# Tryptophan photooxidation promoted by new hybrid materials prepared by condensation of naphthalene imides with silicate by the sol–gel process

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## ABSTRACT

Three novel hybrid organic/inorganic materials were synthesized from 4-substituted ($NO_2$, Br, H) 1,8-naphthalene imide-$N$-propyltriethoxysilane by the sol–gel process. These materials were obtained as a xerogel and partially characterized. The ability to photosensitize the oxidation and degradation of tryptophan indole ring by these materials was studied through photophysical and photochemical techniques. Although the derivatives containing Br and $NO_2$ as substituent do not cause efficient tryptophan photodamage, the hybrid material obtained from 1,8-naphthalic anhydride is very efficient to promote tryptophan photooxidation. By using laser flash photolysis it was possible to verify the presence of naphthalene imide transient radical species. The presence of oxygen causes an increase of the yield of radical formation. These results suggest that the mechanism of photodegradation of tryptophan occurs by type I, i.e. the transient radical ($TrpH^{\bullet+}$) formed by the direct reaction of the triplet state of the naphthalene imide moiety with tryptophan. Thus a inorganic–organic hybrid material that can be used to promote the oxidation of biomolecules was obtained.

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## 1. Introduction

The development of photoactive materials with ability to cause photodamage in selective sites of peptides and proteins is important to elucidate particular aspects of protein structure and function and to furnish information about molecular interactions [1–4]. The choice of the materials and of the associated molecule is also important to photodynamic therapy and for the development of materials capable of causing photodamage to cellular biomolecules [5,6]. The naphthalene imides are good candidates to be incorporated into those materials due to their ground and excited states electron donor and acceptor characteristics and furthermore usually the triplet excited state is a very reactive species. The triplet excited state of naphthalene imides can react with triplet oxygen giving rise to singlet oxygen [7]. Also, their singlet or triplet state can directly react with biomolecules to create transient radical cation and anion. The mechanism of photodamage has been shown to depend on the naphthalene imides chemical structure and on media reaction conditions such as the presence of dis-

solved oxygen and the concentration of the naphthalene imides [1,8–10]. Kelly and co-workers studied the mechanism of interaction of naphthalene imides with proteins and amino acids and they observed that the targets to photodamage are specific biomolecule sites such as the tryptophan residue in peptides and proteins and the guanine moiety in DNA [1,9]. However, when naphthalene imides are in high concentration its own triplet state can react with its ground state creating a very long lived transient radical species that can compete with the reactions of the triplet state with biomolecules [1,8–10]. Therefore, the design and syntheses of materials based upon naphthalene imides that can avoid the self-aggregation and thus this secondary photoreaction are an initial concern. In fact, self-aggregation is a general problem that reduces photosensitizer efficiency [11]. The yield of photodamage can be improved by the naphthalene imide immobilization to hinder their diffusion in the bulk and consequentially the stacking with their ground state species [12–14]. Organic molecules can be included in solid structures by using hybrid organic–inorganic materials obtained by the sol–gel process [15]. In addition the use of these materials can also reduce the toxicity of naphthalene imides that occurs by the reaction or association (e.g. by intercalation in DNA) of their ground state with biomolecules. Materials obtained by the sol–gel process have been shown to be biocompatible and with potential application for drug delivery in medical and agricultural fields [16]. However the use of

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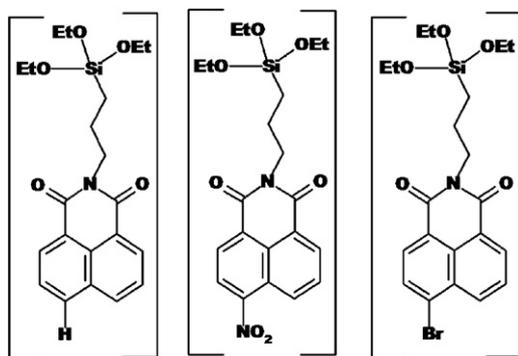
**Scheme 1.** The chemical structures of the monomeric units of the NISiH, NISiNO$_2$ and NISiBr hybrid materials.

these materials is still neglected as agents that make damage of biomolecules.

In this work three new organic–inorganic hybrid materials having naphthalene imide moieties with different substituents (i.e. H, NO$_2$ and Br) on the naphthalene ring were synthesized by the sol–gel process and the obtained non-crystalline materials were characterized. The photophysical and photochemical properties of these new hybrid materials were studied to elucidate the mechanism of photodamage of tryptophan as a model to further studies with peptides and proteins.

