配置並混合均匀

4. 依序放入测光管並用拭鏡紙將表面水分擦乾

5. 螢光之參數須依實驗步驟指示設定

數據紀錄:

- 1. 實驗中需影印『激發(Ex)』波長與『放射(Em)』波長圖譜, 為節省紙資源,正反面列印。
- 表格需填寫完整後給助教檢查簽名,無誤後才可清洗器材, 整理清潔實驗桌面與器材後,始可簽退離開。

螢光分光光譜儀操作

₩ 1	Analysis Method	
F-2500 FL Sp	General Instrument Monitor Processing Report	
900-4	Measurement Wavelength scan	G
800-	Operator: User	1
700-	Instrument F-2500 FL Spectrophotometer	A
600-	Sampling: None	Pr
500-	Comments:	
400-		Me
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200-	Use sample table	
100-	(Set measurement sample)	1
- The second sec		CI

選擇畫面右工具列上的method general (上方分頁) 設定: measurement: wavelength scan

•以1ppm的Quinine作為測量最大吸收與放射波長的標準液

螢光分光光譜儀操作

FL Solutions -	F-2500 FL Spectroph	otometer on C	OM1				
e <u>E</u> dit <u>V</u> iev	Spectrophotometer	<u>Tools</u> <u>U</u> til	lity <u>W</u> indow	Help			
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	inalysis Method	11.	a 1 m 1			×	
F-2500 FI	General Instrument	t Monitor P	rocessing Re	port			Method
000 4	Scan mode:	Excitation		EX Slit:	nm	1.033	
500-	Data mode:	Fluorescend	e 💽	EM Slit:	▼ nm	nm	Sample
800-	EM WL:	450	nm	PMT Voltage:	- ▼	nm	ABC
700-	EX Start WL:	300	nm	Response:	T s	nm nm	Pre Scan
600-	EX End WL:	600	nm	Corrected spectra			2:5
500-	EX WL:	348	nm				Measure
400-	EM Start WL:	300		Replicates:	1		
100	EM End WL:	600	TITL	Cycle time:	0 🚽 min		STOP
300-7	Scan speed:	1500	💌 nm/min				
200-	Delay:	0	∃ s				Close
100-						E	BOB
0				確定取消		<u> </u>	
300	350	400	450	500 550	600 nm		

instrument設定:

Scan mode : excitationData mode : fluorescenceEm WL : 450nmEx Start WL : 300nmEx End WL : 600nmScan speed : 1500設定完後按確定 選擇畫面右工具列的Measure進行掃描

螢光分光光譜儀操作



在此視窗下選擇上方工具列 <u>色</u> 自動範圍調整並找出最大的peak height的波長,紀錄Apex欄位的數值,即為最大激發波長並抄下

螢光分光光譜儀操作

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Scan mode	Emainion	EX She	-	
Data mode	Finozeicence	EM Sht	- nm	
EMANL	430	PMT Voltage	- v	
EX Area Th	300 775	Response	-	1 6
EX EAS TH	600	Corrected spectra		
EX WL	340 80			1 (
EM Shut WL	300 Jun	Replicates 1		
EM End WL	600 mm.	Code tax	-1 mm	
Scan speed.	1500 • nav/min		No. of Concession, No.	
Delay:	0 +			

回到掃描視窗 選擇畫面右上的method instrument 設定: Scan mode: emission Data mode: fluorescence EXWL:輸入最大激發波長(輸入前面所記錄的最大波長值) EM Start WL: 300nm EM End WL: 600nm Scan speed: 1500

螢光分光光譜儀操作



在此視窗下選擇上方工具列 <u>免</u> 自動範圍調整並找出最大 peak height的波長,紀錄Apex欄位的數值,即為最大激發波 長並抄下

螢光分光光譜儀操作

nalyzzs Method	nalysis Method	General Quantitation Instrument Monitor Report		
General Quantitation Instrument Monitor Repo	General Quantitation Instrument Monitor Report	Data mode: Emoretcence		
Measurement Cholometry	2	Wavelength mode: EX WL Fixed		
Operator user	Quannation type: Wavelength Y Number	EX. EM		
Instrument F-2500 FL Spectrophotometer	Calibration None 💌	WL 1. 402 4511 nm		
		WL 2. 400 400 nm		
Sampling: Note		WL 3: 500 500 nm		

按 method

general(上方分頁)設定:measurement:photometry quantitation(上方分頁)設定:calibration:none instrument設定: wave length mode:Ex WL Fixed Fixed WL EX:輸大激發波長 WL 1 EM:輸入最大放射波長 按確定,回測定畫面

螢光分光光譜儀操作

F-2500 FL Spectrophotometer on COM1	- IX Method
Samp.No. 348.0/451.0 1 1.904 2 421.0 3 230,1	Fluorescence: 1259 EX: 3480 nm EM 4510 nm EX Sht 100 nm EM Sht 100 nm
Sample (F4) D Blank (F5) Interrupt (F7) Remeasure (F8) End (F9)	Shutter: Open Lamp: ON Sample: 3 Replicate: 1

放入blank後,選擇上方工具列金歸零需約1分鐘,再選擇畫面右下角的**Measure**,跳出測定畫面按**sample**(**F4**)鍵測量Blank值,抄下數據。之後依序量測溶液1-7之數值。

螢光分光光譜儀數值計算

- •將數據表格填寫完整後給助教檢查簽名
- •以標準溶液之螢光強度 vs. 濃度作圖,求出校正曲線及 unknown濃度