Journal of Optoelectronics and Advanced Materials Vol. 1, No. 1, March 1999, p. 63-68

OPTICAL ABSORPTION STUDY OF THE STABILITY MODIFICATIONS OF AQUEOUS SOLUTIONS OF 5-AMINOLEVULINIC ACID INDUCED BY THE VARIATIONS OF THE URINARY pH

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The photodynamic therapy of bladder cancer can be achieved via the administration of 5-aminolevulinic acid (ALA), which is naturally converted into the photoreactive substance, the protoporphyrin IX. This study analyses the stability of aqueous solutions of ALA in different concentrations (0.5%, 1% and 2%) having in view the influence of pH modification (pH range from 5 to 9) induced by the urine provided by patients with urinary bladder cancer. Patient's alimentation influences the values of urinary pH that induce high modifications of ALA aqueous stability. These results are important for the future strategies in administration of ALA and in photodynamic therapy.

(Received October 15, 1998; accepted February 8, 1999)

1. Introduction

Photodynamic therapy (PDT) is a relatively new modality for the treatment of neoplasm disorders, as well as other pathologic conditions that are characterised by excessive cellular proliferation such as intimate hyperplasia, psoriasis, rheumatoid arthritis and age-related macular degeneration [1]. The principle of the PDT consists in its double selectivity: 1) a photosensitising agent is preferentially accumulated and/or retained by the proliferating cells and 2) the diseased tissue is irradiated with light wavelengths specifically absorbed by the photosensitizer.

So far PDT has been widely applied in the oncology field: several thousands of patients with tumours were treated by PDT. This could make the basis of a new photochemotherapy.

The efficacy of PDT depends on the structure of the photosensitizer, the administration modality, the light source, and the treatment procedure.

A large number of photosensitising agents has been tested both *in vitro* and *in vivo* PDT experiment, but there is not still found a photosensitizer with ideal properties [2]. The main classes of photosensitizers are exogenous (porphyrin derivatives, phtalocyanins and porphycenes) and endogenous ones such as protoporphyrin IX. Protoporphyrin IX is believed to be the predominant porphyrin metabolites induced by exogenous ALA [3].

For the treatment of the gastro-intestinal tract, and of cerebral or bronchopulmonary tumours, the compounds are orally or intravenously administrated. For the treatment of the skin tumours the drugs are applied mainly topically and for the bladder carcinomas by intravesical instillation [4].

The most widely used light systems are the argon ion pumped dye laser (argon-PDT–630 nm), the gold vapour laser and the incoherent light source emitting light close to the absorption peaks of the sensitizer. Diode lasers emitting at long wavelengths of 780 nm to 904 nm represent a promising development in PDT.

The aim of our work was to establish the optimal conditions of ALA administration in PDT of urinary bladder cancer. In this respect, our study analyses the stability of aqueous solutions of ALA in different concentration (0.5%, 1.0% and 2.0%) having in view the influence of pH modification (pH range from 5 to 9).

2. Materials and methods

The absorption spectra of aqueous solutions of ALA and urinary test were recorded by using UV - VIS absorption spectrophotometry method at different times: 0 min, 10 min, 20 min, 30 min, and 60 min from the initial moment of solution preparation.

To study the dependence of the ALA reaction on pH and concentration we prepared a stock solution of ALA at 2% concentration and two other buffered diluted solutions: 1.0%, and 0.5% concentrations. These solutions were prepared in PBS buffer at pH: 5.8, 7.0, and 8.0.

3. Results

The optical absorption spectra of aqueous ALA solutions show one or two peaks, depending on the concentration (Fig. 1).

