

## PARAMAGNETIC INTERMEDIATES IN THE PHOTOLYSIS OF PURINE FREE BASE IN FROZEN AQUEOUS GLASSES

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### Summary

UV irradiation of purine free base used as a model for the purine bases in deoxyribonucleic acid, in aqueous glassy solutions, produces stable photo-products, as well as reactive paramagnetic intermediates such as triplet states, trapped electrons, purine radical cations and anions and solvent radicals. Electron spin resonance techniques and parameters were used to characterize the intermediates produced during the UV irradiation of purine in aqueous glasses at 77 K at different pH values (8 M NaClO<sub>4</sub>, 12 M LiCl, 5 M H<sub>2</sub>SO<sub>4</sub>, 6 M H<sub>3</sub>PO<sub>4</sub> and 8 M NaOH). Biphotonic ionization of the base through a triplet intermediate is one of the principal photodestruction paths in basic and neutral glasses. The quantum yields for the production of trapped electrons in 8 M NaOH and 8 M NaClO<sub>4</sub> glasses are 0.02 and 0.0023 respectively. In 12 M LiCl, 5 M H<sub>2</sub>SO<sub>4</sub> and 6 M H<sub>3</sub>PO<sub>4</sub> glasses, energy transfer from the purine molecules to the surrounding solvent molecules is observed. This transfer of energy produces Cl<sub>2</sub><sup>-</sup>, SO<sub>4</sub><sup>-</sup>, SO<sub>3</sub><sup>-</sup> and HPO<sub>4</sub><sup>-</sup> radical ions.

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### 1. Introduction

The purine bases are part of the light-absorbing system in deoxyribonucleic acid (DNA). Studies of the photochemistry of nucleic acid constituents have concentrated mainly on the photoreactivity of pyrimidines [1], and photochemical information on the purines is scarce [2]. The importance of the role of the purine bases in the interaction of light with nucleic acids has been recognized [2 - 4]. It has been shown that purines, as well as pyrimidines, undergo light-induced reactions with alcohols, amines and ethers [2, 3]. UV irradiation of samples of oriented DNA [5], containing 30% water, at 77 K produces free radicals which have been found to consist mainly of the thymine radical anions and guanine radical cations. The quantum yield for free-radical formation for irradiation at 300 ± 10 nm has been estimated to be 10<sup>-4</sup>.

From electron spin resonance (ESR) studies [6] it has been determined that, in aqueous ethanol solutions of purine at 77 K, UV light ionizes the molecule producing trapped electrons which can be detrapped by illumination with visible light. In these frozen alcoholic solutions, photosensitization of alcohol occurs producing ethanol-derived radicals. It has been suggested that photoejection of electrons and photosensitization of ethanol are competitive processes. A biphotonic absorption process involving the lowest triplet state has been postulated for both processes. No quantum yields have been reported for the production of triplet states or solvent radicals or for the photoionization process.

Sevilla [7] has generated the purine radical anion by electrolysis in dimethylformamide at low temperatures (from  $-34$  to  $-55$  °C). The ESR spectrum of the purine anion, which consists of 16 lines and extends over a range of 34 G, was moderately resolved. McLachlan self-consistent field-molecular orbital calculations [7] predict positions N(3), H(6) and H(8) to be the positions of largest spin density: 0.162, 0.436 and 0.220 respectively.

The UV photolysis of purine in 2-methyltetrahydrofuran (MTHF) glass [8] at 77 K produces trapped solvent radicals with a quantum yield of 0.009. The rate of formation of matrix radicals is approximately proportional to the square of the incident light intensity. Arce and Ramírez [8] had suggested that these solvent radicals are produced by sensitized decomposition which may occur via resonant energy transfer to the solvent from a highly excited state of the purine molecule. No signals attributable to trapped electrons or cations were observed in this glass.

The work reported here was carried out to obtain further information on the nature of the reactive intermediates formed during the UV irradiation of purine and its ionic forms in aqueous glasses at 77 K. We were particularly interested in the determination of quantum yields of the intermediates to compare the different channels for UV energy utilization by purine and its different ionic forms.

## 2. Experimental details

### 2.1. Chemicals and sample preparation

Purine (Aldrich; purity, 99%), NaClO<sub>4</sub> (Fisher; certified reagent), NaOH (Fisher; certified reagent), LiCl (Fisher; certified reagent), *o*-H<sub>3</sub>PO<sub>4</sub> (Fisher; 85%), H<sub>2</sub>SO<sub>4</sub> (Fisher; certified reagent) and galvinoxyl and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals (Aldrich) were used as received. Aqueous glasses were formed by adding a high concentration of the inorganic salts or acids to triply distilled water and cooling to 77 K.

