MALAYSIAN JOURNAL OF SCIENCE
MJS
ISSN 1394-3065

Regular Issue

Malaysian Journal of Science 44 (1): 87-92 (March 2025) https://mjs.um.edu.my

Enhancing the Spectroscopic Properties of Rhodamine B Via the Nano-Concentration Effect

Hanan Auda Naif[1a]*, Ruba Al-Obaidi[2a] and Oday M. Abdulmunem[3a]

Abstract: Developing nano-liquid materials with ultra-low concentrations leads to new or improved methods, as well as resolving issues in previous studies. Nano-liquid materials require special treatment because they are very sensitive materials. Many techniques have been developed, including the optical cavity technique, which depends on increasing the path length of the light beam between two dielectric mirrors to obtain more accurate and sensitive measurements. This method also provides beneficial information about the chemical composition. In this study, broadband cavity-enhanced absorption spectroscopy was used at a range of visible wavelengths to obtain spectra of rhodamine B ($C_{28}H_{31}ClN_2O_3$) at room temperature. The liquid phase of rhodamine B was chosen because it is the most complicated and volatile phase. The spectral analyses showed the fine structure of the aqueous solution of rhodamine B and the different molecular dynamics. The processes of the electron dynamics inside the molecules also changed at the ultra-low sample concentrations achieved working at the nanomolar scale. Combining experimental and data analysis via simulation programs has many benefits, such as reducing the time needed to study the materials, as it presents a typical design with fewer issues. In addition, costly, scarce, or difficult-to-store materials should be studied at low concentrations, and these combined studies can yield results without using these materials. The novelty of our research is the successful study of low concentrations of liquid samples. The high quality of the data, demonstrated by the goodness-of-fit parameters, allows for further analyses. Spectral analysis of nano-concentrations of rhodamine B shows new multiphoton absorption processes that drive the shifts in peak intensity. The solvent interaction effects caused changes in the binding energy states of the molecular structure of the sample. Here, we present a new spectral analysis of rhodamine B in aqueous solution using the broadband cavity-enhanced absorption spectroscopy (BBCEAS) technique.

Keywords: *Broadband cavity, nano-concentrations, Rhodamine B, enhanced absorption spectra, simulation program*

1. Introduction

Earlier investigations have focused on studying molecular structures with optical cavity techniques, such as cavity ring-down spectroscopy (CRDS), cavity-enhanced absorption spectroscopy (CEAS), and broadband cavity-enhanced absorption (BBCEAS), which is the main improvement obtained using a broadband light source. Engeln et al. (1998) have investigated using a narrow band light source with a high-finesse optical cavity to obtain high-resolution optical absorption spectra through the accidental synchronisation of the laser frequency with the cavity frequency, which led to extracting the absorption and polarisation rotation. This study presented the spectra of oxygen, ammonia, and water in a cell and the spectra of molecular oxygen and ammonia in a slit-jet expansion (Engeln et al., 1998). In 2003, Fiedler and co-workers demonstrated a new highly sensitive technique depending on incoherent broad-band cavity-enhanced absorption spectroscopy (IBBCEAS). They have used this technique to measure the weak transitions in molecular oxygen, which were found between 15865 and 1593 cm^{-1}, as well as the absorption spectrum of gaseous azulene in the region 628–670 nm, where several vibronic transitions appeared (Fiedler et al., 2003). Another BBCEAS study has been reported by Qu et al. in 2013. Using a Griess assay, they used this technique to measure Rh6G dye at 527 nm and determine the nitrite concentration. A low-cost webcam was used as a detector, reducing the cost of this technique (Qu et al., 2013). In general, a BBCEAS system consists of a broadband light source, an optical cavity formed by two high-quality dielectric mirrors, and a multiplex detector, such as a CCD spectrometer, to monitor the absorption spectra depending on the variation in the wavelength (Engeln et al., 1998; Islam et al., 2007, Naif et al, 2024).

