DETECTION OF FORMALDEHYDE USING FLUORAL-P.

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Abstract: Formaldehyde is a volatile organic compound classified by the International Agency for Research on Cancer as a carcinogen, doses of 14.96 ppm of formaldehyde in rats are capable of developing tumors in their biological structure. The concern with this aldehyde is due to the health problems that can be generated by it when exposed to humans and also to contribute to the degradation of marine fauna. In view of this, the aim of this project is to develop a prototype for detecting formaldehyde by reacting it with fluoral-p to form 3,5-diacetyl-1,4-dihydrolutidine (DDL). The development of this work is based on the analysis of the fluorescence emission wavelength of the luminescent spectrum of the reaction between fluoral-p and formaldehyde present in seawater samples, and this fluorescent light emission is the indicator for the detection of this aldehyde.

Keywords: Sea water; Fluoral-p; Formaldehyde; DDL; Marine ecosystem.

DETECÇÃO DE FORMALDEÍDO ATRÁVES DE FLUORAL-P

Resumo: O formaldeído é um composto orgânico volátil classificado pela Agência Internacional de Pesquisa em Câncer como cancerígeno, doses de 14.96 ppm de formaldeído em ratos são capazes de desenvolver tumores em sua estrutura biológica. A preocupação com este aldeído é devido aos problemas de saúde que possam ser gerados por ele quando exposto ao ser humano e também contribuir para degradação da fauna marinha. Diante disso, o presente projeto possui como objetivo desenvolver um protótipo de detecção do formaldeído a partir da reação com fluoral-p para a formação do 3,5-diacetil-1,4-dihidrolutidina (DDL). O desenvolvimento deste trabalho leva como base análises do comprimento de onda da emissão de fluorescência do espectro luminescente da reação entre fluoral-p e formaldeído presente em amostras de água do mar, sendo essa emissão luminosa fluorescente o indicativo para a detecção desse aldeído.

Palavras-chave Água do mar; Fluoral-p; Formaldeído; DDL; Ecossistema marinho.

1. INTRODUCTION:

The ocean plays a crucial role in the Earth's dynamics, contributing to global climate regulation [2] and supporting a diverse range of fauna and flora through various physico-chemical interactions. The presence of contaminants in marine environments represents a significant environmental concern due to their potential adverse effects on marine life and human health. Among the chemical pollutants of interest, formaldehyde (HCHO) has emerged as a highly relevant substance. Formaldehyde is a highly reactive and volatile species, often associated with the degradation of organic matter and atmospheric oxidation of hydrocarbons.

The occurrence in marine waters may arise from natural sources, such as the decomposition of organic material, but it is also frequently introduced by anthropogenic activities, including industrial discharges, untreated sewage, and the use of chemicals in vessels. Additionally, formaldehyde can be present as a byproduct of other chemical compounds or because of complex photochemical processes involving the interaction between atmospheric pollutants and seawater [2-4].

The precise and sensitive detection of formaldehyde in marine environments is essential for monitoring water quality and assessing the impact of human activities on marine ecosystems, superficial formaldehyde concentration was 15 mg L^{-1}; The concentration along the water column ranged between 4.5 to over 40 mg L^{-1} [2]. In this context, fluorescence spectroscopy has emerged as a promising analytical technique, enabling the rapid and selective determination of organic compounds in various environmental samples, with its current phase having a detection range of 50 mg L of formaldehyde using fluoral-p.

The use of acetylacetone in presence of ammonia (also called Nash reagent) to estimate the amount of formaldehyde was first time published in 1952 by Nash to report the neutralization of formaldehyde in living bacterial cultures [5]. This reaction proceeded very quickly at pH 6 and the yellow color obtained is due to the formation of diacetyl-dihydro-lutidine (DDL). The use of the Fluoral-p reagent in fluorescence spectroscopy has been under investigation due to its high specificity in reacting with formaldehyde, forming a very stable UV/VIS sensitive compound which exhibits a maximum absorbance at a wavelength of 410 nm a stable fluorescent complex [6]. This reaction emits fluorescence around 510 nm upon been excited with UV light (~410 nm). Moreover, the technique of fluorescence spectroscopy with the Formal P reagent offers advantages, such as simplicity in sample preparation and low interference from other compounds present in seawater.

