STUDY OF THE PHOTODEGRADATION OF METHYLENE BLUE: POTENTIAL APPLICATION IN ANTIBACTERIAL PHOTODYNAMIC THERAPY

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ABSTRACT

Photodynamic Therapy (PDT) is an ancient technique that uses light as a therapeutic agent, with roots in ancient civilizations such as Egypt, India, and China, although its scientific understanding developed in the early 20th century. Niels Finsen, a Danish physician, innovated by applying red and ultraviolet light in the treatment of smallpox and cutaneous tuberculosis, earning him the Nobel Prize in 1903. PDT, introduced by von Tappeiner, is based on the use of light-activated photosensitizers to damage target cells, receiving FDA approval to treat esophageal cancer in 1995. The photochemotherapy technique combines photosensitizers with light in the tissues where they are present, with applications dating back to ancient times, such as the use of psoralens by indigenous people to treat vitiligo. PDT has found applications in various medical fields, such as cancer, dermatology, ophthalmology, dentistry, and more. Methylene blue is a common photosensitizer in PDT, generating reactive oxygen species when activated by light, being used in the treatment of cancer and infections, as well as improving tissue visualization in medical procedures. In this work, we studied the photodegradation of methylene blue when irradiated with different energies at a wavelength of 661 nm.

Keywords: Photodynamic therapy; Methylene Blue; UV-VIS Spectrophotometry.

INTRODUCTION

The history of light therapy dates back to ancient civilizations such as Egypt, India, and China, which used sunlight to treat various health conditions. Photodynamic therapy (PDT) began in the late 19th century when Danish physician Niels Finsen developed phototherapy and received the Nobel Prize for its use in treating skin diseases. PDT evolved over time, transitioning from conventional lamps to lasers due to their ability to emit monochromatic light. It is applied in various medical fields, including oncology, dermatology, ophthalmology, dentistry, gynecological oncology, treatment of vascular lesions, bacterial, fungal, and protozoan infections, inflammatory diseases, and vascular therapy. Methylene Blue (Figure 1), a photosensitizer, is used in PDT to damage target cells when activated by light at specific wavelengths. Its versatility, low cost, low toxicity, and antimicrobial action make it suitable for various clinical applications, such as the treatment of cancer and infections, but its use should

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be supervised by qualified healthcare professionals, adapting to the specific needs of each patient. (KESSEL, 2019; ALGORRI et. al, 2021; WU et. al, 2019; TANG et. al, 2020; FANG et. al, 2021; YOU et. al, 2023;)

Figure 1 - Structure and model for a methylene blue dye molecule.