BBCEAS has been used to make many advances, especially in the study of the liquid phase, the most complex and changeable liquid molecular phase. One such study was performed by Fiedler et al. in 2005 to determine the weak transitions in the liquid phase (Franck–Condon inhibited absorption of the fifth C-H stretching overtone in liquid benzene) using a modified double-beam UV-visible spectrometer. This study is the first to use BBCEAS in the liquid phase (Fiedler et al., 2005). In 2007 and 2009, Islam et al. and Seatohul et al. used BBCEAS with an LED light source in the liquid phase with 2-mm and 20-cm wide cells, respectively. These

Authors information:
[a]Mustansiriyah University, College of Science, Physics Department, Baghdad, IRAQ. E-mail: h.naif@uomustansiriyah.edu.iq[1]; ruba@uomustansiriyah.edu.iq[2]; munem@uomustansiriyah.edu.iq[3]
*Corresponding Author: h.naif@uomustansiriyah.edu.iq

Received: May 31, 2023
Accepted: November 22, 2023
Published: March 31, 2025

DOI:https//doi.org/10.22452/mjs.vol44no1.10
Malaysian Journal of Science 44(1): 87-92 (March 2025)

studies produced the most sensitive liquid phase absorption measurements (Islam et al., 2007; Seatohul et al., 2009). Moreover, in the work of Bajuszova et al. (2017), the higher sensitivity of BBCEAS was combined with liquid phase stopped-flow kinetics to measure fast reactions by slowing down the reaction rate using a lower concentration of reagents (Bajuszova et al., 2017). Naif et al. (2021) have reported the spectral behaviour of very low concentrations of coumarin dye and showed applications of these measurements in photonics (Naif et al., 2021).

In previous studies, the spectral properties of rhodamine B have been studied on sliver surfaces by Rai et al. (Rai et al., 1988), and the molecular structure of these dyes has been studied using the near-infrared fluorescence technique (Grzybowski et al., 2018; Lian et al., 2019; Wu et al., 2018; Xia et al., 2019). The influence of solvents on the absorption and fluorescence spectra of rhodamine B at different concentrations at room temperature has been studied by Ali et al. in 2012 (Ali et al.,2012). The current study studied the molecular structure and the fast and ultrafast molecular dynamics of rhodamine B ($C_{28}H_{31}ClN_2O_3$) using the BBCEAS technique.

2. Materials and Methods

Sample Preparation

To determine the molecular dynamics and structure of rhodamine B, nine concentrations from 4.70 to 47.01 nM were prepared from 0.0225 g of rhodamine B (Sigma Aldrich). First, the rhodamine B was dissolved in 100 mL of deionised water to obtain a stock solution of 4.7mM. Then, the samples were prepared by taking 0.001, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007, 0.008, 0.009, and 0.01 mL from the stock solution and then dissolving each in a 100-mL volumetric flask with deionised water.

Apparatus

This study used the BBCEAS technique to study the molecular structure and dynamics. A BBCEAS system includes three parts: 3 W white LED (Lumileds-SR-12, USA), which is used as a light source with an output power equal to ~5mW; the optical cavity, which contains two dielectric high reflectivity mirrors (R ≥ 0.99) (Layertec, Germany); and an Andor spectrometer (Andor Shamrock 163 Czerny-Turner spectrograph, UK), which was connected to the optical cavity using a 2m fibre optic cable (Ocean optics, USA). A quartz cuvette (Hellma, UK) of 1 cm thickness and 4 cm length was used to hold the sample.

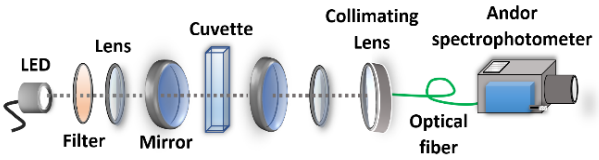

Figure 1. A schematic diagram of BBCEAS setup.

Molecular structure

Rhodamine B was chosen for study due to its important uses in medicine and other fields. Rhodamine B belongs to a group of dye molecules with a large molecular weight of approximately 479.02 g/mol. The molecular system contains a conjugated double-bond structure (see Figure 2). The chemical formula of the molecule is $C_{28}H_{31}ClN_2O_3$.

