Study on the Photochemical Reaction Process of 4-methyl-7-Hydroxycoumarin and Its Mechanism by Multi-Spectroscopic Technologies

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Abstract. The photochemical reaction process of 4-methyl-7-hydroxycoumarin (7H4MC) was studied by UV-Vis spectrometer and fluorescence spectroscopy. The results have shown that the characteristic peak of the UV-Vis spectra at 323 nm of 7H4MC undergoes a significant decrease in absorbance and a slight blue shift when illuminated; the absorbance of the absorption peak at 279 nm rises, and there is a tendency for new peaks to appear. May be, there are structural changes or new materials generation. The addition of imidazole, VC, 3-indole methanol, GSH and other substances also have different effects on the photochemical reaction of 7H4MC. Among them, only imidazole has a certain inhibitory effect on the process of its photochemical reaction.

1. Introduction
Photosensitive substances have received more and more attention from scholars due to their unique light-absorbing properties and play irreplaceable roles in many fields, such as antibacterial and anti-inflammatory, photoelectric conversion, catalyzing organic reaction processes, and fighting tumors. As a new type of choleretic drugs and the core of drug synthesis, 7H4MC plays an important role in the field of medical research. In recent years, coumarin and its derivatives have become a research hotspot in anti-tumor, especially malignant tumors. In 2015, Yu Hao used 7H4MC as the mother nucleus to synthesize copper 7-hydroxy-4-methylcoumarin valine phenanthroline, which has a good killing effect on breast cancer MCF-7 cells. Killing and inhibiting effects have been verified in mice [1].

As early as 1974, Hiroki Mizoguchi’s team investigated the proton transfer of the reaction between 7H4MC and tertiary amine by ultraviolet, fluorescence and nuclear magnetic spectroscopy. The results showed that the 7H4MC of the carbonyl group was hydrogen-bonded, and the tautomer anion was stable in the ground state at room temperature. In the presence of weakly basic arginine, pyridine and triethylamine, the hydroxyl group of 7H4MC will connect with tertiary amine to form a complex [2]. In 1994, Li Longdi discovered that the photosensitivity of 7H4MC not only varies with the nature of the solvent, but also varies with the concentration of 7H4MC [3]. In 2000, J. Seixas de Melo’s team studied the acid-base balance of the ground state, the lowest energy singlet and the triplet excited state
of 7H4MC [4]. In the same year, Masanori Hoshiyama et al. used ultraviolet spectroscopy, circular dichroic spectroscopy, fluorescence spectroscopy and NMR spectroscopy to study the complexation and proton dissociation behavior of 7H4MC in the presence of β-cyclodextrin[5]. In 2015, J. Sergio Seixas de Melo et al. had studied the proton transfer of 3CL7H4MC under light-induced conditions from the perspective of excited state protons [6].

At present, most of the research on 7H4MC focuses on the calculation of its kinetics and the synthesis of anti-tumor drugs. The photosensitivity mechanism and the external substances that affect its photosensitivity are not sufficiently discussed. Further analysis of the reaction mechanism has important research significance.

2. Experimental

2.1. Reagent and Instrument
Reagent. 7H4MC, purity ≥ 98%, was purchased from Aladdin Company, dissolved in absolute ethanol, configured into 2.5×10⁻⁵ mol·L⁻¹ solution. Here, the oxidant was H₂O₂, the reducing agent was Na₂S₂O₄, GSH, imidazole, 3-indole methanol and VC configured into 0.1 mol·L⁻¹ solution.

Instruments. FP-6500 fluorescent spectrophotometer and V-560 UV-visible Spectrophotometer (Jasco, Japan, F-12 cooling and heating circulator (Julabo, Germany).

2.2. Experimental Methods
The absorption spectra were recorded at room temperature (25 ºC) with 1.0 cm quartz cells at a scanning speed of 400 nm/min and bandwidth for 2 nm. The fluorescence emission spectra were recorded with 1.0 cm quartz cell in the wavelength upon excitation at 374 nm at a scanning speed of 500 nm/min and bandwidth for both excitation and emission were 3 nm. At the same time the synchronous fluorescence spectra were obtained, Δλ=35.