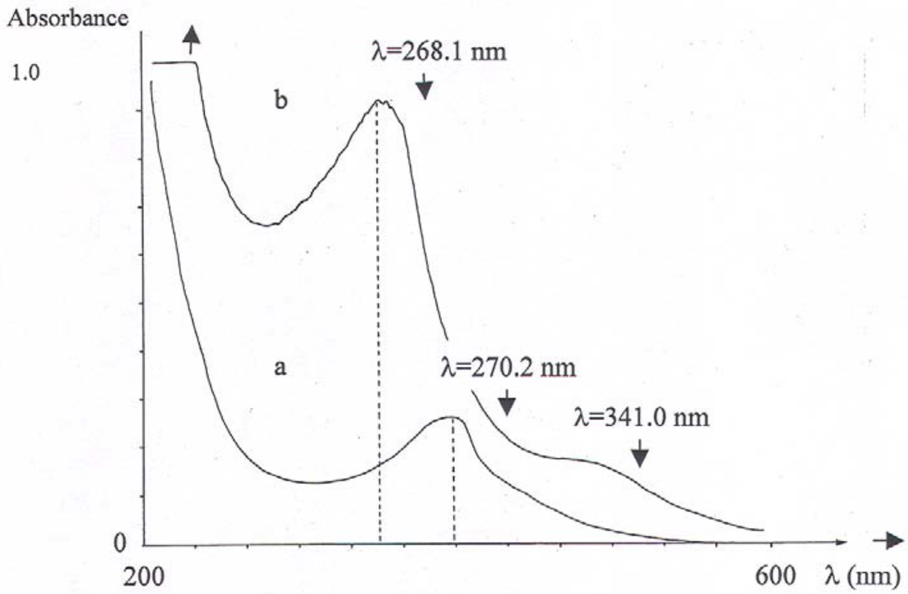

Fig. 1 The optical absorption spectra of aqueous ALA solutions at pH=7.0 recorded immediately after preparation a) c = 0.5%, b) c = 2%.

As it results from Fig.1, the aqueous ALA solution with the concentration 0.5%, exhibits one absorption band at $\lambda = 270.2$ nm while the other ALA solution (c = 2.0%) has two absorption bands at $\lambda_1 = 268.1$ nm and $\lambda_2 = 341.0$ nm. In this study we analysed only the modifications on the first absorption band situated in the UV domain induced by pH and concentration.

In order to study the pH dependence of the UV absorption band, we prepared the different stock solutions (c = 0.5%, 1.0% and 2.0%) at pH: 5.8, 7.0, and 8.0 and we measured their absorption spectra at $\lambda = 268.0$ nm, and at different reaction times. The absorption due to the concentration of hydrogen ion is presented in Fig. 2.

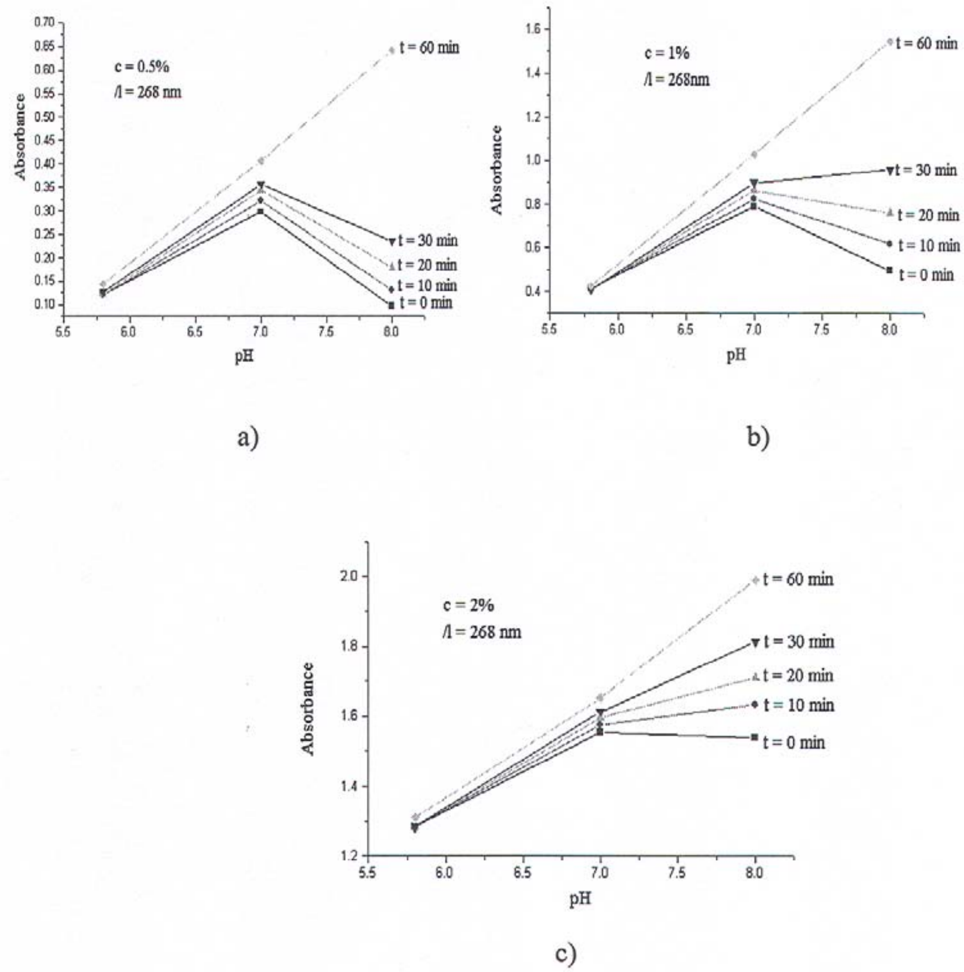

Fig. 2 Time dependence of ALA absorption with the pH of solution
a) c = 0.5% b) c = 1.0%; c) c = 2.0%.