## 2. Experimental

### 2.1. Synthesis of the hybrid materials, 1,8-naphthalene imide-N-propyltriethoxysilane (NISiH), 4-nitro-1,8-naphthalene imide-N-propyltriethoxysilane (NISiNO$_2$) and 4-bromo-1,8-naphthalene imide-N-propyltriethoxysilane (NISiBr)

The compounds 1,8-naphthalic anhydride, 4-bromo-1,8-naphthalic anhydride and 4-nitro-1,8-naphthalic anhydride were obtained from Aldrich and used without further purification. One millimole of the respective 1,8-naphthalic anhydrides was kept under reflux with 3-aminopropyltriethoxysilane (APTES, 1.0 mmol), in dry ethanol (20 mL) during 4 h to carry out the imide synthesis (Scheme 1), and the reaction time course was monitored by TLC and GC–MS (Shimadzu 14B/QP5050A). In all cases, after completing the reaction the solvent was evaporated under reduced pressure and the materials was obtained as non-crystalline gels. Following that, an amount (3 mL) of deuterated chloroform was added to the solid materials and aliquots were removed for $^1$H NMR and MS analysis. The analytical characterization of the products is as follows.

1,8-Naphthalene imide-N-propyltriethoxysilane (NISiH): $^1$H NMR (CDCl$_3$, ppm): 0.7 (t, 2H, Si–CH$_2$), 1.3 (t, 9H, CH$_3$), 1.8 (quintet, 2H, CH$_2$), 3.8 (q, 6H, O–CH$_2$), 4.2 (t, 2H, N$_{(imide)}$–CH$_2$), 7.7 (t, 2H, Ar), 8.2 (d, 2H, Ar), 8.6 (d, 2H, Ar). MS m/z: 401 M$^+$, 355, 341, 314, 300, 286, 272, 258, 198, 180, 152.

4-Nitro-1,8-naphthalene imide-N-propyltriethoxysilane (NISiNO$_2$): $^1$H NMR (CDCl$_3$, ppm): 0.8 (t, 2H, Si–CH$_2$), 1.2 (t, 9H, CH$_3$), 1.8 (quintet, 2H, CH$_2$), 3.8 (q, 6H, O–CH$_2$), 4.2 (t, 2H, N$_{(imide)}$–CH$_2$), 7.9–8.0 (m, 2H, Ar), 8.3–8.1 (m, 3H, Ar). MS m/z: 446 M$^+$, 400, 385, 359, 343, 327, 212, 163, 119, 79.

4-Bromo-1,8-naphthalene imide-N-propyltriethoxysilane (NISiBr): $^1$H NMR (CDCl$_3$, ppm): 0.8 (t, 2H, Si–CH$_2$), 1.2 (t, 9H, CH$_3$), 1.7 (quintet, 2H, CH$_2$), 3.8 (q, 6H, O–CH$_2$), 4.2 (t, 2H, N$_{(imide)}$–CH$_2$), 7.7–7.9 (m, 2H, Ar), 8.4–8.6 (m, 3H, Ar). MS m/z: 480 M$^+$, 434, 420, 406, 392, 376, 360, 290, 259, 232, 207.

The obtained silanes were then transferred to an Erlenmeyer containing 60 mL of ethanol and 12 mL of NH$_4$OH and the mixture was stirred at room temperature for 12 h. The hybrid materials
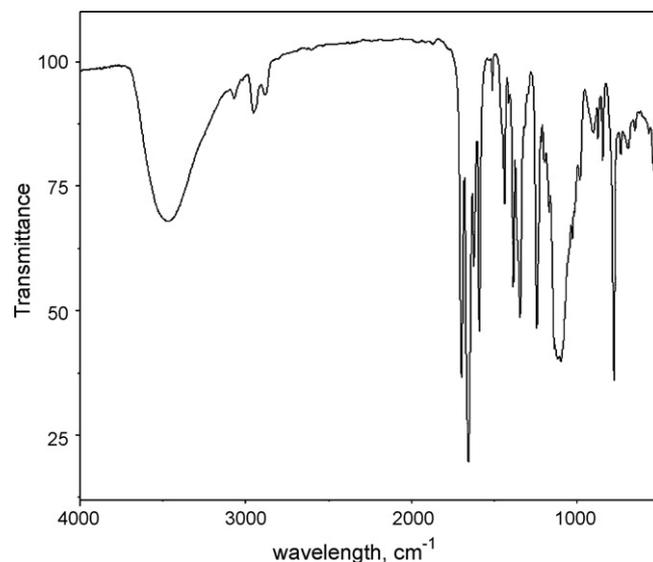


**Fig. 1.** Infrared spectrum of the NISiH hybrid material. The measurement was conducted with powdered materials by using KBr.

obtained were filtered and washed with water and ethanol and submitted to drying at 100 °C. The structures of the obtained imides are presented in Scheme 1 and the synthetic route to obtain the hybrid materials NISiH, NiSNO$_2$ and NISiBr is presented in Scheme 2.