3. Results and Discussion

3.1. UV-Visible Spectra of Chemical Redox and Photochemical Reactions of 7H4MC
In order to judge the photochemical change mechanism of 7H4MC induced by light, it was compared with the chemical redox situation. Add H₂O₂ and Na₂S₂O₄ at a ratio of 1:50, and detected in the dark, as shown in Figure 1A, B. A full-wavelength xenon lamp as the excitation light source was used to detect the UV-Vis spectra before and after 7H4MC illumination, as shown in figure 1C.

![Figure 1. UV-Vis absorption spectra of 7H4MC.](image)

(A: chemical oxidation; B: chemical reduction; C: xenon illumination, illumination time of from a to j: 0, 3, 5, 10, 15, 20, 25, 30, 35, 40 min, respectively)

It can be seen from the UV-Vis spectra that 7H4MC has two steady-state absorption peaks, the first characteristic absorption peak at 279 nm is the B band, which is mainly produced by the Π→Π* transition of the benzene ring in 7H4MC. The second absorption peak is at 323 nm, which is the strongest characteristic absorption peak and is produced by the entire conjugated structure. Under dark
conditions for 30 minutes, the UV-Vis spectra are almost unchanged, which indicate that its structure and function are relatively stable. The 7H4MC UV-Vis spectra are almost unchanged with the increase of reaction time added Na₂S₂O₃ and H₂O₂ at a ratio of 1:50. It can be seen that the redox reaction is not easy to occur with conventional oxidizing agents and reducing agents. When irradiated with a full-wavelength xenon lamp, it can be seen from the UV-Vis spectra that the two characteristic absorption peaks of 7H4MC undergo significant changes, especially at 323 nm, a large decrease in absorbance occurs with a slight blue shift. A new peak appears at 279 nm, which can be inferred that it underwent structural changes or new substances are formed.

3.2. 7H4MC Fluorescence Spectrum Analysis

The electron-donating group (-CH₂, -OH) connected to the benzene ring of 7H4MC have strong endogenous fluorescence. The results show that the best excitation wavelength for 7H4MC is 374 nm, and the best emission wavelength is 448 nm. It can be seen from the fluorescence spectra and the synchronous fluorescence spectra of Δλ=35 nm that the fluorescence intensity gradually decrease after the xenon lamp is irradiated. When excited at 374 nm, the optimal excitation wavelength has a slight red shift, and the characteristic peak at 375 nm of the synchronous fluorescence spectra of Δλ=35 nm before and after illumination also has a slight red shift. As we all know, fluorescence spectroscopy can detect material structure change information, we can infer that 7H4MC undergo a photochemical reaction after being irradiated, and is accompanied by a structural change. This result is consistent with the conclusion of the ultraviolet spectra.

![Fluorescence spectra of 7H4MC](image)

**Figure 2.** Fluorescence spectra of 7H4MC. 
(A: 374 nm excitation; B: synchronous fluorescence spectra Δλ=35 nm)

3.3. Effect of Addition of External Substances on the Photochemical Reaction of 7H4MC

3.3.1. Effect of Imidazole on the Photochemical Reaction of 7H4MC. As an excellent coordination substance, imidazole has great research significance for its influence on the photochemical reaction process of 7H4MC. The spectra almost have no change added imidazole at a ratio of 1:1 for 30 minutes in the dark., which it can be determined that imidazole does not occur with 7H4MC under non-illumination conditions. Figure 3A shows that the degree of change in absorbance at 279 nm is very small, and the trend of generating new peaks is not obvious after adding imidazole. Figure 3B shows that the addition of imidazole makes the slope smaller under light conditions compared with dark condition, which obviously inhibits the rate of decrease in the absorbance of the characteristic peak of 7H4MC. Because there is a lone pair of electron on the nitrogen atom of imidazole, it is easy to occur electron transfer in the conjugated system of imidazole, which makes the hydrogen atom on imidazole easily leave in the form of protons, and the hydrogen proton are very active in the solution and are very easy to interact with the hydroxyl groups of ethanol. 7H4MC can react with ethanol with the help of light excitation. Therefore, it is speculated that the reason why imidazole inhibits the photochemical reaction of 7H4MC is the competitive reaction of free hydrogen protons with alcohol.
The reason for the photochemical change of 7H4MC to produce the structural change is that the ester group-like part of 7H4MC undergoes photolysis and reacts with ethanol. The free hydrogen protons of imidazole affect the reaction of 7H4MC with ethanol, so the photochemical change process of 7H4MC is inhibited.