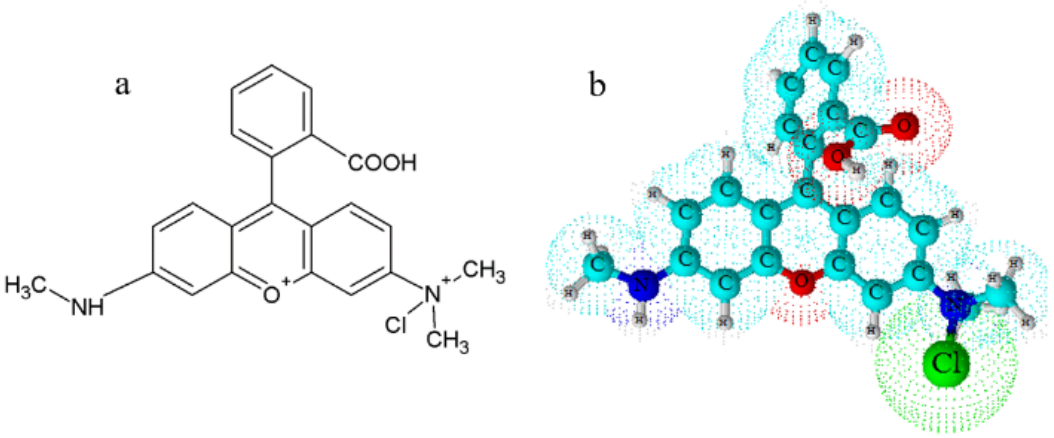

Figure 2: (a) Rhodamine B molecule and (b) a 3-D plot of $C_{28}H_{31}ClN_2O_3$ molecular structure.

Absorption and Transmission Spectra Detection

For the absorption assay, the spectra of rhodamine B in aqueous solution were measured using a broadband light source technique, BBCEAS. Different sample concentrations were prepared to study the effects of ultra-low concentrations (nanomolar scale). The absorption spectra were collected at excitation wavelengths between 500 and 600 nm. The absorption peak at 556 nm dominates the spectra due to the high absorption intensity of the incident photons. The liquid phase analyses of the representative transmission spectra were measured under the same conditions as the absorption spectra. The experimental settings were kept the same to maintain the same environment for measurement, and the LED source was installed between 500 and 600 nm.

DOI:https//doi.org/10.22452/mjs.vol44no1.10
Malaysian Journal of Science 44(1): 87-92 (March 2025)

3. Results and Discussion

The measured absorption spectra of rhodamine B are shown in Figure 3. To improve the detection statistics, representative absorption spectra were measured every 2 minutes as the accumulation signal and for 60 minutes for all recorded spectra. The detected peak amplitude decreased at low and ultra-low sample concentrations due to the small number of molecules per unit volume, which changes at different sample concentrations. Figure 3 shows the collective absorption spectra of rhodamine B aqueous solution measured at 4.70 to 47.01 nM.

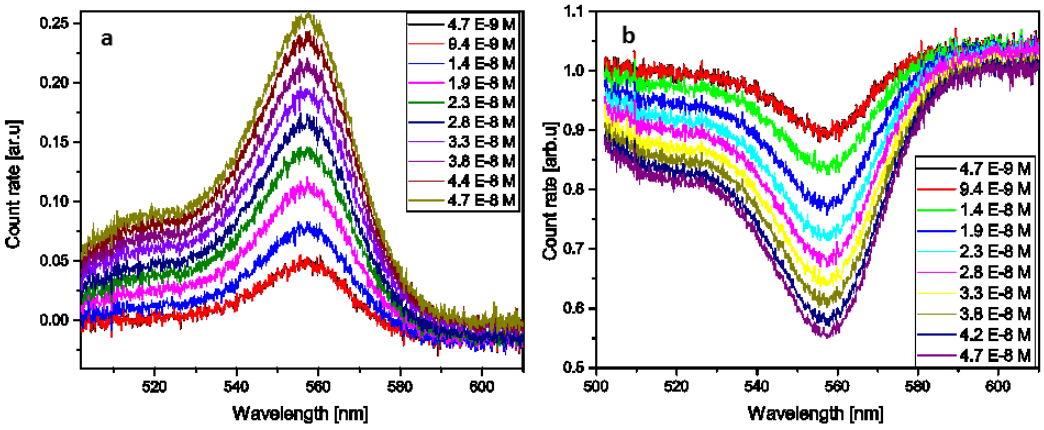

Figure 3. (a) Absorption and (b) transmission spectra of an aqueous solution of rhodamine B at different concentration ranges. The concentration (4.7 E-9M) disappeared behind the concentration (9.4E-9M) because their values were close together

For high sample concentrations, the intensity of the absorbed photons at the approximate peak position of 565.590 nm is high but reduced for lower sample concentrations. High sample concentrations resulted in a high molecular density per unit volume of solution, which changed the absorption cross-section and increased the number of absorbed incident photons. Consequently, the number of excited electrons per incident photon increased, changing the molecular dynamics. At low concentrations, the spectra show reduced intension of the absorption peaks observed in rhodamine B. The decrease occurs between 535 and 588 nm (Figure 3).