The hybrid materials obtained were then characterized by infrared spectroscopy and by elemental analysis. Typical infrared spectrum of one of the synthesized xerogels (NISiH) is presented in Fig. 1. The bands at 1100 and 3467 cm$^{-1}$ are due to the silicate matrix, the first one corresponds to the Si–O stretching and the second one to the O–H stretching. The stretching bands at 950 and 870 cm$^{-1}$ (O–Si–O) are overlapped by the large band at 1100 cm$^{-1}$ [17]. The peaks at 1700 and 1656 cm$^{-1}$ are attributed to the 1,8-carbonyl group, where the first is the symmetric stretching and the second is the asymmetric one [12]. These results indicate that there are no remaining naphthalene anhydride reactants and that the xerogel was successfully formed by polymerization of the imide intermediary formed by the reaction between the respective anhydride and APTES. The results obtained by elemental analyses (Table 1) are in accordance with the formation of polymeric materials from their monomers (Scheme 2).

### 2.2. Absorbance and photoluminescence spectra of NISiH, NISiNO$_2$ and NISiBr hybrid materials

Photoluminescence spectra of the particles suspended in water were recorded by using diffuse reflectance with a USB 200 photometer (Ocean Optics). MgO was used as the reflectance standard. A Puma lamp of low pressure with wavelength set at 365 nm was used to perform the spectra acquisition. When the particles were irradiated their emission spectra were recorded by using the USB 2000 system. In all the assays the same distance between the light

**Table 1**
Percentage obtained for C, N and H by elemental analysis for the NISiH, NISiNO$_2$ and NISiBr materials. The results for NISiH were obtained before the photooxidation of tryptophan and after the irradiation as indicated.

| Particles | % C | % N | % H |
|---|---|---|---|
| NISiH | 57.11; 56.78[a] (56.94) | 4.60; 4.52[a] (4.43) | 4.40; 4.31[a] (4.46) |
| NISiNO$_2$ | 47.27 (46.84) | 7.63 (7.44) | 4.41 (4.28) |
| NISiBr | 46.87 (46.84) | 3.43 (3.93) | 3.43 (3.41) |

[a] Data obtained after tryptophan photooxidation (140 min of irradiation).
The data between parentheses are calculated data.
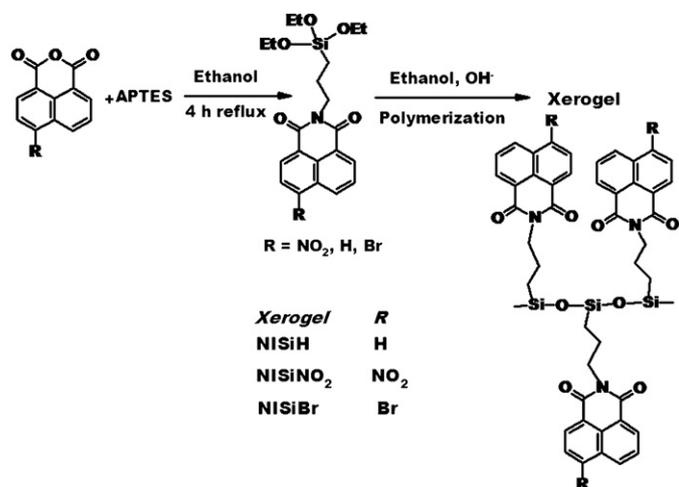
**Scheme 2.** Syntheses pathway to obtain the NISiH, NISiNO$_2$ and NISiBr hybrid materials.



**Fig. 2.** Photoluminescence spectra of the NISiH (-.-), NISiBr (. . .) and NISiNO$_2$ (_). Organic–inorganic hybrid particles suspended in water. The spectra were recorded by using a diffuse reflectance methodology and with excitation wavelength at 365 nm (USB 200 photometer, Ocean Optics).

source and the sample was used for the data acquisition.

### 2.3. Study of photooxidation of tryptophan by NISiH, NISiNO$_2$ and NISiBr hybrid materials

For the photooxidation experiment an aqueous solution of Trp (at $3.0 \times 10^{-5}$, $5.6 \times 10^{-5}$, $1.06 \times 10^{-4}$ and $1.48 \times 10^{-4}$ mol L$^{-1}$) was added to a 10 mm path length cuvette together with 0.0012–0.0025 g of the NISiH, NISiNO$_2$ and NISiBr polymeric materials. Mixtures were irradiated by using a 300 W halogen lamp. Experiments were conducted at 25 °C during the irradiation time. UV–vis absorbances were recorded after 15, 45, 115 and 145 min of irradiation. The tryptophan photooxidation products were characterized by using LDI-ToF (i.e. by laser desorption ionization without matrix addition) mass spectrometry by using a Bruker Ultraflex II spectrometer. In all cases the measurements were done in acidic media.

