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THE EFFECT OF SUNLIGHT AND HPLN MERCURY FLUORESCENT LAMP ON PHOTODEGRADATION OF RIBOFLAVIN IN AQUEOUS SOLUTIONS

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ABSTRACT
The study is conducted to evaluate the effect of sunlight (SL) and mercury HPLN fluorescent lamp (ML) on riboflavin (RF) aqueous solution containing. The pure solutions of RF (5 x 10^{-5} M) were exposed to SL and ML for 20 min. The pure solutions of RF possessed more photodegradation, in presence of SL as compared to ML. Moreover, the photolysis of pure solutions of RF possessed first-order rate constant. The values of first-order rate constant (k_{obs}) of RF irradiated in SL and ML were obtained, 2.503×10^{-2} min^{-1} and 8.545×10^{-3} min^{-1}, respectively, from slope of the plot of log concentration of RF against time at pH 7.4. The shelf-lives of the photolyzed solution of RF in SL and ML were 27.68 and 81.10 min. The concentration of RF in solution was found 31.58% and 65.90% after 20 minutes exposure in SL and ML, respectively. In the present study, it was found that the rate of degradation of RF in aqueous solution in SL is increased upto two-fold as compare to ML because of the formation of excited species due to the intensity and nature of wavelengths absorbed by RF solutions. The present study revealed the effect of intensity of light along with wavelength emitted by SL have possessed more photodegradation of RF in aqueous solution as compared to ML.

Keyword: Riboflavin; light effect; photolysis; kinetics

1. INTRODUCTION
Vitamin B_2 is a hydrophilic, thermosensitive and photosensitive vitamin, commonly known as riboflavin (RF) (Fig. 1). It is abundantly found in animal and plant-based foods. RF regulates a number of biological processes in body. It is a major factor of coenzymes involved in production of energy by metabolism of carbohydrates, proteins, fats into glucose for energy. It also acts as antioxidant and plays an important role in suitable functioning of immune, nervous and digestive systems. The medicinal use of RF includes better skin and hair growth, decrease the oxidative stress and inflammation of nerves [1].

RF is an extremely sensitive to light compound and is widely used as a drug component in aqueous vitamin preparations and parenteral nutrition solutions. RF is one of the most extensively considered compounds to study the photostability and its degradation in aqueous and organic solvents. The organic solvent has possessed more photolytic degradation of RF than aqueous solution [2]. The photodegradation of RF in aqueous solution is mainly occurred by an intermediate product, formylmethylflavin (FF) which is further degraded by hydrolysis to lumichrome (LC) and lumiflavin (LF) as one of the major photoproducts [3].

The aqueous solution of RF is sensitive to light [4]. Some workers have carried out studies on the photodegradation of RF [3,5-8]. Several workers have noted the increase in the photostability of RF by addition of substrate like liposomes [4], disodium ethylenediamine [9], caffeine [10], cyclodextrin [11], cyanocobalamin [12], famotidine [13], norfloxacin [14] and bovine serum albumin [15].

Yousif et al [16] have found the maximum degradation was occurred in acidic and alkaline medium, whereas, at near to neutral the degradation rate was minimized. Ahmad and co-workers observed that the photodegradation of RF in acidic medium is followed second-order kinetics, whereas, in alkaline medium followed first-order kinetics [3]. The photolytic degradation of RF in sulfate anions is faster as compared to phosphate anionic due to electronegativity and complex formation was reported at pH 7.0 in 1.0 molar buffer solutions [17].

The present wok has studied on the photodegradation of RF in phosphate buffer saline (PBS), 0.005 molar buffer at pH 7.4. RF. The RF solution was irradiated by sunlight (SL) and ML (ML). The main aim of the study is to evaluate the intensity and magnitude of photochemical degradation at different wavelengths of light. It will help to critically control the rate of photodegradation.

2. MATERIALS & METHODS

Riboflavin (RF), lumichrome (LC) and lumiflavin (LF) were purchased from Sigma– Aldrich, USA. All solvents were of analytical grade. The distilled water was freshly prepared. The physiological buffer PBS (0.005 M) was used for pH 7.4.

2.1. pH Measurements

The pH of the pure solution of RF were determined by Elmetron LCD display pH meter (Model–CP 501 sensitivity ±0.01 pH unit, Poland) by using a combination pH electrode calibrated by phthalate (pH 4.008) and phosphate (pH 6.865) buffer solutions.

2.2. Spectral Analysis

All spectra and absorbance readings of the pure RF and photolyzed solutions were carried out by using Shimadzu UV–1601 recording spectrophotometer using quartz cells of 10–mm path length.