For a more detailed analysis, the data were fitted to the asymmetric double sigmoidal (ADS) function, where y_o is the offset, A is the peak amplitude (normalized), W_1 is the full width at half maximum, and W_2 and W_3 are the width and variance of the low- and high-energy side, respectively (see equation below):

$$y = y_o + A\frac{1}{1+e^{-\frac{(x-x_c)+W_1/2}{W_2}}}\left(1-\frac{1}{1+e^{-\frac{(x-x_c)+W_1/2}{W_3}}}\right)$$

The effect of the concentration of rhodamine B on the position of the central peak is shown in Figure 4(a). Increasing sample concentration shifted the peak centre to higher wavelengths, indicating changes to the structure of rhodamine B. The liquid phase of rhodamine B in high concentrations shows different molecular dynamics properties. At high concentrations of rhodamine B, the molecules absorbed photons of long wavelengths (low-energy photons), and the central peaks shifted to 565.590 nm at a concentration of 4.715E−8 M. This phenomenon is driven by molecular processes, such as the multiphoton absorption process (Chen et al., 2006). The high number of molecules per unit volume raised the rate of the absorption photons, which is reflected in Figure 4(b) as a sharp rise in the peak amplitude due to increasing sample concentration. In Figure 4(c), the stability of the FWHM (width W_1) of the peaks obtained from the ADS function agrees with the sample concentrations. The FWHM of the peaks changed slightly with increased sample concentration.

The large error bars of some points in the figure are due to the fitting process; low-concentration spectra did not match the experimental data exactly. In Figures 4(d) and (e), the variance of the photon absorption rate increased with the rate of the signal of the high and low sides of the peaks. These conflict actions of the rhodamine B molecules appear at lower wavelengths, from approximately 500 to 540 nm, where the high side of the peak tail rises, and at the low sides of the peak tails between 560 and 600 nm. Moreover, the behaviour of the low and high sides of the peak tails (widths W_3 and W_2) relate to different absorption processes of the rhodamine B molecules, where the orientation of the molecules plays a significant role in changing the binding energy of the molecules (Millan et al., 2016). On the low side of the peak tails, the molecules absorb photon energy between 2 and 2.2 eV), and on the high side, the molecules absorb photons of 2.3 to 2.5 eV.

DOI:https//doi.org/10.22452/mjs.vol44no1.10
Malaysian Journal of Science 44(1): 87-92 (March 2025)

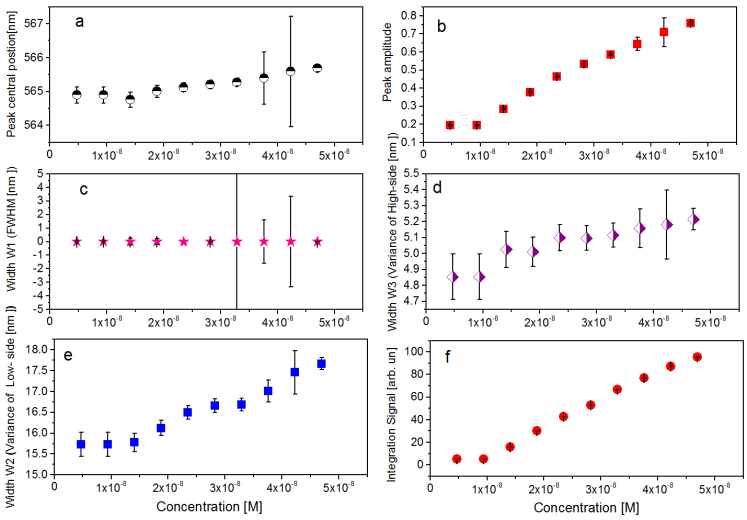

Figure 4. Absorption spectra of aqueous solutions of rhodamine B ($C_{28}H_{31}ClN_2O_3$) at different concentrations.

The peaks in Figures 4 (a) and (b) show the effect of the rhodamine B concentration on the peak centre and peak amplitude, respectively. Figures 4 (c) and (d) show the full width at half maximum of the peaks (FWHM) with the variance on the low side of the peak tails (W_3) and the high side of the peak tails (W_2), with the integration absorption signal indicated in Figures 4 (e) and (f).

The changing absorption processes are also related to the water molecules, which change the solvation binding energy (Jensen, 2015). The analyses from the ADS fits have small error bars, as shown in Figure 4. The unique structure of the molecules results in the spatial properties of the sample. The integration signal of the detected photons was calculated for every spectrum measured in the sample and channel F from Figure 4. The increase in the integrated signal with increasing sample concentration is due to the high absorption rate of the high-concentration molecules.