From Fig. 2 it is noticeable that in acid medium the UV absorption band intensity shows a slow increase with pH modification irrespective of concentration and time.

For 0.5% and 1.0% ALA solutions, the absorption band intensity decreases strongly in the first half an hour elapsed, in alkaline medium. After an hour from the solution preparation, the absorbency bands intensity increases irrespective of concentration and pH.

It is, also found that, at pH = 5.8, irrespective of the time moment, the absorbance is practically constant. At the same value of pH (namely, 5.8) arises the least wavelength shift corresponding to the maximum of absorption (Fig. 3)

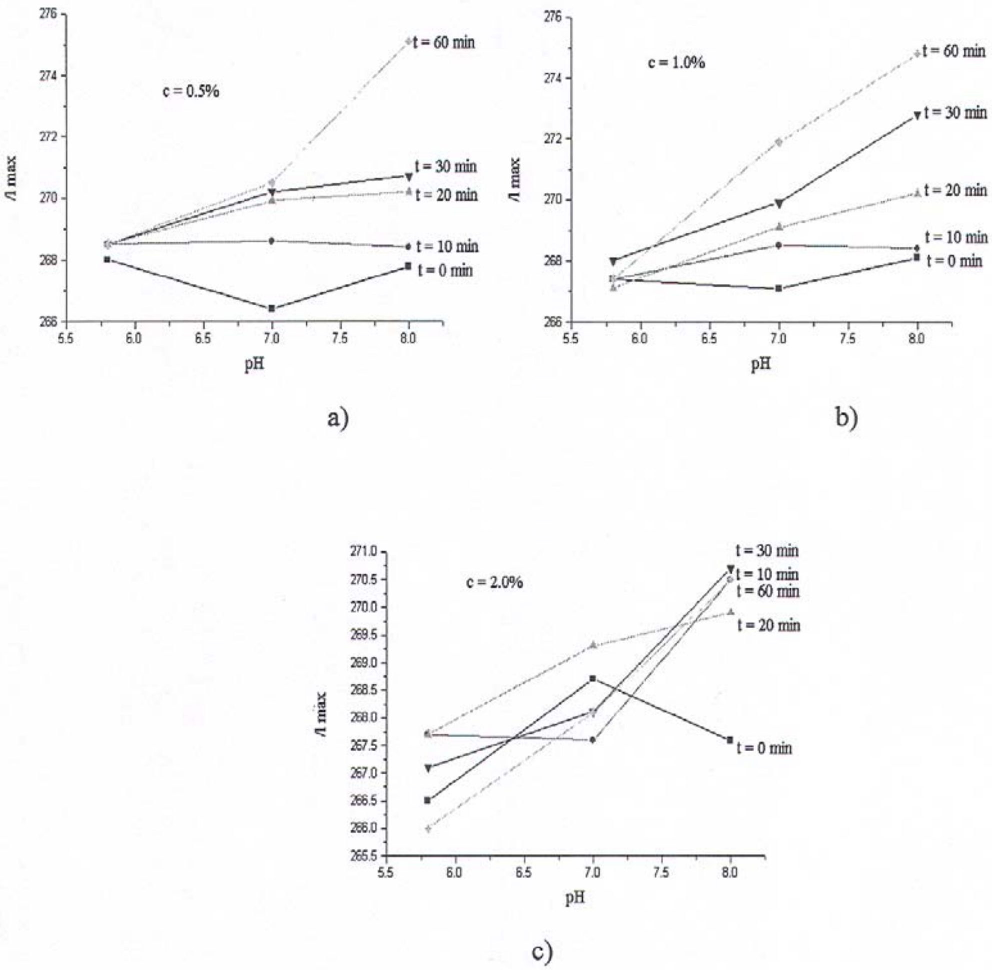

Fig. 3 Dependence on pH of the absorption band shift of aqueous ALA solutions in the UV domain a) c = 0.5% b) c = 1.0% c) c = 2.0%.

In alkaline medium, the absorption band presents a significant change of the place from the highest value of λ. After an hour from the solution preparation, the shift of λ_{max} is large (i.e. for ALA solutions: 0,5% concentration and pH = 8.0, $\Delta\lambda_{max}$ = 8.9 nm, and for 2% ALA solution at the same pH, $\Delta\lambda_{max}$ = 4.9 nm). Note that, $\Delta\lambda_{max}$ is smaller at higher than at lower concentrations.

To establish the concentration values of ALA solutions that can be used in the treatment of urinary bladder cancer, we have drawn the absorption spectra of urine sample from the affected patients, before and after the addition of ALA solution in various concentrations (0.5%, 1.0% and 2.0%). The spectra are represented in Fig. 4.