The aim this study was to develop a fluorimetric sensor for detecting formaldehyde in aquifers and marine environments. To validate the approach, the sensitivity of the experimental apparatus to different concentrations of formaldehyde in sea water was studied. This article demonstrates the results obtained for samples with known formaldehyde concentrations in the range of 50-400 mg L^{-1} using a traditional UV/VIS absorption spectrometry and compares the results with those found in the literature.

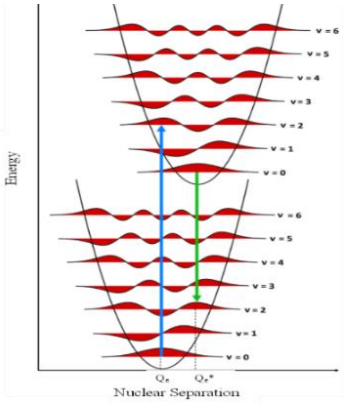
1.1 Fluorescence Spectroscopy

Environmental contamination in marine ecosystems has become a pressing global issue, prompting the urgent need for efficient and sensitive detection methods to monitor and assess pollutant levels. Among the arsenal of analytical techniques available, fluorescence spectroscopy has emerged as a powerful tool for identifying and quantifying various contaminants in environmental samples. In this article, one of

the goals of this study is to provide an in-depth exploration of the concept, instrumentation, and application of fluorescence spectroscopy, with a focus on its use in developing sensors for detecting contaminants in marine environments.

Fluorescence spectroscopy is a non-destructive analytical technique that exploits the unique optical properties of fluorescent molecules. When exposed to excitation light of a specific wavelength, certain molecules absorb the energy and undergo an electronic transition to higher energy levels Figure (1). Subsequently, they emit light of a longer wavelength (lower energy) in a process known as fluorescence. The emitted fluorescence intensity and spectral characteristics can be correlated to the concentration and identity of target analytes, making fluorescence spectroscopy extremely sensitive and selective [7].

Figure 1: Energy levels of electrons in a molecule, represented as a function of nuclear coordinates Q. The horizontal lines correspond to the vibrational levels of the nuclei of the molecules. The arrows refer to: (Blue) light absorption; (green) fluores.



Fluorescence spectroscopy instruments typically consist of three main components: a light source, a sample compartment, and a detection system. The light source emits excitation light of a specific wavelength, for this work, we used a Led at 405 nm. The sample compartment houses the environmental sample of interest, where fluorescence is induced upon excitation. A four-sided transmission cell was used because in fluorescence measurements, the direction of the incident beam must be at 90° from the emitted beam, as the emitted fluorescence has a much lower light intensity than the transmitted one. Thus, it is possible to let the transmitted light pass without interfering with the fluorescence signal [8]. A set of filters or monochromators is employed to isolate the emitted fluorescence from the excitation light, ensuring accurate measurements. Finally, the detection system, which may involve photomultiplier tubes (PMTs) or charge-coupled devices (CCDs), records the fluorescence emission signal for subsequent analysis.

Fluorescence-based sensors have gained prominence in environmental monitoring due to their high sensitivity, rapid response times, and ability to detect a wide range of contaminants. In the context of marine environments, fluorescence sensors can be employed to detect pollutants such as petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), heavy metals, and pesticides, among others. These sensors are designed to selectively interact with target analytes, generating fluorescence signals proportional to the contaminant concentration [9,10].

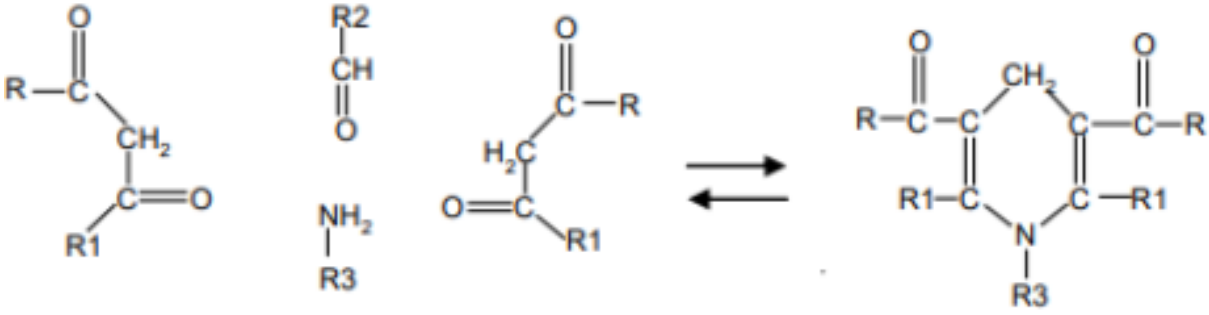
The development of fluorescence-based sensors for environmental contaminant detection involves careful selection of fluorophores or fluorescent probes that exhibit high affinity and specificity for the target pollutants. Additionally, advancements in