The spectra in Figure 5(a) indicate the different behaviours of the molecules and the shifts in the centres of the peaks with increasing sample concentrations. Figure 5(b) shows the reduction of the peak amplitude of the transmission spectra due to the increased sample concentrations due to the increased number of transmitted photons at a specific wavelength. In Figure 5(c), the FWHM (W_1) were quasi-stable with high sample concentrations, as also seen in the absorption spectra analysis. From these analyses, different absorption processes happened within the inner molecular orbitals.

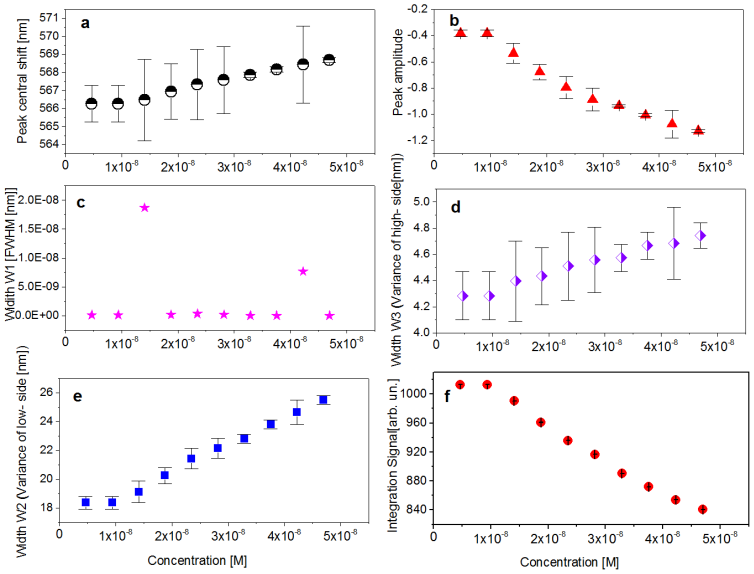

Figure 5. Transmission spectra of aqueous solutions of rhodamine B at different concentrations.

In Figure 5(a), peaks are fitted to the ADS function. The analyses show the effect of the concentration of the liquid phase of rhodamine B on the position of the peak centres. The peak amplitude is shown with the FWHM in Figures 5(b) and (c). The variance of the low side of the peak tails (W_3) and the variance of the high side of the peak tails (W_2) are illustrated in Figures 5(d) and (e), with the integration absorption signal given in Figure 5(f). In Figures 5 (d) and (e), the low and high sides of the peak tails

DOI:https//doi.org/10.22452/mjs.vol44no1.10
Malaysian Journal of Science 44(1): 87-92 (March 2025)

(W_2 and W_3) of the obtained spectra were fitted to the ADS function. These analyses showed that the high and low sides of the tails of the peaks increased with higher sample concentrations. The high and low sides of the peak tails indicate a change in the structural dynamics of the molecules. This effect was also achieved when peaks were detected by absorption and when the integration transmitted signal was reduced by increasing the sample concentration (Figure 5(f)).

4. Conclusion

The cavity-enhanced absorption spectroscopy technique has been demonstrated using an LED-based light source. Furthermore, the effect of ultra-low sample concentrations was studied. The experimental setup was developed to achieve optimum overlap with the reflectivity of the cavity mirrors for the largest differential absorption cross-section of a target molecule. High-quality broadband BBCEAS spectra were obtained under conditions with a substantial mismatch in the peak wavelength of the LED output (500–600 nm). Quantitative absorption and transmission spectra measurements were collected at ultra-low sample concentrations. The spectra were fitted to the ADS function, and analysis indicated a change in the molecular structure and the dynamics when the sample concentrations reached ultra-low nanomolar scale concentrations. This shifted in the central peak position and changed the peak amplitude, FWHM, and integration signal of both absorption and transition spectra. These indicators can be explained by changes in the absorption and transition processes of the rhodamine B molecule due to slight changes in the binding energy of the energetic states of the molecules. The vibrational and rotation energies of molecules also change due to the solvent effect, leading to changes in the electronic transitions. As a result, the molecular dynamics rotations, vibrations, and transitions of rhodamine B also change slightly due to molecule–molecule interactions from changes to the molecular distance between the sample and the solution in high and low concentrations. This was observed from the change in the spectra fit to the ADS function for every sample concentration.

5. Acknowledgements

The authors acknowledge the Physics Department, College of Science, Mustansiriyah University. Baghdad, Iraq.