2.3. Photolysis of RF Solution

The photodegradation of the pure RF (5×10^{-5} M) was studied. A 10 ml of RF pure solutions were placed in Pyrex glass beakers (50 ml) and exposed in SL. The SL experiments were carried out at Karachi in December 2023, whereas, and the pure solutions of RF were irradiated with a Philips HPLN 125W high pressure mercury vapor fluorescent lamp (ML) fixed at a distance of 25 cm from the center of the beakers. The samples irradiated in SL and ML were withdrawn after every 5 minutes interval for a period of 20 minutes. The temperature of the RF solutions was maintained at 25 ± 1°C during irradiation. The selected λ_{max} of RF is 445 was used to analyze the remaining photodegradation of RF in pure RF solutions by two-component spectrophotometric method.

3. RESULTS

3.1. Spectral Characteristics

RF shows absorption maxima at 445, 373, 267 and 223 nm in aqueous solution at the region of visible and UV (Fig. 2). The exposure of pure RF solutions in the SL and ML for 20 minutes, may affect the rate of photolysis of the RF. The spectra of photolytic degradation of RF aqueous solution in SL (Fig.

3) and ML were shown in Fig. 4. It has been observed there is a steady decrease in the absorbance change at around 445 nm with an increase in absorbance at 356 nm with the increase in light exposure. This has shown the photodegradation of RF. LC, a photoproduct of RF has shown its peak at 356 nm.

3.2. Choice of Analytical Wavelengths.

The photodegraded aqueous solutions of RF were extracted to chloroform for the segregation of LC. The photodegraded LC and pure RF have been determined by a two-component spectrometric assay at 356 and 445 nm, respectively. These wavelengths belong to the absorption maxima of these compounds and are most suitable for their assay by a spectrometric method [18].

3.3. Assay of RF and Photoproducts

The assay of RF and its photoproducts LC were determined by two–component spectrometric method as described by Ahmad and Rapson [19]. It has already been used for the analysis of the photodegradation of RF [3,4,18], photodegradation of RF in phosphate buffer [17], effect of caffeine [10] and effect of acetate and carbonate buffer [20].

The photolyzed RF was assayed at pH 2.0 (KCl–HCl buffer, 0.1 M HCl) for the accurate determination of RF and intermediate photoproduct FMF. Then the photolyzed solution is extracted three times with chloroform and evaporate it and diluted with acetate buffer, pH 4.5 (0.5 M). The two–component spectrometric assay was carried out at particular peaks of RF (445 nm) in aqueous solution and LC (356 nm) in chloroform extracts. The peaks of the components with molar absorptivities of RF and photoproducts were described by Sheraz et al. [2].

The standard curve obeyed the Beers-Lambert law as the concentration of RF was reduced the absorbance of RF at its λ_{max} was also decreased. The standard curves formation was easily accomplished at 445 nm from a range of 1.0×10^{-4} M to 1.0×10^{-6} M RF solutions (Fig. 5). This range is highly sensitivity in aqueous solutions and buffering by a common buffering system of PBS. PBS provided stable readings of pure RF and photolyzed solutions of RF. The equation of the line was y = 12345x - 0.004 and with a coefficient of determination $R^2 = 0.999$. The photolytic degradation of RF has shown decrease in absorbance from 445 nm with an increase in absorbance at 356 nm. The rate of photolysis of RF in SL was observed much faster than ML (Fig. 6 and 7).

3.4. Kinetics of Photolysis

The photolysis of RF in aqueous solution is followed first-order kinetics. The rate of reaction of the photolysis of RF in aqueous solution (pH 7.4; 0.005 M PBS) in SL was determined 2.503×10^{-2} min^{-1} and in ML was 8.545×10^{-3} min^{-1}. The comparison of the rate of the photolysis of RF in SL and ML has shown that the degradation of RF in SL was noted far much faster that the ML.

4. DISCUSSION

PN solution is highly concentrated nutrients usually administered in very low volume [21]. A number of photosensitive nutrients are added in PN solution. The PN solutions must be protected from light by packing in ethylene vinyl acetate [22].

4.1. Two-Component Spectrophotometric Assay

The two–component spectrometric assay provided nearly accurate assay of RF and its photoproducts by compliance of data with apparent first–order kinetics. The assay has been validated for its application of RF and its photoproducts [18,19]. The two-component spectrophotometric method has shown consistently increasing values of LC with nearly constant molar balance, with time. It has indicated a good reproducibility of the method. LF, a minor photoproduct of FMF, accounting to less than 5%, does not interfere with the assay method (Table 1). A kinetic plot for the photolysis of RF in liposomes has proved that LC is the final product in PBS of 0.005M at pH 7.4 [4].