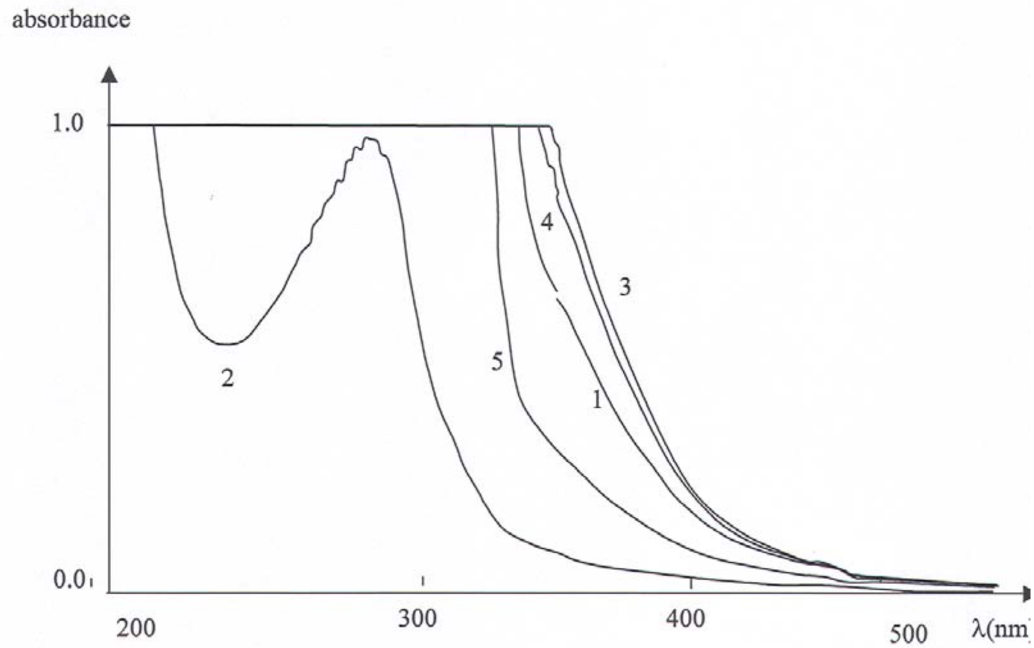

Fig. 4 The optical absorption spectra of urine sample from a patient with urinary bladder cancer; 1-urinary absorption spectrum; 2- ALA absorption spectrum; 3, 4 and 5- absorption spectra of urinary samples with ALA solutions in different concentrations.

Having in view that a strong interaction occurs between aqueous ALA solutions and urine, which leads to modifications of the optical spectra, it is necessary for clinical applications, to use concentrated solutions of ALA (more than 2.0%) and a small volume of urine.

4. Conclusions

The mechanism of preferential intratumoral uptake of the precursors and photosensitizers is still not fully understood. In the case of ALA, the active transport is the most likely explanation of uptake, but passive diffusion may be operative as well [2].

The properties of aqueous ALA solutions were studied with the aim to find the optimal condition for their administration to patients. The stability of ALA solution, to be used for instillation into the urinary bladder was investigated. There was experimentally established that the absorption spectrum of aqueous solutions of ALA presents two absorption bands at $\lambda \cong 268$ nm and $\lambda \cong 341$ nm. The effect of various parameters, such as concentration, pH and time on the stability of ALA solution was studied. These studies were performed by using UV-VIS absorption spectrophotometric method

At pH = 5.8 the absorbency of ALA is not variable in time. At alkaline pH = 8.0 the absorbency increases only after one hour from the solution preparation. At neutral pH = 7.0, the absorbency increase is small.

The absorption maximum is localised at $\lambda_{max} \cong 267$ nm in acid medium (pH = 5.8, c = 2.0%, t = 0 min), but presents a shift of the wavelength to highest value. In alkaline solutions $\lambda_{max} \cong 268$ nm (pH = 8.0, c = 2.0%, t = 0 min).

It is possible that in ALA solutions could be present more than one compound, or could take place some interference, or could be ruled a new chemical process [5].

In the presence of ALA solutions, in the urine are taking place some interactions and some new compounds appear. In the case of intravesical instillation, the ALA concentration must be higher than 2% in order to observe an efficient action of this therapy.

Further studies might give us the optimal moment of aqueous ALA solution instillation in urinary bladder as a function of urinary metabolism and the importance of their pH in the photodynamic therapy.

In spite of these positive results, here outlined, there are several limitations regarding the clinical applications:

a) chemical instability (a new reaction can be initiated)

b) high concentration needed to induce the tumour photosensitization

c) prolonged persistence and a long clearance time

To overcome these limitations further studies are needed.

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