6. Declaration of Interest Statement

We, the co-authors in the tagged research "Enhance the Optical Properties of Rhodamine B via Nano concentrations Effect", acknowledge and recognize that there is no intersection of interests between researchers participating in the research or the institution to which we belong.

7. References

Ali RA, Abdul-Munem OM, Abd AN. 2012. Study the spectroscopic characteristics of Rhodamine B Dye in Ethanol and Methanol mixture and Calculation the Quantum Efficiency. Baghdad Science Journal 9

Bajuszova Z, Naif H, Ali Z, McGinnis J, Islam M. Cavity enhanced liquid-phase stopped-flow kinetics. Analyst. 2018; 143(2): 493-502.

Chen S, Liu B-F, Fu L, Xiong T, Liu T, et al. 2006. Continuous wave-based multiphoton excitation fluorescence for capillary electrophoresis. Journal of Chromatography A 1109:160-6

Engeln R, Berden G, Peeters R, Meijer G. 1998. Cavity enhanced absorption and cavity enhanced magnetic rotation spectroscopy. Review of scientific instruments 69:3763-9

Fiedler SE, Hese A, Ruth AA. 2003. Incoherent broad-band cavity-enhanced absorption spectroscopy. Chemical physics letters 371:284-94

Grzybowski M, Taki M, Senda K, Sato Y, Ariyoshi T, et al. 2018. A Highly Photostable Near-Infrared Labeling Agent Based on a Phospha-rhodamine for Long-Term and Deep Imaging. Angewandte Chemie International Edition 57:10137-41

Islam M, Seetohul LN, Ali Z. 2007. Liquid-phase broadband cavity-enhanced absorption spectroscopy measurements in a 2 mm cuvette. Applied spectroscopy 61:649-58

Jensen JH. 2015. Predicting accurate absolute binding energies in aqueous solution: thermodynamic considerations for electronic structure methods. Physical Chemistry Chemical Physics 17:12441-51

Lian X, Wei M-Y, Ma Q. 2019. Nanomedicines for near-infrared fluorescent lifetime-based bioimaging. Frontiers in Bioengineering and Biotechnology 7:386

Millan S, Satish L, Kesh S, Chaudhary YS, Sahoo H. 2016. Interaction of lysozyme with rhodamine B: a combined analysis of spectroscopic & molecular docking. Journal of Photochemistry and Photobiology B: Biology 162:248-57

Naif HA, Abbas AM, Al-Kadhemy MFH (2024) Investigate spectroscopic experimental and theoretical model for hemoglobin nanoscale solution. Baghdad Sci J 21(2):0465–0465. https://doi.org/10.21123/bsj.2023.7775

Naif HA, Saeed AA, Al-Kadhemy MFH. Spectral Behaviour of the low concentrations of Coumarin 334 with Broadband Cavity Enhanced Absorption Spectroscopy. Baghdad Sci J. 2022; 19(2): 0438.

DOI:https//doi.org/10.22452/mjs.vol44no1.10
Malaysian Journal of Science 44(1): 87-92 (March 2025)

Qu Z, Engstrom J, Wong D, Islam M, Kaminski CF. 2013. High sensitivity liquid phase measurements using broadband cavity enhanced absorption spectroscopy (BBCEAS) featuring a low cost webcam based prism spectrometer. Analyst 138:6372-9

Rai V. 1988. Optical properties of Rhodamine B and Rhodamine 6G on silver surfaces. Pramana 31:313-22

Fiedler SE, Hese A, and Ruth A A.2005. Incoherent brand-band cavity- enhanced absorption spectroscopy of liquids. Rev. Sci. Instrum. 76, 023107.

Seetohul LN, Ali Z, Islam M. 2009. Liquid-phase broadband cavity enhanced absorption spectroscopy (BBCEAS) studies in a 20 cm cell. Analyst 134:1887-9

Wu Z, Yuan X, Zeng G, Jiang L, Zhong H, et al. 2018. Highly efficient photocatalytic activity and mechanism of Yb3+/Tm3+ codoped In2S3 from ultraviolet to near infrared light towards chromium (VI) reduction and rhodamine B oxydative degradation. Applied Catalysis B: Environmental 225:8-21

Xia S, Fang M, Wang J, Bi J, Mazi W, et al. 2019. Near-infrared fluorescent probes with BODIPY donors and rhodamine and merocyanine acceptors for ratiometric determination of lysosomal pH variance. Sensors and Actuators B: Chemical 294:1-13

DOI:https//doi.org/10.22452/mjs.vol44no1.10
Malaysian Journal of Science 44(1): 87-92 (March 2025)