### 2.4. Photophysical studies of transient species by laser flash photolysis (LFP)

The flash photolysis experiments were conducted by using Nd-YAG laser (Spectron Laser Systems; England) coupled to a mLFP-111 Luzchem miniaturized equipment. The signal from the monochromator/photomultiplier detection system was captured by a Tektronix TDS 2012 digitizer (Beaverton, OR). The laser system and the digitizer were connected to a personal computer via General Purpose Instrumentation Bus (GPIB) and serial interfaces that control all the experimental parameters and provide suitable processing and data storage capabilities. The software package was developed in the LabVEW environment from National Instruments (Austin, TX) and compiled as a stand-alone application. The measurements were conducted with laser bandwidth of 20 MHz with excitation at 355 nm. Relaxation decay measurements were monitored at 400 or 490 nm. The transient spectra were obtained with 20 mV/division and 50 μs/division. The measurements were performed in neutral media with the NISiH, NISiNO$_2$ and NISiBr (0.0012–0.0025 g) suspended in tryptophan solution ($3.5 \times 10^{-4}$ mol L$^{-1}$) or in water in the presence of N$_2$ or O$_2$.

## 3. Results

With the aim of obtaining a material capable to cause photodamage to biomolecules by a straightforward synthetic strategy and with high efficiency the hydrid organic–inorganic materials NISiH,
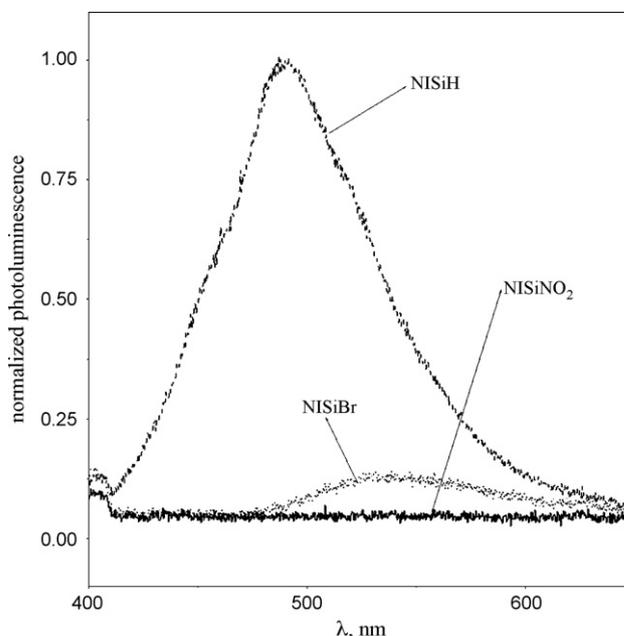
NISiNO$_2$ and NISiBr were synthesized as presented in Scheme 2. In Fig. 2, the photoluminescence spectra of the NISiH, NISiNO$_2$ and NISiBr hybrid materials (when suspended in water) are presented. Bands of very low intensity were observed at 380 nm for the NISiH, NISiNO$_2$ and NISiBr hybrid materials. An emission peak above 500 nm is observed in high intensity for the spectrum of the NISiH material. On the other hand, it is almost not observed for the NISiBr particle and could not be observed for the NISiNO$_2$ particle. The luminescence emission of naphthalene imides in their monomeric form appears as a band around 380 nm. The emission above 500 nm have been observed for naphthalene imides in very concentrated solution (above 1 mol L$^{-1}$), such as when they are constituents of dendrimers or in the solid state [13,18] and those peaks are attributed to the formation of a charge transfer complex [13,18].

### 3.1. Photooxidation of tryptophan by the NISiH, NISiNO$_2$ and NISiBr particles

To investigate the effect of the particles in the photodegradation of tryptophan, that is one of the major targets of the biomolecule oxidation, NISiH, NISiNO$_2$ and NISiBr particles (0.0012–0.0025 g) were suspended in a solution of tryptophan and the suspension was irradiated with a halogen lamp (Scheme 3). The absorbance spectra were recorded after 15, 45, 115 and 145 min of irradiation. A clear indication of tryptophan photodegradation is observed in the experiment with the NISiH hybrid material (Fig. 3). A decrease in the tryptophan absorbance maxima at 270 nm and a concomitant increase in the absorbance in the far UV region are observed (Fig. 3).