4.2. Effect of Light on Photolysis of RF

The irradiation of SL exerts much greater effect on drug degradation than that of the ML. The lamp has an inner quartz tube containing the mercury vapor discharge. This is enclosed by an outer glass bulb that filters out harmful short-wavelength ultraviolet (UV) radiation [23]. It was found that after 20 minutes irradiation of RF solutions in SL and ML has photolyzed 68.42% and 34.10% RF in solutions, respectively. It has been noted that the rate of photodegradation of RF is much faster in SL than ML. The increase in photolysis of RF in SL is mainly due to rainbow of colors known as complete spectrum, whereas, fluorescent lamp emits slightly different spectrum not a complete spectrum of visible light. Furthermore, the intensity of brightness of SL is far much greater than fluorescent light bulb (Fig. 8) [24].

The bulbs have an inner quartz tube containing the mercury vapor discharge. This is enclosed by an outer glass bulb that filters out harmful short-wavelength ultraviolet (UV) radiation [23]. The fluorescent bulb light possesses different properties due to composition and temperature. The mercury atoms have specific wavelength of light with precise transition to specific spectral lines in the emission in its gas discharge spectra at 365 nm (ultraviolet), 405 nm (violet-blue), 436 nm (blue), 546 nm (green), and 578 nm (yellow-orange) (Fig. 9) [25].

4.3. Photoproducts of RF

The assay of RF and its photoproducts (FF, LC and LF) is formed by oxidation of ribityl side chain and determined by two–component spectrometric assay. It helps in correction in irrelevant absorption of RF, FF, LC and LF. The method is validated and applied to evaluate the kinetics of photodegradation in aqueous solution [3,18,19]. It was observed that the rate of photodegradation of RF in aqueous solutions yields mainly LC (Table 1). The percentage of loss of RF and the formation of photoproducts of RF after 20 min. irradiation in SL and ML is given in Table 2.

FF is protonated at pH 2.0 and distinct its peak at 385 nm instead of 445 nm. RF can be easily assayed in presence of FF, which is the intermediate product and converted into LC and LF as final product. Moreover, LC and LF were extracted from photolyszed solution by chloroform and assayed at pH 4.5. The accuracy of assay in molar balance of RF, FF, LC and LF can be achieved. LF is considered as minor photoproduct at pH 7.4 [4]. Thus, FMF and LF is not greatly affected on total molar concentration of initial RF. LC is formed through the mediation of FF, an intermediate in the photolysis of RF [20]. LC is a major photoproduct of RF formed through excited singlet state and triplet state through FMF [26,27]. The other minor products like β–keto acid and flavovoilet are formed by cleavage of isoalloxazine ring in high alkaline pH [3]. The identification of photoproducts has been confirmed by spectrometric methods [28].

4.4. Kinetics of Photolysis of RF

The photolysis of cyanocobalamin and folic acid is followed first order of kinetics [29,30,31]. RF in aqueous solution is followed first-order Kinetics [3,4,15,18,20]. The values of first-order rate constant (k_{obs}) of RF irradiated in SL and ML were obtained, 2.503×10^{-2} min^{-1} and 8.545×10^{-3} min^{-1}, respectively, from slope of the plot of log concentration of RF against time at pH 7.4. The shelf-lives of the photolyzed solution of RF in SL and ML were 27.68 and 81.10 minutes. The concentration of RF in solution was found 31.58% and 65.90% after 20 minutes exposure in SL and ML.

RF act as a photosensitizer in visible and UV light [32]. The type I and type II photosensitized oxidation pathways were elaborated by Ionita and Matei [32] and Baptista [33]. The kinetics of photostability of RF, norfloxacin, cyanocobalamin were also studies [4,14,15,30,33,34]. The photodegradation of RF is depends on its redox behavior [3]. RF is photodegraded in different pathways by intramolecular photoreduction, photoaddition and photodealkylation. The basic mechanism of photodegradation of RF is through photoreduction in aqueous solution by the generation of radical species. The detailed mode of photo, thermal and chemical degradation of RF is discussed by Sheraz et al. [2].

5. CONCLUSION

The present study revealed the effect of intensity of light along with wavelength emitted by sunlight (SL) and HPLN mercury fluorescent lamp (ML) on the photodegradation of RF in aqueous solution. RF is used in combination of vitamin B complex syrups and parenteral nutrition solutions. The mode of photodegradation of RF in aqueous solution is mainly leads by its redox behavior. Redox reaction is the continuous process, if the reaction is initiated it would be destabilized the nutrients and drugs even inside the body and produced toxic degraded products. It was found that the rate of degradation of RF in aqueous solution in SL is increased up to two-fold as compare to ML because of the formation of excited species due to the intensity and nature of wavelengths absorbed by RF solutions. The wavelengths emitted by SL and ML plays important role in initiating the specific photodegradation reaction. The role of packaging may also effects on stability of drug [36-39]. The study supports in optimization in formulation of light sensitive drug products and to evaluate the photostability of pharmaceutical products during development.

6. CONFLICT OF INTEREST

No conflicts of interest are disclosed by the authors.