Source: ZHANG et al., 2011

METHODOLOGY

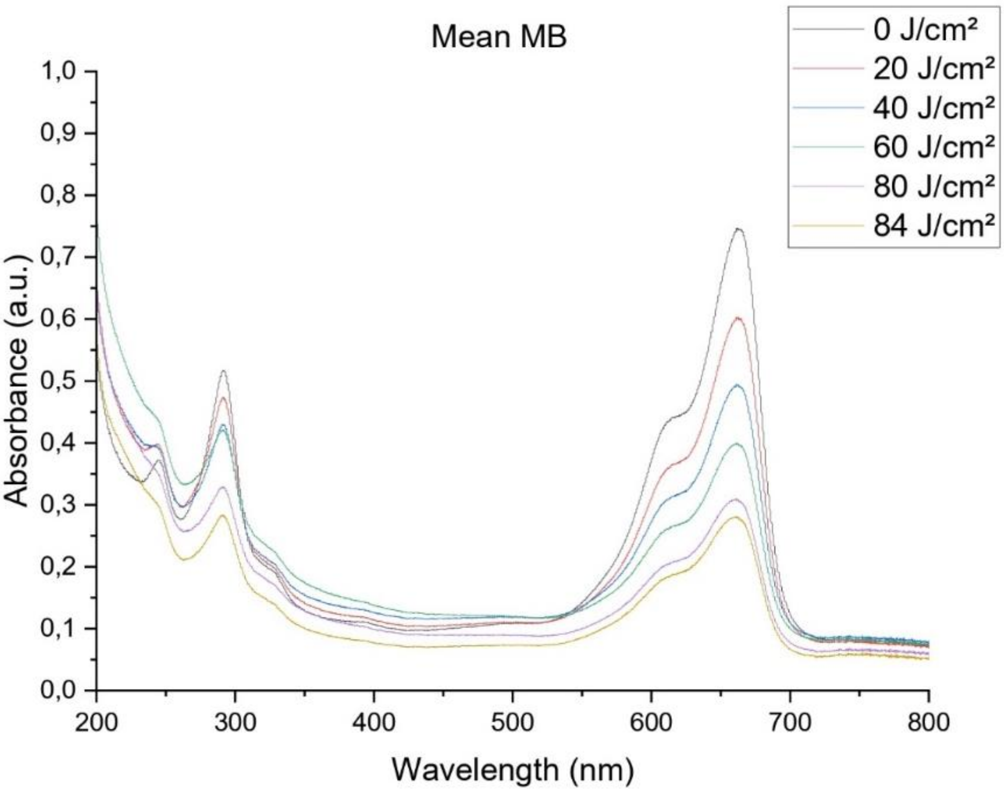
In this study, an LED light source with a specific wavelength of 660 nm was employed to activate methylene blue in Photodynamic Therapy (PDT). The analysis of the samples was carried out using a Perkin Elmer Lambda 35 UV-VIS spectrophotometer, along with quartz cuvettes that allow for precise analysis of elements in the UV spectrum. This spectrophotometer uses Beer’s Law to separate organic and inorganic compounds based on wavelength. The data obtained were processed using the Origin software, which is a graphical tool for data analysis and statistics. During PDT, photosensitizers, such as methylene blue, interact with light to generate reactive oxygen species, resulting in the photodynamic effect, as illustrated in the Jablonski diagram. Photosensitizers can transition to excited states, singlet or triplet, triggering type I and type II reactions that damage target cells. The choice between these reactions depends on the characteristics of the photosensitizer and the specific clinical objectives, such as the selective destruction of cancer cells or pathogenic microorganisms.

1125 µM of methylene blue in 0.1 L of distilled water were weighed on an analytical balance in a 100mL volumetric flask. After preparation, it was diluted to 20 µM, where the degradation of methylene blue was analyzed under 660 nm light exposure at the respective times: t=0J/cm2, t=20J/cm2, t=40J/cm2, t=60J/cm2, t=80J/cm2, and t=84J/cm2. For the execution of the protocol, a sterile cell culture plate, made of polystyrene with a flat bottom, and a device that emitted 660 nm light were used. To have a better view of the degradation, it was analyzed in triplicate, each with 3mL of methylene blue at a concentration of 20 µM.

RESULTS

The photobleaching of methylene blue, an indicator of the photosensitizer's efficacy in photodynamic therapy, was observed at all evaluated energies, with an average degradation of 12% (Table 1). The difference in degradation between the non-illuminated samples and those illuminated with 84J/cm² was 62%. Previous studies reported greater degradation with the addition of catalysts. The change in the color of methylene blue after exposure to light indicated its degradation.

Figure 2 - Average of Methylene Blue Samples



Source: Own elaboration.

Table 1 - Average of Methylene Blue Samples

Average Degradation Percentage						
Energy per Pulse	Nm	Abs	%		Average (%)	Total degradation (%)
0 J/cm²		0,746667	100%	-		
20J/cm²		0,603	81%	19%		
40J/cm²	661	0,495	66%	14%	12%	62%
60J/cm²		0,4	54%	13%		
80J/cm²		0,310333	42%	12%		
84J/cm²		0,281333	38%	4%		

Source: Own elaboration.

CONCLUSION

Our results demonstrated that the degradation of methylene blue increased with the increase in light dose, with an average degradation of 12% for all evaluated energies. Furthermore, variations in the spectral properties of methylene blue were observed, including different maximum absorption wavelengths. In summary, this study provided insights into the degradation of methylene blue under different light doses, relevant for applications such as photodynamic therapy.

THANK YOU

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