In Fig. 4 one can see the time course of the absorbance (at 240 nm) obtained for a tryptophan solution ($1.48 \times 10^{-4}$ mol L$^{-1}$) in the presence of the NISiH (♦), NISiNO$_2$ (■), NISiBr (▲) hybrid materials in the presence of oxygen and with NISiH particles without O$_2$ (●). Since tryptophan photooxidation pathway is already known, the products were searched for and identified by mass spectrometry (Fig. 5) for the reaction in the presence of NISiH hybrid materials.
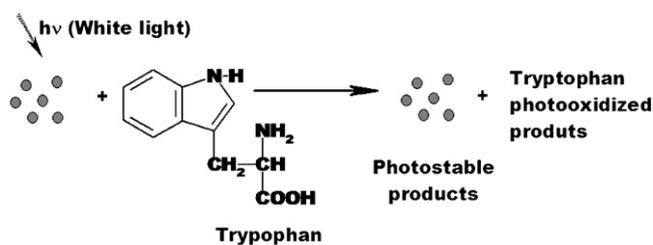
**Scheme 3.** Pictorial representation of photodegradation of tryptophan by NISiH hybrid material.
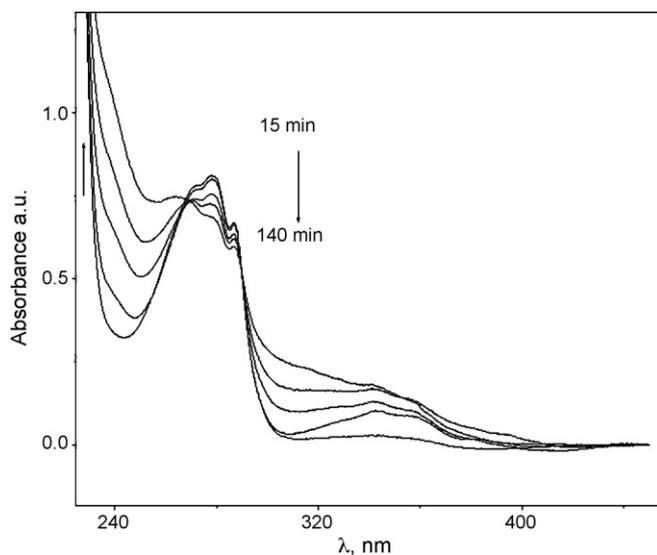


**Fig. 3.** Normalized absorbance spectra obtained for tryptophan photodegradation. The normalization factor was 0.7. Tryptophan ($1.48 \times 10^{-4}$ mol L$^{-1}$) as dissolved in water with a suspension (21.2 mg) of NISiH particles. The NiSiH hybrid materials were irradiated for 15, 45, 115 and 145 min with a 300 W halogen lamp and then the spectra were recorded.
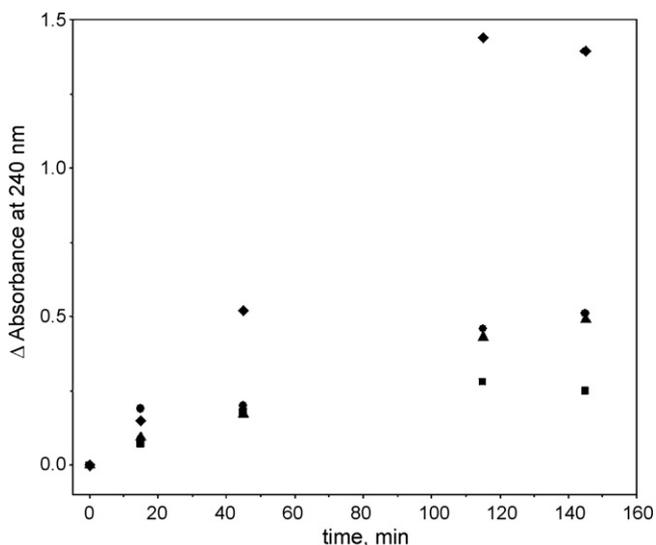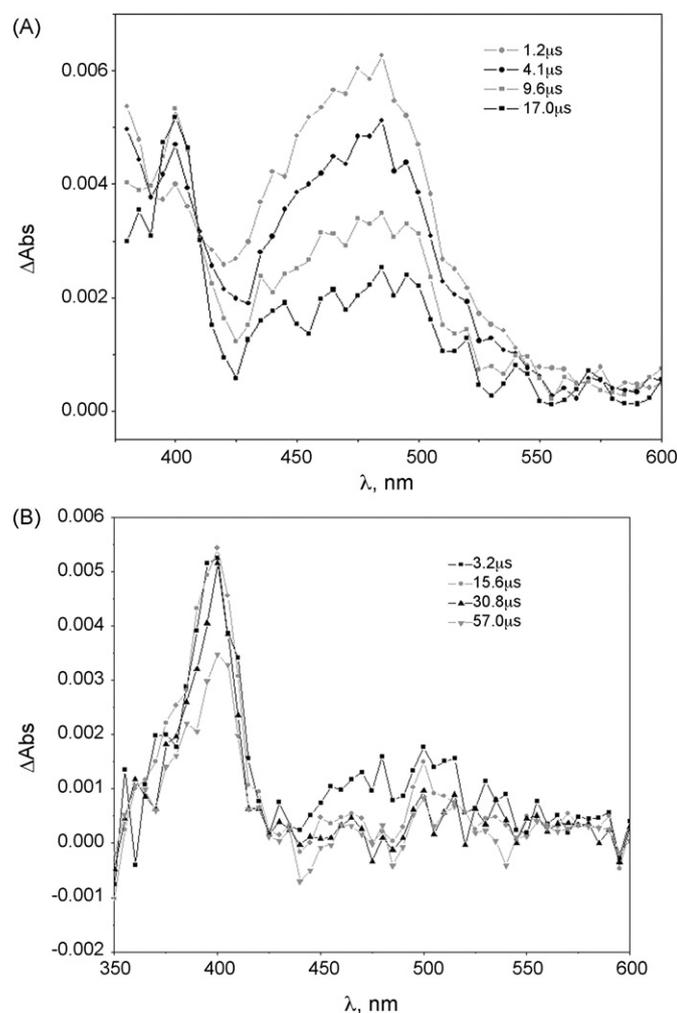