7. AUTHOR'S CONTRIBUTION

Nida Ghani, Adeel Arsalan, Mirza Tasawer Baig, Kiran Qadeer, Hina Abrar, Hina Yasin, Kaneez Fatima, Maria Ashfaq

N.G.: Methodology, Data curation, Investigation.
A.A.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – Original draft, Writing – Review & Editing, Supervision.
M.T.B.: Writing – Review & Editing.
K.Q.: Writing – Review & Editing.
H.A.: Methodology.
H.Y.: Visualization.
K.F.: Visualization.
M.A.: Methodology.

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Table 1: Concentration of RF, FF, LC and LF after photolysis of 5×10^{-5} M RF solutions[a]

Time (min.)	RF	FF	LC	LF	Total[x]
0	5.000	0.000	0.000	0.000	5.000
5	3.999	0.314	0.581	0.111	5.005
10	3.369	0.472	0.995	0.159	4.995
15	2.254	0.616	1.899	0.227	4.996
20	1.579	0.704	2.479	0.239	5.001

Time (min.)	RF	FF	LC	LF	Totally
0	5.000	0.000	0.000	0.000	5.000
5	4.483	0.111	0.338	0.070	5.002
10	4.031	0.195	0.675	0.103	5.004
15	3.669	0.250	0.968	0.120	5.007
20	3.295	0.316	1.268	0.123	5.001

[a] Values are the mean of five determinations.
[x] Aqueous solution of RF irradiated in sunlight
[y] Aqueous solution of RF irradiated in HPLN mercury fluorescent lamp

Table 2: Percentage of RF, FF, LC and LF after photolysis of 5×10-5 M RF solutions[a]

RF	FF	LC	LF	Total	t^{90} (min.)
31.58%[x]	14.08%	49.58%	4.78%	100.02%	27.68 [x]
65.90%[y]	6.32%	25.36%	2.46%	100.04%	81.10 [y]

[a] Values are the mean of five determinations.
[x] Aqueous solution of RF irradiated in sunlight
[y] Aqueous solution of RF irradiated in HPLN mercury fluorescent lamp