Purine solutions of known concentration, ranging from  $10^{-4}$  to  $10^{-3}$  M, were prepared using standard weighing and volumetric techniques. An aliquot of the solution was introduced in a square Suprasil cell with an optical path length of 3 mm. The cells were connected to a vacuum line and the solutions were degassed by several freeze-thaw cycles using a (dry ice)-acetone mix-

ture to avoid cracking of the cell. Finally, the solution was sealed under vacuum. Because frequent cracking of the cell resulted when the  $\text{NaClO}_4$  samples were immersed in liquid nitrogen or in a (dry ice)-acetone mixture, these samples were deoxygenated by bubbling argon through the solution for 15 min.

### 2.2. Irradiation conditions

Irradiation of samples at 77 K were performed using a low pressure mercury helical lamp (Hanovia) having a Vycor tube inside the lamp as a filter. The sample cells were immersed in liquid nitrogen in an ESR liquid nitrogen Dewar (S-816 Scalco Company) and the Dewar was positioned inside the Vycor tube.

After UV irradiation, the samples were photobleached for 5 min using a 300 W tungsten halogen lamp in front of which was a 3-67 Corning filter, which transmits at wavelengths longer than 540 nm.

The dependence of the photoionization rate on the light intensity was determined by varying the light intensity of the helical lamp by almost an order of magnitude by introducing into the lamp neutral density filters made of wire screens. Frozen 8 M  $\text{NaClO}_4$  and 8 M NaOH solutions of equal initial purine concentration were UV irradiated for equivalent time intervals (25 s and 10 s respectively) at 77 K. The peak-to-peak heights of the ESR signal were used as a concentration index.

### 2.3. Electron spin resonance measurements

ESR measurements were performed with a Varian E-3 spectrometer using 100 kHz modulation. The splitting factors or  $g$  values were measured using the comparison method [9] and the standard was a minute sample of finely powdered DPPH [10] ( $g = 2.0036$ ) or pitch in KCl ( $g = 2.0028$ ).

Power saturation studies were carried out with a Varian E-9 spectrometer which can operate at low microwave powers (0.01 mW).

The concentration of purine radical cations and anions, trapped electrons and triplet states was determined from ESR measurements. The first derivative ESR signal was integrated using a computer program of numerical double integration [11]. The procedure followed to determine the concentration of paramagnetic species from ESR data has been described previously [8].

### 2.4. Actinometry and quantum yield determination

The light intensity incident on the samples was measured with a ferrioxalate actinometer [12, 13] both before and after photolysis of each sample. The average of the two light intensity determinations was used in the quantum yield calculation. Actinometry was carried out using the same experimental conditions as those for the samples photolyzed inside the helical lamp.

The quantum yields for radical R production were calculated using the relation

$$\Phi = \frac{6.023 \times 10^{20} V[R]}{I_0(1 - 10^{-OD})t}$$

where [R] is the molar concentration of the sample radical,  $V$  is the volume of the photolyzed solution,  $I_0$  is the incident light intensity and  $t$  is the irradiation time. The optical density OD of the purine solution was measured at 254 nm (mercury resonance line) with a Cary 14 instrument at room temperature.

### 3. Results and discussion

Purine free base PuH could exist in different ionic forms ( $\text{PuH}_2^+$ ,  $\text{Pu}^-$ ) depending on the pH of the medium. To compare the photoreactivity and intermediates resulting from the UV irradiation of the different ionic forms, ESR studies were carried out in neutral (8 M  $\text{NaClO}_4$ , 12 M LiCl), acid (6 M  $\text{H}_3\text{PO}_4$ , 5 M  $\text{H}_2\text{SO}_4$ ) and basic (8 M NaOH) aqueous glassy solutions at 77 K.

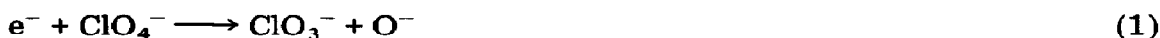
#### 3.1. Intermediates in neutral glasses

##### 3.1.1. 8 M $\text{NaClO}_4$

In 8 M  $\text{NaClO}_4$  glasses containing purine, a light blue–purple coloration characteristic of trapped electrons develops after a few seconds of UV irradiation. The ESR spectrum taken after 25 s of photolysis appears in Fig. 1, curve A. The spectrum consists of a broad low field component with  $g = 2.054$ , an unresolved asymmetric singlet at a higher field with  $g = 2.004$  and a very weak intensity doublet 500 G apart not shown in this figure. This doublet results from trapped hydrogen atoms.