**Fig. 4.** Time course for absorbance variation (non-normalized) at 240 nm after irradiation of tryptophan with a 300 W halogen lamp ($1.06 \times 10^{-4}$ mol L$^{-1}$) in the presence of the following hybrid materials: NISiH (♦), NISiNO$_2$ (■), NISiBr (▲) in the presence of O$_2$ and NISiH in the absence of O$_2$ (●). The particles were suspended in water. To obtain the values of △absorbance for NISiH the original absorbance values from Fig. 3 were used and 0.1135 (referent the absorbance at 240 nm without irradiation, i.e. zero irradiation time) was subtracted from the original values.
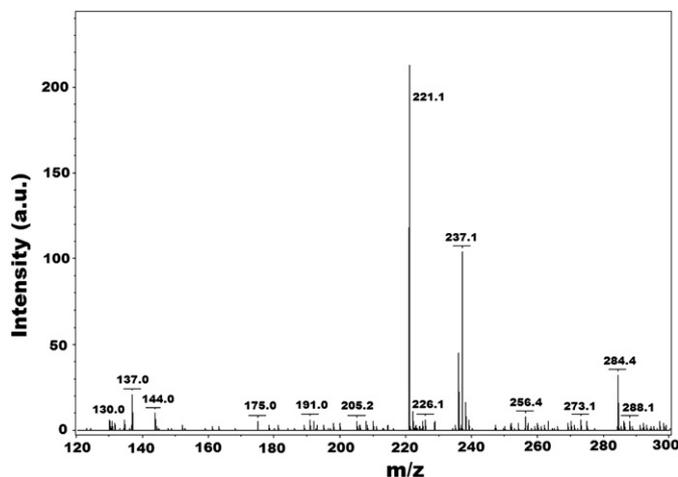


**Fig. 5.** Laser desorption mass spectrometry of photoirradiation products (300 W halogen lamp, 145 min) of tryptophan obtained in the presence of NISiH hybrid particles. Freeze-dried sample was solubilized in aqueous solution acidified with 0.5% (by volume) formic acid. The spectra was obtained by laser irradiation on an UltraFlex II MALDI-TOF/TOF Mass Spectrometer (Bruker Daltonics, Billerica, MA). A control experiment was performed for non-irradiated (white light) tryptophan and the photooxidation products were not observed.

It is possible to infer that the NISiNO$_2$ and NISiBr particles do not cause considerable photooxidation of tryptophan since the absorbance spectra and the time course for increments of absorbance (at 240 nm) do not change when compared to the same data obtained for tryptophan irradiated in the absence of any of the non-crystalline solids (data not shown). Thus, direct tryptophan irradiation caused negligible photodamage when compared to the most effective photoactive naphthalene imide material. These results are in accordance with the photoluminescence data (Fig. 2), where the luminescence intensity that is referent to the formation of the charge transfer complex [13] is much more pronounced for NISiH than for the other materials (Fig. 4). As expected, the oxygen presence is also important for the process of tryptophan photodegradation since in the absence of oxygen the variation of the absorbance at 270 nm is smaller than in the presence of oxygen.

To the mass spectra data acquisition, laser desorption ionization was conducted without conventional matrix addition since tryptophan and its derivatives could probably play the role as matrixes. Indeed, when α-cyano-4-hydroxycinnamic acid was added the signals due to the tryptophan photoproducts are quenched by the $m/z$ signals of the own matrix products up to 400 Da (omitted data). However, when the ionization was conducted in the absence of α-cyano-4-hydroxycinnamic acid it was possible to observe the presence of several molecular ions that correspond to tryptophan photooxidation products. Although the intensity of the signals is quite low, probably because tryptophan is not efficient as matrix as α-cyano-4-hydroxycinnamic acid, the presence of ions is clear. The products of $m/z$ = 221.1 is attributed to the 3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrroloindole, a known tryptophan photoproduct [19]. The ion with (M+H)$^+$ = 237.1 may correspond to a mixture of 3a-hydroperoxy-1,2,3,3a,8,8a-hexahydropyrroloindole and of N-N-formylkynurenine with probably a major contribution of the former compound that is a secondary amine and thus ionize better than N-formylkynurenine. The species with $m/z$ = 130.1 and $m/z$ = 175.1 are radical cations formed in the process of ionization by laser [20] whereas the ion with $m/z$ = 205.2 is the precursor tryptophan. The products of photooxidation found here are in accordance with compounds already described by Davies et al. [21,22] and by Nakagawa et al. [20]. Nevertheless, this is the first report of the use of laser desorption ionization mass spectrometry as an efficient tool