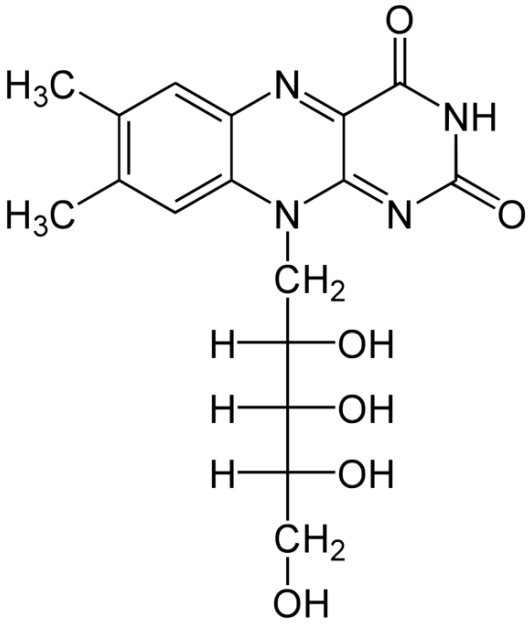

Fig. 1: Chemical Structure of RF

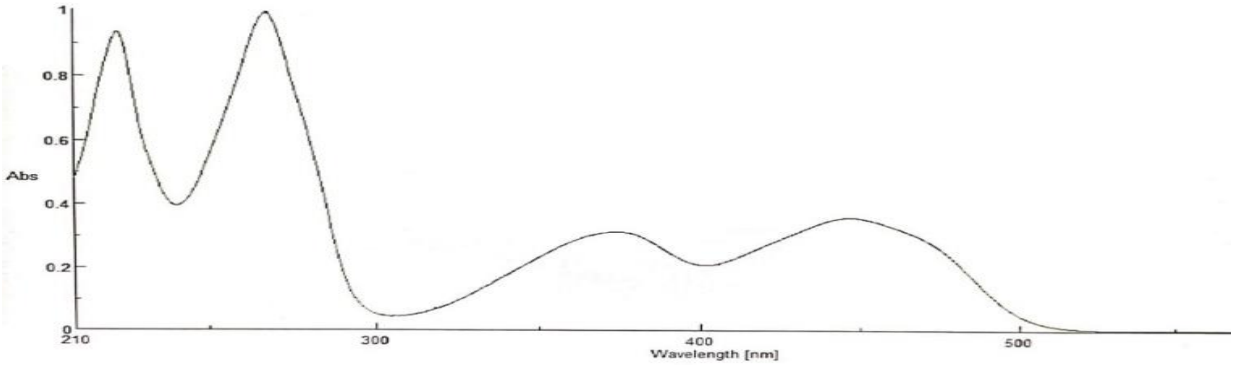

Fig. 2: UV-visible absorption spectrum of RF

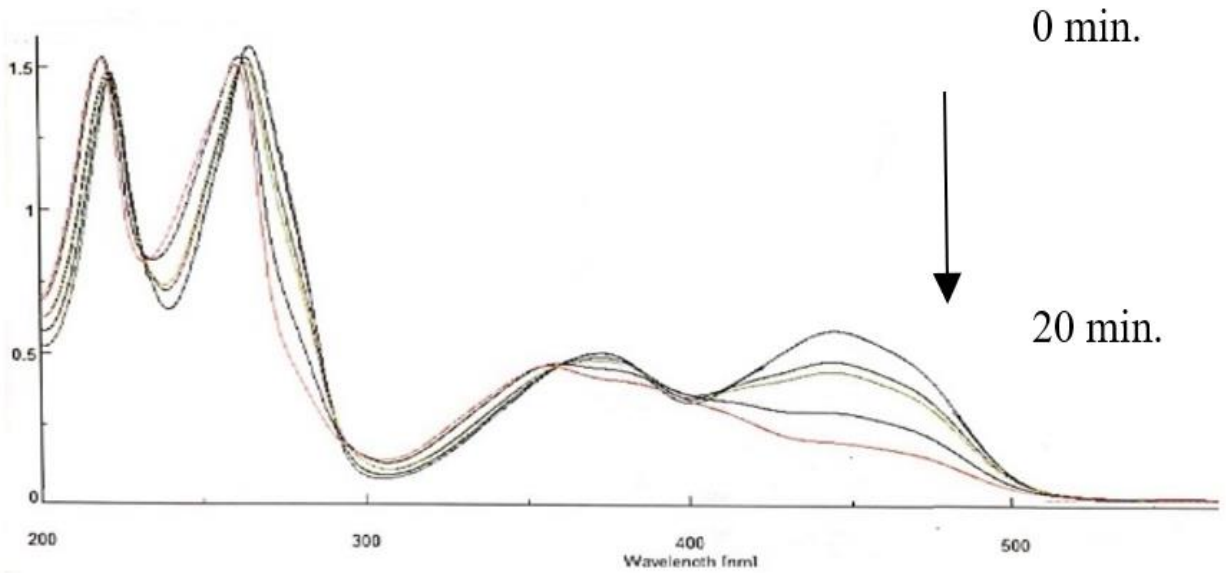