The ESR spectrum taken after the sample had been photobleached with visible–IR light is shown in Fig. 1, curve B. The spectrum is similar to that presented in Fig. 1, curve A, except that the singlet peak with  $g = 2.004$  has a reduced intensity while the intensity of the singlet with  $g = 2.054$  has increased. Subtraction of spectrum B from spectrum A (point by point) gives a singlet with a linewidth of 16 G, extending over a range of 60 G with a  $g$  value of 2.005. This singlet which disappears on photobleaching could contain contributions from the ESR signals of trapped electrons and/or purine radical cations.

Perchlorate ions can act as scavengers of electrons [14] producing  $\text{O}^-$  radicals:



By UV photoionization of potassium ferrocyanide ( $\text{K}_4[\text{Fe}(\text{CN})_6]$ ) in 8 M  $\text{NaClO}_4$  glasses, Sevilla *et al.* [15] have generated the ESR spectrum of the  $\text{O}^-$  radical free of other radicals. The  $\text{O}^-$  spectrum shows a broad doublet, one component with  $g_{\parallel} = 2.003$  and the other with  $g_{\perp} = 2.055$ . The broad low field component with  $g = 2.054$  observed in the ESR spectra of UV-photolyzed purine solutions in 8 M  $\text{NaClO}_4$  closely resembles the low field line of the  $\text{O}^-$  spectrum reported by Sevilla and coworkers [14, 15].

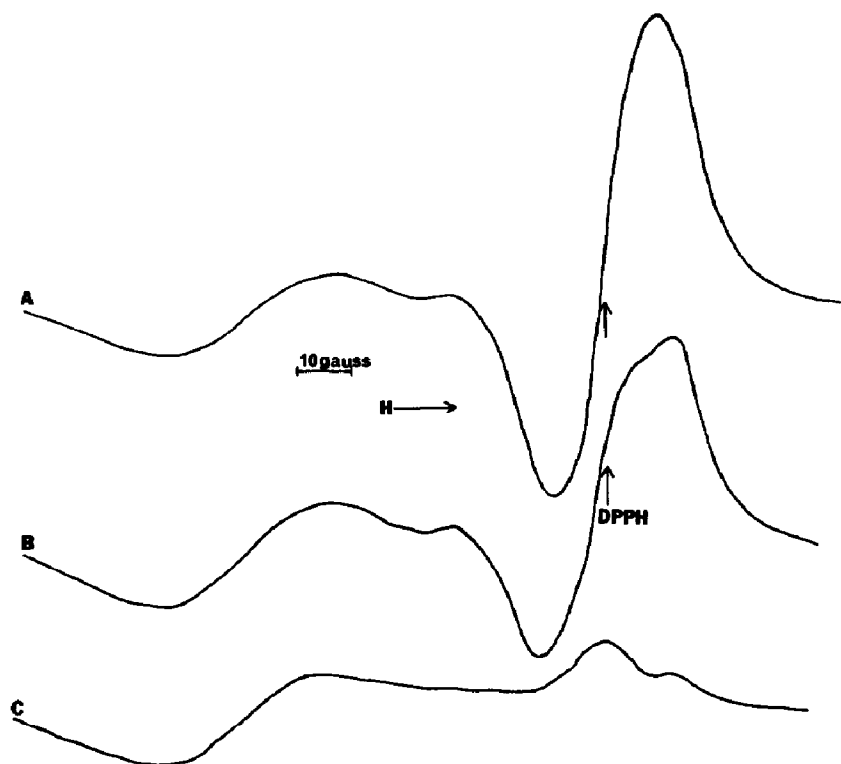


Fig. 1. ESR spectra of 8 M NaClO<sub>4</sub> glass containing purine illuminated for 25 s with 254 nm light (spectrum A), purine in 8 M NaClO<sub>4</sub> after photobleaching for 5 min (spectrum B) and O<sup>-</sup> in 8 M NaClO<sub>4</sub> produced by UV photolysis (15 s) of K<sub>4</sub>[Fe(CN)<sub>6</sub>] at 77 K (spectrum C).