**Fig. 7.** Transient decay for the NISiH hybrid material obtained by laser flash photolysis with laser excitation at 355 nm and emission measurement at 400 nm. Spectra of transients of NISiH in the absence of tryptophan (...), in the presence of tryptophan under N$_2$ atmosphere (–■–) and in presence of tryptophan under O$_2$ atmosphere (–▲–).
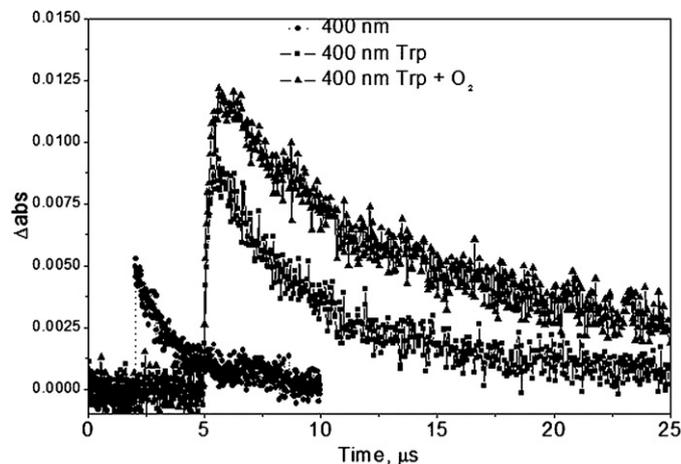
**Fig. 6.** Absorbance spectra of transient species recorded by laser flash photolysis with laser excitation at 355 nm. (A) Spectra of NISiH particles suspended in water, obtained at different times after a laser pulse. (B) Spectra of NISiH particle suspended in an aqueous tryptophan solution ($3.5 \times 10$ mol L$^{-1}$), obtained at different times after a laser pulse.

for the rapid identification of those products of photooxidation of tryptophan.

It is important to state that the NISiH hybrid material was stable to the process of tryptophan photooxidation. The elemental analysis result for the NISiH particle obtained after 140 min of irradiation, in presence of tryptophan, is almost equal to the data obtained for the particle without any irradiation (Table 1). Despite this being a promising result concerning the material applications, additional studies are necessary for future discussion about the stability of the naphthalene imide moiety in the silica particles.

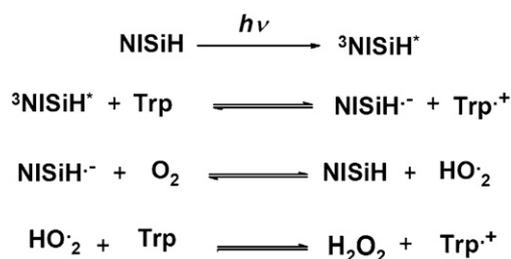## 4. Laser flash photolysis studies

Fig. 6A presents the spectra of transients of NISiH in a nitrogen atmosphere obtained by laser flash photolysis. It is possible to observe that the peak at 490 nm decreases and a small increase in peak intensity at 400 nm occurs with the measurement time course after the laser pulse. These results show that transient species with a shorter lifetime is being consumed, giving rise to new product with longer lifetime. These transients were already characterized as the naphthalene imide triplet state, which absorbs at 490 nm, and radical anion of naphthalene imide, with a peak at 400 nm [23]. This radical anion is assigned as a product of reaction of its triplet

state with its own ground state [14]. On the other hand, the transients for NISiNO$_2$ and NISiBr were not observed in the experiment of flash photolysis in the wavelength range and in the time course of this study. These results are in agreement with those obtained by steady-state fluorescence of the materials (Fig. 2). Naphthalene imides with a nitro group do not show photophysical or photochemical reactions and their excited states decay by a non-radiative pathway [24]. In the case of bromine substituent an effect of heavy atom may occur and an increase in the inter-system crossing is favored and consequently an increase of the yield of triplet state is expected. However, laser flash photolysis data showed that the naphthalene imide with bromine in the fourth position led to a very low intensity transient with a long wavelength absorption [8], around 650 nm. For the hybrid materials in suspension, such as NISiBr, the absorbance for the transients is expected to be lower than that for the soluble organic compounds and thus the lack of excited state reactions for the NISiBr particle is predictable. Fig. 6B presents the absorbance of transient species when NiSiH particles are suspended in tryptophan solution. Tryptophan causes a quench in the absorbance of transient species at 490 nm, but the presence of the radical anion of imide of NISiH, absorbent at 400 nm, is evident when tryptophan is present (Fig. 6B). The tryptophan transients above 490 nm are not observed in the time interval of the experiment [25,26]. These results clearly indicate the presence of a deactivation pathway for the tryptophan transients. In fact the absorbance spectra and mass spectrometry show the formation of stable photoproducts, i.e., compounds in their fundamental state (Figs. 2 and 5). These results indicate that NISiH is an efficient photosensitizer of tryptophan oxidation and degradation and thus it is a promising material for applications involving photooxidation of proteins.