Fig. 3: Spectra of RF after exposure in sunlight for 20 min.

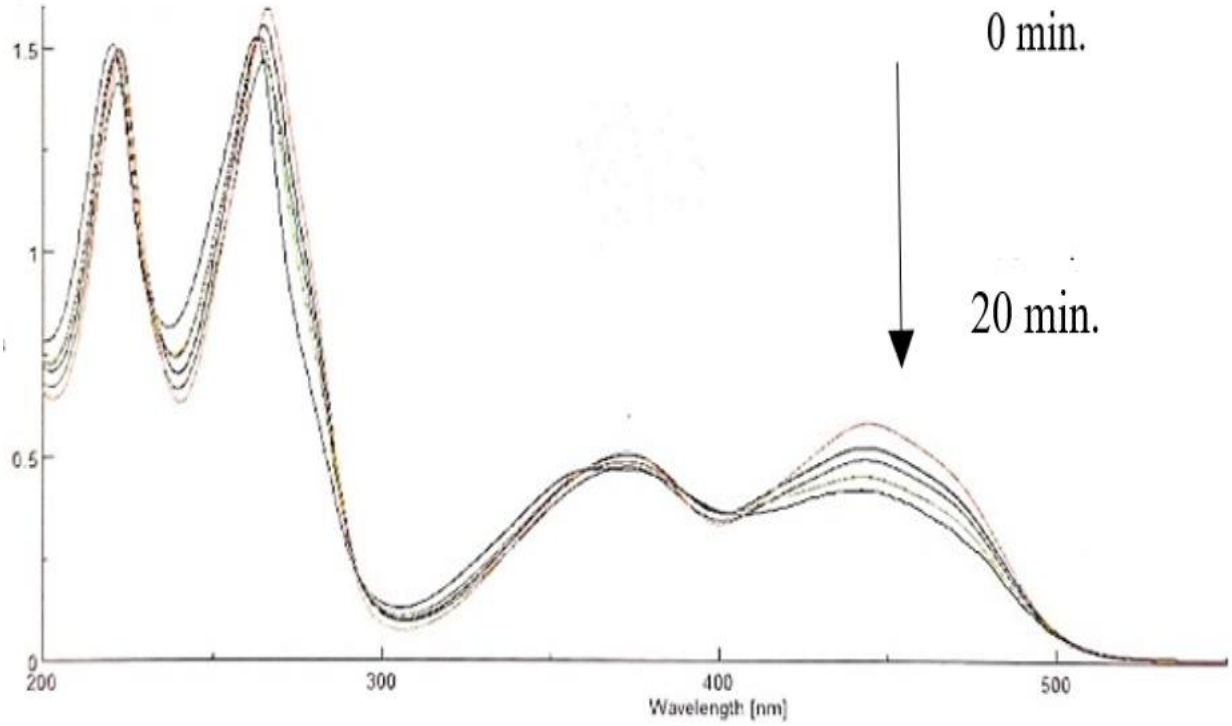

Fig. 4: Spectra of RF after exposure in HPLN mercury fluorescent lamp for 20 min.