sensor design, miniaturization, and incorporation of microfluidics have allowed for the creation of portable and real-time monitoring devices, enabling on-site and in-situ detection [11-13].

1.2 Nash Reaction:

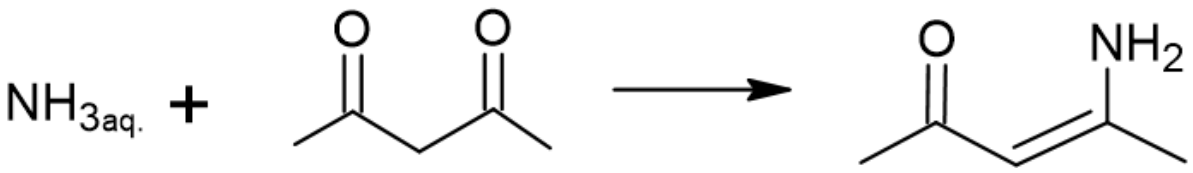
The reaction of neutralization of formaldehyde in bacterial cultures was introduced by Nash for the photometric determination of formaldehyde. 2,4-Pentanedione and ammonium acetate (which serves both as a source of ammonia and a buffering agent) are used in a specific way for the determination of formaldehyde, giving rise to 3,5-diacetyl-1,4-dihydro lutidine (DDL) [14]. The Nash colorimetric procedure, based on the Hantzseh reaction between acetylacetone, ammonia and formaldehyde with formation of 3,5-diacetyl-1,4-dihydro lutidine (DDL) is shown in Figure (2).

Figure 2. Hantzseh reaction



The application of Nash's reagent for the determination of formaldehyde in continuous flow systems was found to be unsuitable for several reasons. Nash's reagent, 2,4-pentanedione in concentrated ammonium acetate (2M), reacts with formaldehyde to produce 3,5-diacetyl-2,6-dihydro lutidine (DDL). It has a high viscosity, usually requires the use of 2M ammonium acetate and is difficult to mix with other liquids such as the mobile phase normally used in reversed phase HPLC systems. Despite the above difficulties, this method exhibits high selectivity and sensitivity, which favors its application [15]. During studies of aldehyde detection systems it was discovered that in Nash's reagent there is a specific constituent, the product of the reaction between 2,4-pentanedione and ammonia, which is easily isolated and stable and can be stored as an analytical reagent [15] is shown in Figure (3). This compound (4-amino-3-penten-2-one), which was named fluoral-p in reagent form, was used as a reagent for the photometric determination of aldehydes. It should be noted that this reagent forms colored compounds with aldehydes, but only forms fluorescent compounds with formaldehyde [14].

Figure 3. Fluoral-p formation



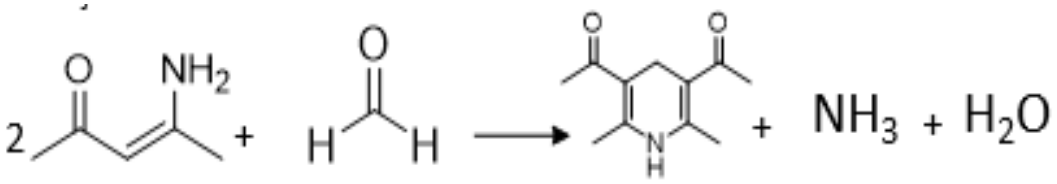
The resulting fluorescence signal allows for precise and real-time quantification of formaldehyde concentrations, making it a promising technique for environmental monitoring in marine settings.