Figure 3. Effect of imidazole on the photochemical changes of 7H4MC.

(A: xenon light spectra containing imidazole B: contrast line chart with imidazole)

3.3.2. Effect of Glutathione on the Photochemical Reaction of 7H4MC. GSH is a macromolecule with important biochemical effects, commonly found in organisms. It is a common antioxidant that can scavenge free radicals, and it is also prone to photochemical changes. Therefore, the effect of GSH on the photochemical changes of 7H4MC is helpful to summarize the mechanism of light-induced photochemical changes of 7H4MC. The UV-Vis spectra of 7H4MC has been measured with GSH added to the 7H4MC solution at a ratio of 1:10 under the condition of irradiating with a xenon lamp, and compare with without GSH. The results are shown in figure 4.

Figure 4. The effect of glutathione on the photochemical changes of 7H4MC.

(A: xenon light spectra containing CSH B: contrast line chart with GSH)

The UV-Vis spectra almost has no change in the dark for 30 minutes. It shows that 7H4MC hardly react with GSH under non-light conditions. Figure 4A shows that the characteristic peak of 7H4MC at 323 nm has a significant change, and there is a greater downward trend. From figure 4 B, it can be found that the slope is very large the first 10 minutes of the reaction after adding GSH, indicating that the absorbance of the characteristic peak of 7H4MC decreases faster at this time, and after 10 min, the trend of the broken line is gentle, and the slope becomes smaller, but it is still enhancement. Because GSH is excited by light, it gains energy to generate cationic free radicals and active oxygen. Active oxygen can promote the reaction between ethanol and 7H4MC, accelerate the photolysis of ester-like groups in 7H4MC, change the structure, and promote the process of 7H4MC photochemical reaction.
The addition of GSH promotes the photochemical reaction. It shows that in the photochemical change of 7H4MC, active oxygen will accelerate the break of the "C-O" bond in 7H4MC and promote the reaction process, as shown in figure 4B.

3.3.3. Effect of VC on the Photochemical Reaction of 7H4MC. After being kept in the dark for 30 minutes, UV-Vis spectroscopy was carried out. It was found that there was almost no change in the spectra, and it could be confirmed that VC did not react with 7H4MC under non-illuminated conditions. Since VC itself has a strong characteristic absorption peak at 264 nm, it will overlap with the characteristic absorption peak of 7H4MC at 279 nm, as shown in Figure 5A. As shown in Figure 5B, the addition of VC promotes the photochemical change process of 7H4MC, which is similar to the promotion effect of GSH. It changes significantly in the first 10 minutes and slows down after 10 minutes. The difference is that GSH has always been a promotion. However, VC loses its promoting effect in the late stage of the reaction. This is because VC is sensitive to light. Under light conditions, photochemical changes will occur to produce active oxygen, which promotes the combination of 7H4MC with ethanol, and accelerates the photolysis of the ester-like part of 7H4MC. However, because VC itself is not resistant to light and heat, it may be photo-damaged in the late stage of the reaction and lose its role in promoting the photochemical changes of 7H4MC.

![Figure 5](image5.png)

**Figure 5.** Effect of VC on the photochemical changes of 7H4MC.

(A: xenon light spectra containing VC B: contrast line chart with VC at a ratio of 1:1)

3.3.4. Effect of 3-indole Methanol on the Photochemical Reaction of 7H4MC

3-Indole methanol is used in many photochemical reactions due to its own electron transfer, so it may also have a certain impact on 7H4MC photochemical reactions. Add it to the 7H4MC solution at a ratio of 1:1, and the results are shown in figure 6.

![Figure 6](image6.png)