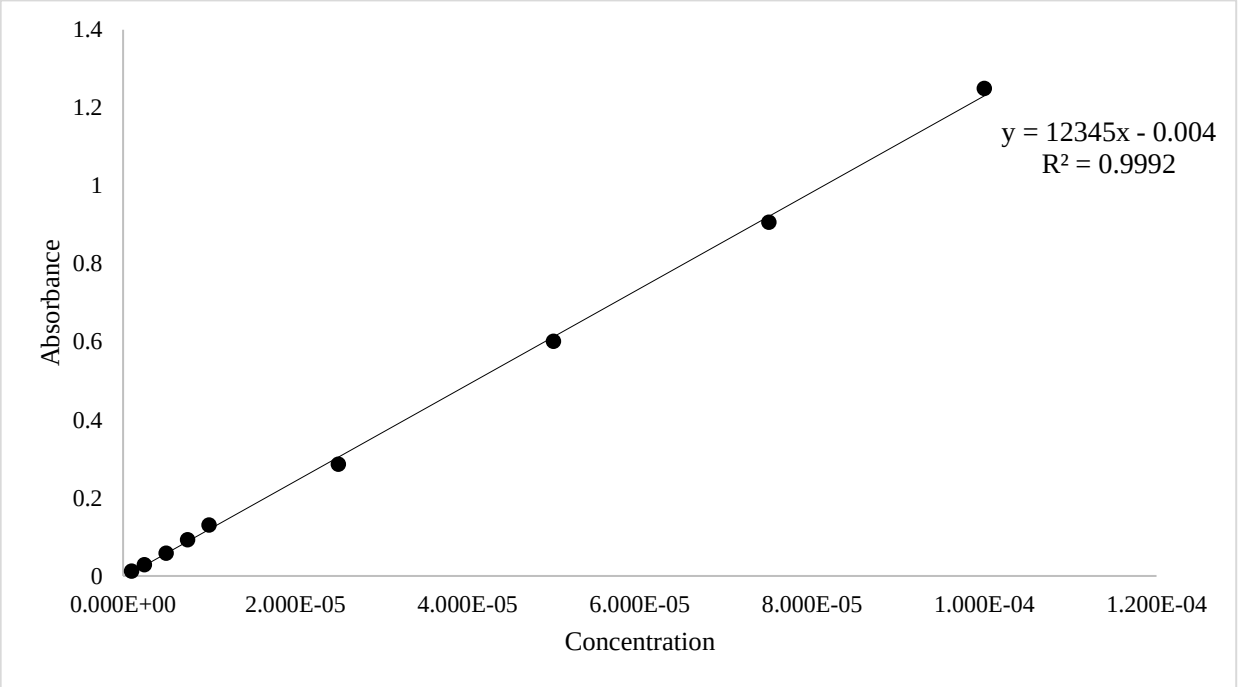

Fig. 5: Calibration curve of RF at 445 nm

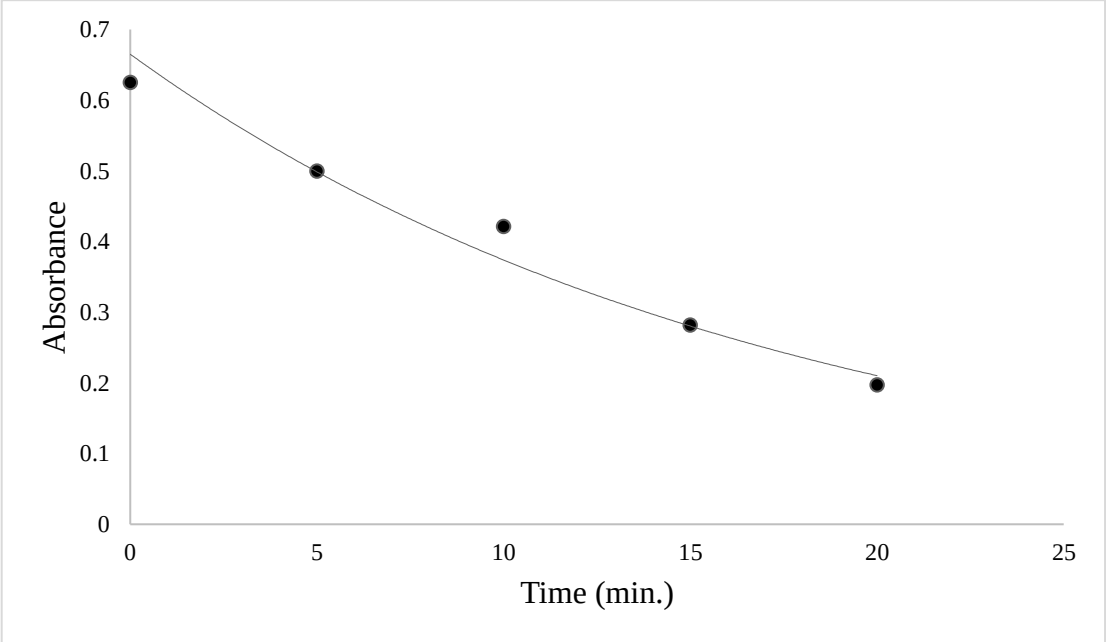

Fig. 6: Loss of absorbance loss at 445 nm versus time for the photolysis of RF (5×10^{-5} M) in sunlight

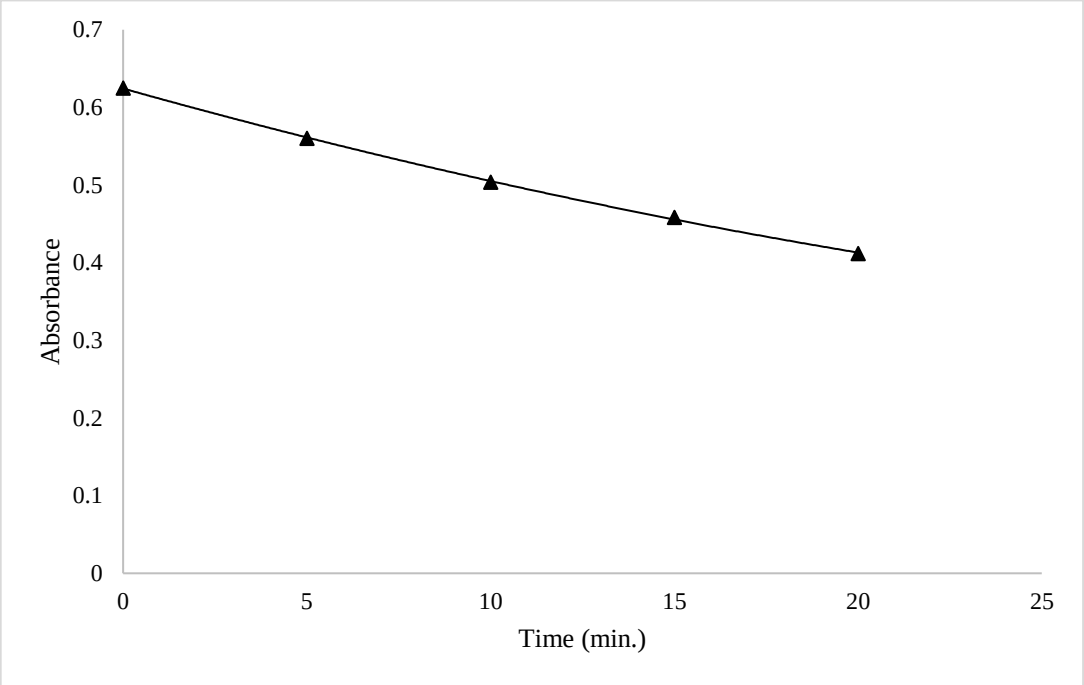

Fig. 7: Loss of absorbance at 445 nm versus time for the photolysis of RF (5×10^{-5} M) in HPLN mercury fluorescent lamp