2. METHODOLOGY:

The chemical supplies and reagents used were Fluoral-p (realized by the authors); Formaldehyde P.A (–Vetec Química fina); Ammonium acetate; Acetylacetone P.A (NEON); Acetic acid glacial 99,8% P.A (NEON); Ethanol 95% P.A./ACS (NEON); Led 405nm M405 FP1(ThorLabs); Spectrometer 3648 (Avantes); 1 pair of M28L01 optical fiber (Thorlab); Tcube led driver (ThorLabs); Sample holder QPpode2e Quantum (North West); Cuvettes; Glass filter GF / F 47mm (WHATMAN).

The fluoral-p used was prepared in the laboratory by reacting 0.2 mL of acetylacetone with 15.4 g of ammonium acetate in the presence of 0.3 mL of acetic acid, the final volume being adjusted to 100 mL with 50% (v/v) ethanol [16]. This process is repeated every time an optical analysis is performed, always using a reagent prepared on the same day as the analysis. After the preparation of the reagent, the step of fluorescence analysis of the reaction between 1mL of the fluoral-p reagent and 1 mL of the seawater sample containing different concentrations of formaldehyde (50 mg L^{-1}, 100 mg L^{-1}, 200 mg L^{-1}, 300 mg L^{-1}, 400 mg L^{-1}) is carried out, this reaction will form the DDL which is represented in the Figure (4). This analysis is carried out in plastic cuvettes with a volume of 3.75 ml inserted in the sample holder connected to the spectrometer and simultaneously coupled to the computer with the Avasoft software to obtain the reaction spectrum.

Figure 4. DDL formation

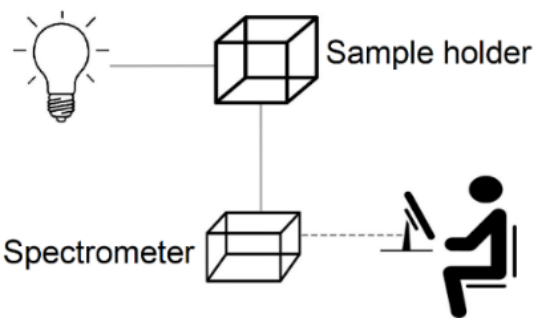


Fluoral-p Formaldehyde DDL

Seawater was collected at Itapuã beach, located in the city of Salvador, Bahia, Brazil, (Lat. 12° 57 '24.9"S-Long. 38°23'09.1"W), in the fall of 2023 and stored in a polyethylene bottle. A day after collection, the seawater was filtered using a glass filter GF / F 47mm WHATMAN.

The configuration consisted of a 405 nm LED, as a light source, directed through an optical fiber towards the sample in the sample holder. The emitted fluorescence light is directed at a 90-degree angle through an optical fiber to the Avantes Spectrometer which is connected to a computer. The AvaSpec software records the intensity values at different wavelengths,thereby generating the fluorescence spectrum of the analyzed sample Figure (5).