Decay of NISiH transients without tryptophan addition and also in the presence of tryptophan under nitrogen (–■–) or under oxygen (–▲–) with measurements conducted at 400 nm are presented in Fig. 7. The transient in the absence of tryptophan has a lifetime around 0.8 μs, which is lower than the observed value in the presence of tryptophan under reducing (16 μs) and oxidant atmosphere (25 μs). Thus, it is clear that NISiH transient species concentration is higher in the presence of tryptophan than in its absence, especially in oxidant media. These results are in agreement with photooxidation data previously shown (Fig. 4) where the presence of oxygen has favored the formation of the transient species.

$$NISiH \xrightarrow{h\nu} {}^3NISiH^*$$

$${}^3NISiH^* + Trp \rightleftharpoons NISiH^{\cdot-} + Trp^{\cdot+}$$

$$NISiH^{\cdot-} + O_2 \rightleftharpoons NISiH + HO_2^{\cdot}$$

$$HO_2^{\cdot} + Trp \rightleftharpoons H_2O_2 + Trp^{\cdot+}$$

**Scheme 4.** Proposed mechanism for the photodegradation of tryptophan by NISiH particles.

## 5. Discussion

The pathways for the singlet state deactivation of naphthalene imides can be governed by substituent effects but they are not easily predictable and it is thus necessary to perform photophysical studies to evaluate the contribution of intersystem crossing, radiative and non-radiative decay for new compounds and materials prepared. NISiNO$_2$ and NISiBr are naphthalene imide derivatives that do not cause photooxidation of tryptophan or they promote the oxidation with very low yield. Those imides with Br and NO$_2$ substituent present poor photophysical and photochemical behavior as discussed before [22,23]. On the other hand the NISiH derivative is efficient to form a charge transfer complex in the singlet as well as in the triplet state (Figs. 2 and 6B). These results show that the ability to photo-electron transfer is necessary to cause tryptophan photodamage. As has been described earlier a pathway to cause photodamage to tryptophan by using naphthalene imide as photosensitizing is through electron transfer from tryptophan ground state to the triplet estate of naphthalene imides forming the radical anion of imides and tryptophan radical cation [1].

Tryptophan residue has been shown to be the main target in the process of protein photooxidation and thus it is chemically relevant to understand the details of photodegradation of free tryptophan [1,25,26]. Thus, through the analyses of results presented in this manuscript one can infer about the possible photooxidative mechanism of proteins by using naphthalene imides. The photooxidation of proteins by NI can occur by two mechanistic types. (I) Via naphthalene imide triplet or singlet state by forming radical species as transients, or (II) by singlet oxygen formation through the reaction of naphthalene imide triplet state with ground state oxygen [27]. Considering that no quenching of transient species is observed in the presence of oxygen and that the molecular transient of longer lifetime (i.e. the radical anion) is photosensitized by tryptophan, type I mechanism appears to be the most plausible one for the photooxidation of tryptophan by NISiH. On the other hand the presence of oxygen is important for the pathway of tryptophan photooxidation leading to the formation of stable products. Davies et al. [22] have proposed a mechanism for the oxidation of tryptophan where the transient radical of tryptophan reacted with triplet oxygen giving rise to oxygen radical species that lead to the formation of more stable products. A similar mechanism could be occurring here where the triplet state of NISiH reacts with tryptophan ground state to produce the radical anion of NISiH and radical cation of tryptophan. The radical anion of NISiH can then react with triplet oxygen giving arise to the protonated superoxide radical. A second molecule of the neutral ground state tryptophan can then react with the superoxide leading to hydrogen peroxide and the tryptophan radical cation (Scheme 4).

The photooxidation of tryptophan by NISiH has probably the same final pathway as that observed by Davies et al. [21,22] for the oxidation since hexahydropyrroloindoles and N-formylkynurenine clearly appear in the mass spectrometry analysis of photooxidation products [20,27].

## 6. Conclusion

Although NISiBr and NISiNO$_2$ materials do not cause an efficient photodamage to tryptophan the NISiH hybrid material is very efficient to cause the photooxidation and photodamage of the amino acid. The NISiBr and NISiNO$_2$ materials present a photodeactivation pathway that prevents the formation of transient reactive species of tryptophan. Photophysical data show that the oxidation of tryptophan occurs mainly by type I mechanism but the reaction progress for the formation of tryptophan stable products depending upon oxygen. In this case the radical anion of NISiH, created by the reaction of NISiH triplet state with Trp, reacts with triplet oxygen creating the reactive species of oxygen. LDI-mass spectrometry allowed the identification of the final (ground state) photooxidation product formed.

## References

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