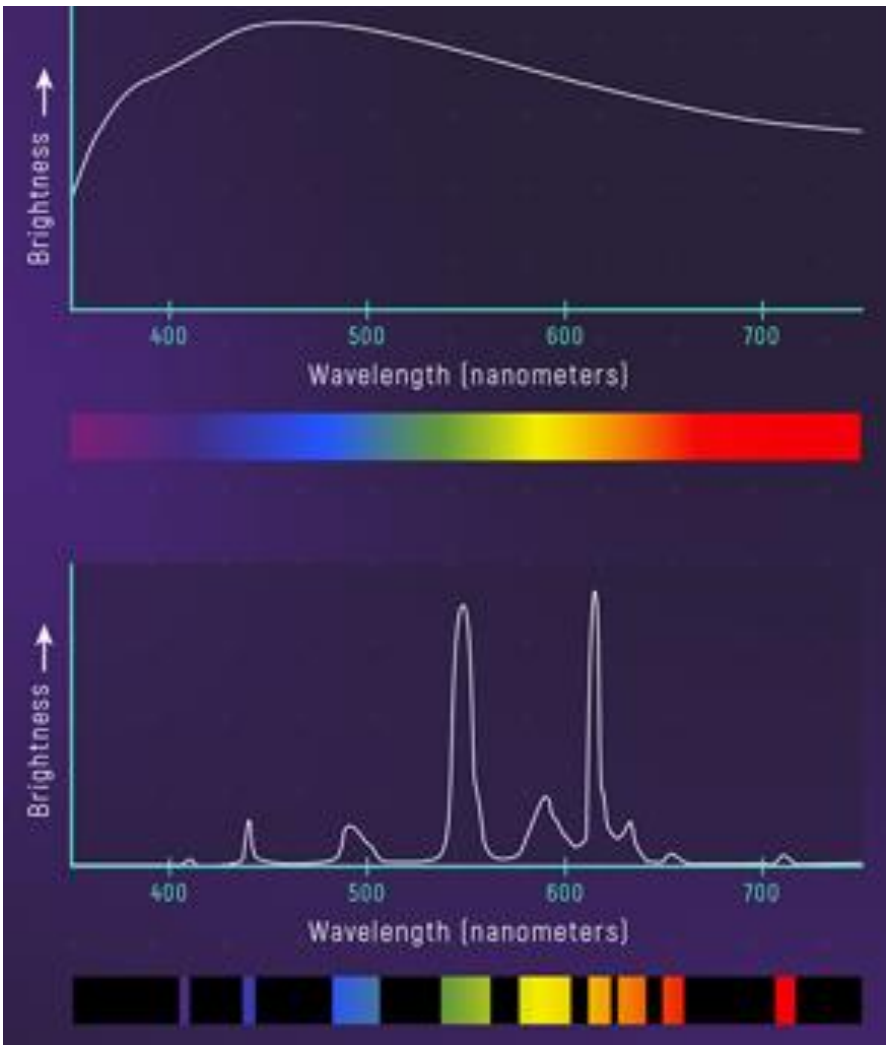

Fig. 8: Intensity of visible wavelength of sunlight and HPLN mercury fluorescent lamp

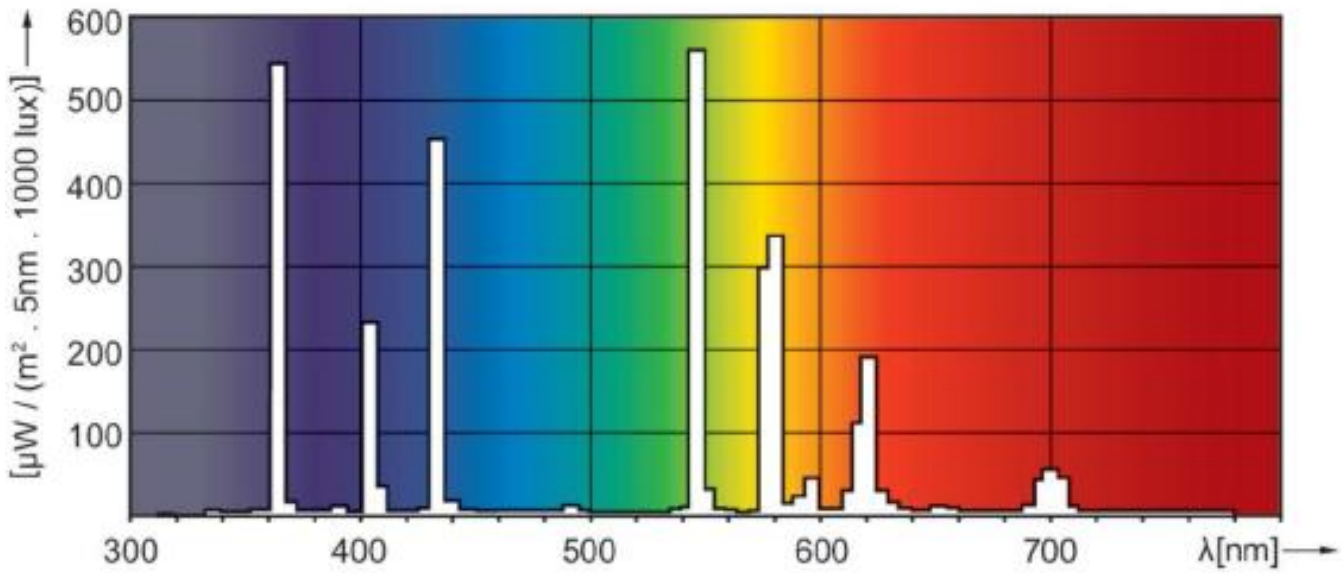

Fig. 9: Spectral power distribution color of high-pressure mercury lamp