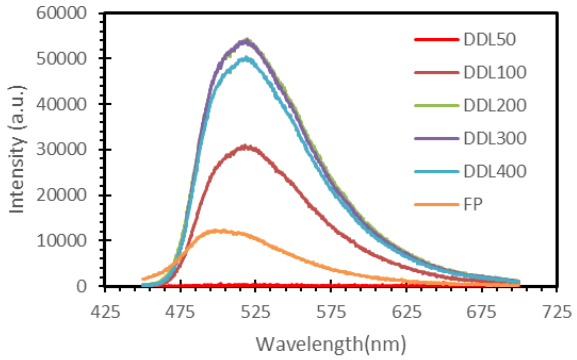
Figure 5. Experimental set up



3. RESULTS AND DISCUSSION:

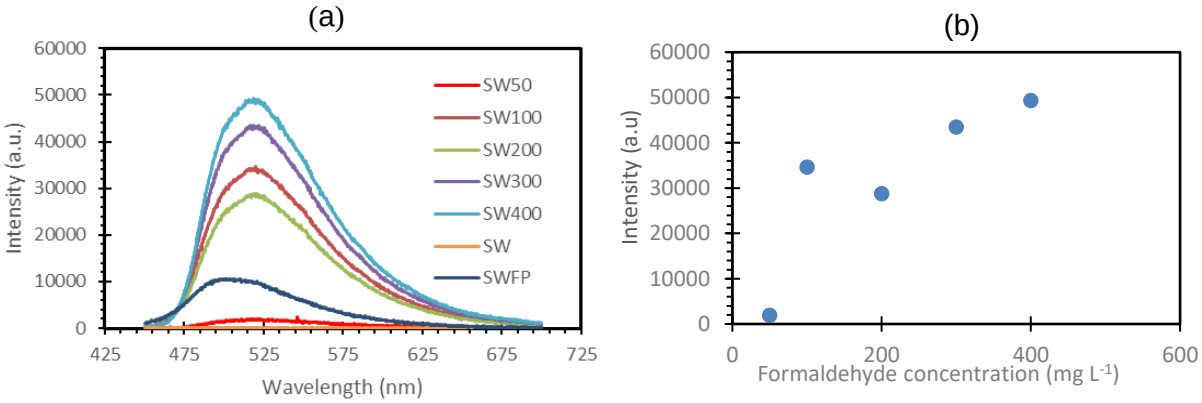
The recording of fluorescence emission data from the formation of the DDL compound from the reaction between formaldehyde and fluoral-p is carried out at this stage of the optical analysis. Figure 6 is the fluorescence spectra of FP/FA 0-400 mg L^{-1}. The DDL sample with different concentrations of FA (formaldehyde) has a fluorescence peak around 518nm; while FP (fluoral-p) shows a blue-shifted fluorescence peak around 500nm, even when mixed in seawater, Figure (7). This means that the seawater collected does not contain any substance that reacts to form fluorescence at the same wavelength as formaldehyde.

Figure 6. Fluorescence spectra of Fluoral-p when reacted with 0-400mg/L FA



Through the spectra in Figure (6) above, the DDL samples with different concentrations of FA show a fluorescence peak around 518 nm; while the FP shows a blue-shifted fluorescence peak around 500 nm, both pure and mixed in seawater, Figure (7) located on the left (a). Figure (7) located on the right (b) represents the fluorescence intensity at 518 nm as a function of the concentration of FA in seawater, confirming the veracity of the data according to the Beer-Lambert law of spectrophotometry.

Figure 7. Fluorescence spectra of Fluoral-p when reacted with 0-400 mg L^{-1} FA into seawater and fluorescence intensity at 518nm versus the concentration of FA in the seawater.



The analysis of the spectra of the substances involved in the reaction is of major importance during the results phase. This critical step allows us to verify the effectiveness of the process and validate the theory based on real-world observations. According to the literature [17], formaldehyde itself does not emit fluorescence, while DDL, the product of the reaction between fluoral-p and formaldehyde, shows

fluorescence in the range of 500 to 545 nm. Throughout our experiments, we made a discovery regarding the detection limit of formaldehyde in seawater, which is up to 50 mg L^{-1}. Below this concentration, there were no indications of DDL formation in the reagent medium. This finding highlights the importance of the prototype in the detection of formaldehyde in marine environments, as it allows researchers to monitor concentrations as low as 50 mg L^{-1}. Further aiming to devote a greater detection range for improved performance and ability to identify formaldehyde levels in seawater, with such enhanced sensitivity it becomes possible to understand the potential impact of formaldehyde on the marine ecosystem and assess its environmental implications. The sample collected on the shore of Itapuã beach did not emit fluorescence and showed no signs of formaldehyde contamination in the tests carried out. The sampling site is an area frequented by bathers and far from submarine outfalls, which suggests that if formaldehyde is present in this area, it must be in quantities of less than 50 mg L^{-1}, so it cannot currently be detected. It is to be expected that environments located near submarine outfalls will have higher concentrations of this aldehyde.

4. CONCLUSION:

In view of the above, it is evident that the development of the proposed research has a significant importance for the scientific, environmental, and social community. Since the ocean with its abundant diversity of fauna and flora, plays a fundamental role in maintaining the stability of the planet. In addition, formaldehyde when in contact with humans can develop cancer and various health problems. Despite its current limitation in detecting concentrations below 50 mg L^{-1} of formaldehyde in water, this tool can contribute invariably to monitoring potential impacts of formaldehyde in both aquatic and social environments. Therefore, for future phases of the project, some changes were considered, such as changes in the LED and the addition of a region for collecting seawater. Regarding the radiation source, to achieve an analysis capable of detecting minute amounts of formaldehyde, changing the current LED to one with greater power is essential. New locations were mentioned to collect samples due to the low pollution in the current region, locations that have sewage terminations should have a higher amount of formaldehyde, thus not needing to add a dilution in the collection sample to simulate contamination.

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