**Figure 6.** The effect of 3-indole methanol on the photochemical changes of 7H4MC.

(A: xenon light spectra containing 3-indole methanol B: contrast line chart with 3-indole methanol at a ratio of 1:1)
The UV-Vis spectra show that 7H4MC hardly reacts with 3-indole methanol 30 minutes in the dark. Because 3-indole methanol has a benzene ring, it has a strong characteristic absorption peak at 281 nm, which has a certain masking effect on the characteristic absorption peak of 7H4MC in the B band, so the experiment mainly studies the characteristic absorption peak of 7H4MC at 323 nm is shown in Figure 6A. It can be seen from Fig. 6B that the addition of 3-indole methanol promotes the process of the 7H4MC photochemical reaction, but the degree of promotion is small, and the reaction is no longer promoted after a period of light. Therefore, it is speculated that 3-indole methanol can react similarly to ethanol at the initial stage of addition, and bind to the "CO" bond, so the reaction will be promoted at the beginning, but 3-indole methanol has two hydrogen donors, hydrogen donor existence makes the hydrogen connected to it easy to leave in an ionic manner, so the generation of hydrogen protons after light excitation will inhibit the reaction from proceeding, thereby inhibiting the photolysis of the 7H4MC ester structure. When 3-indole methanol was added to 7H4MC, the inhibitory effect occurred. It is mainly that the hydrogen ions left by the 3-indole methanol after illumination affect the reaction of 7H4MC with ethanol, which in turn leads to competitive inhibition.

3.4. Light-induced Photochemical Reaction Mechanism of 7H4MC

Comprehensive analysis of the above experimental data can infer the mechanism of light-induced photochemical reaction of 7H4MC. Under light excitation conditions, 7H4MC undergoes photolysis and is accompanied by the formation of new substances. 7H4MC obtains energy to break the "CO" bond in the ester-like group under light conditions [7], reacts with ethanol to form a new substance containing the ester group, realizes photodecomposition, produces a steady-state spectral change, and has new absorption peak is generated, as shown in Figure 7.

![Figure 7. Mechanism diagram of photochemical reaction of 4-methyl-7-hydroxycoumarin.]

4. Conclusion

In this paper, the photochemical reaction process and mechanism of light-induced 7H4MC has been studied. The results showed that 7H4MC did not react with t-H$_2$O$_2$ and Na$_2$S$_2$O$_4$. When irradiated by the xenon lamp, the absorbance of the characteristic absorption peak of 7H4MC at 323 nm decreased significantly with a slight blue shift; the absorbance of at 279 nm gradually increased, and there was a tendency for new peaks to appear. It is speculated that there is a structural change or the formation of new substances. This phenomenon is also confirmed by the slight red shift of the emission peak when excited at 374 nm in the fluorescence spectra. VC, 3-indole methanol, GSH, imidazole and other substances have different degrees of influence on the photochemical reaction of 7H4MC. The addition of imidazole inhibits the reaction to a certain extent, while GSH greatly promotes the progress of the photochemical reaction. It is speculated that the photosensitive mechanism may be due to the breakage of the "C-O" similar to the ester group, which reacts with ethanol to form a new substance containing the ester group.

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References

[1] Yu H, Xiao X D, Xu X S and Li Q Y. 2015 Effect and mechanism of the proliferation and apoptosis of the MCF-7 cells on copper complexes with aromatic aldehyde derivatives in vivo and vitro[J] Journal of Jiangsu University (Medicine Edition) 25 (1) p49.

[2] Mizoguchi H, Kubo K, Sakurai T and Inoue H. 2010 Solvent effects on the proton-transfer reactions of 7-hydroxycoumarin and its 4-methyl derivative with tertiary amines in the ground and excited singlet states [J] Berichte der Bunsengesellschaft fur physikalische Chemie 101 (12) p1914.

[3] Li L D and Jin G Z. 1994 Luminescence properties of 4-Methy-7-hydroxy-coumarin [J] Chinese Journal of Analytical Chemistry 22 (5) p440.

[4] J. Sérgio Seixas de Melo and António L. Maçanita. 2015 Unveiling the Eigen-Weller Ion Pair from the Excited State Proton Transfer Kinetics of 3-Chloro-4-methyl-7-hydroxycoumarin [J] Journal of Physical Chemistry B 12 (1) p198.

[5] Hoshiyama M, Kubo K, Igarashi T and Sakurai T. 2001 Complexation and proton dissociation behavior of 7-hydroxy-4-methylcoumarin and related compounds in the presence of β-cyclodextrin[J]. Journal of Photochemistry and Photobiology A Chemistry 138 (3) p227.

[6] Fernandes J. 2001 Spectroscopy and photophysics of 4- and 7-hydroxycoumarins and their thione analogs[J]. Journal of Molecular Structure 556-557 p69.

[7] Trozzolo A M, Dienes A and Shank CV. 1974 Excited-state reactions of a laser dye. Evidence for a two-step phototautomerism in 7-hydroxy-4-methylcoumarin [J] Journal of the American Chemical Society 96 (14) p4699.