The presence of trapped electrons as evidenced from the ESR and visible absorption spectra of photolyzed purine solutions [16] and from the presence of O<sup>-</sup> radicals produced through reaction (1) is evidence for the photoionization of purine as one of its primary photochemical processes. This implies the formation of a purine radical cation. To obtain the ESR signal corresponding to the purine radical cation alone (Fig. 2), the ESR spectrum of the O<sup>-</sup> radical was produced by photolysis of a  $5 \times 10^{-3}$  M K<sub>4</sub>[Fe(CN)<sub>6</sub>] solution in 8 M NaClO<sub>4</sub> at 77 K for 15 s and by subsequent photobleaching of the sample. The resulting ESR signal which consists of a broad doublet with  $g_{\parallel} = 2.004$  and  $g_{\perp} = 2.054$  (Fig. 1, spectrum C) was subtracted from the ESR signal presented in Fig. 1, spectrum B, which resulted from photobleaching a previously UV-irradiated purine solution. The singlet resulting from this subtraction has been assigned to the purine radical cation (Fig. 2) and has a linewidth of 22 G, extends over a range of 80 G and has a  $g$  value of 2.004. An absorption band with a maximum at 320 nm which is observed for UV-irradiated purine solutions in 8 M NaClO<sub>4</sub> and which disappears on warming was attributed to the radical cation [16].

No evidence for the presence of purine radical anions formed through the reaction of photoejected or detrapped electrons with purine molecules

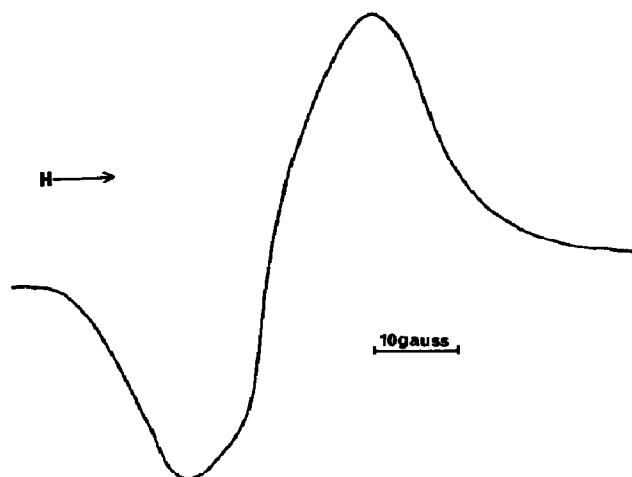


Fig. 2. ESR spectrum of the purine radical cation  $\text{PuH}^{\bullet+}$  in 8 M  $\text{NaClO}_4$  glass obtained by the subtraction of Fig. 1, spectrum C, from Fig. 1, spectrum B.

was found. Although the rate constant for the reaction of an electron with purine is 1000 times higher [17, 18] than that for the reaction of an electron with a  $\text{ClO}_4^-$  ion, the concentration of  $\text{ClO}_4^-$  ions is  $30 \times 10^3$  times higher than the purine concentration. Thus the rate of reaction of  $\text{ClO}_4^-$  with an electron is higher, resulting in an undetectable yield of purine radical anions. From these facts, the latter singlet shown in Fig. 2 has been assigned to the purine radical cation ( $\text{PuH}^{\bullet+}$ ). Thus, the ESR spectrum shown in Fig. 1, curve A, consists of the sum of the individual spectra of three species: the trapped electron, the purine radical cation and the  $\text{O}^-$  radical.

Sevilla and Mohan [19] have calculated  $g$  values for the radical cations of the purine bases, adenine and guanine, in neutral aqueous glasses (12 M LiCl). The  $g$  values corresponding to the cations of adenine (2.0043) and guanine (2.0042) are similar to those found in this work for the radical cation of purine free base (2.004) in 8 M  $\text{NaClO}_4$  glass. Thus, substitution of amine and/or keto groups in the purine ring system does not alter the  $g$  values of radical cations of the corresponding purine DNA bases. The corresponding  $g$  values [19] for the radical anions of adenine and guanine in 12 M LiCl are the same,  $g = 2.0034$ .

The rate of photoionization of purine can be expressed in terms of the incident light intensity by the following general expression:

$$\frac{d[\text{PuH}^{\bullet+}]}{dt} = kI_0^n$$

where  $[\text{PuH}^{\bullet+}]$  is the concentration of the radical cation,  $k$  is a constant and  $n$  is the number of photons absorbed to produce photoionization. The peak-to-peak heights of the high field ESR signal (Fig. 1, spectrum A) were used as a measure of the radical concentration. Since this signal has been assigned to three species, the rate of photoionization is represented by  $d[S]/dt$ , where

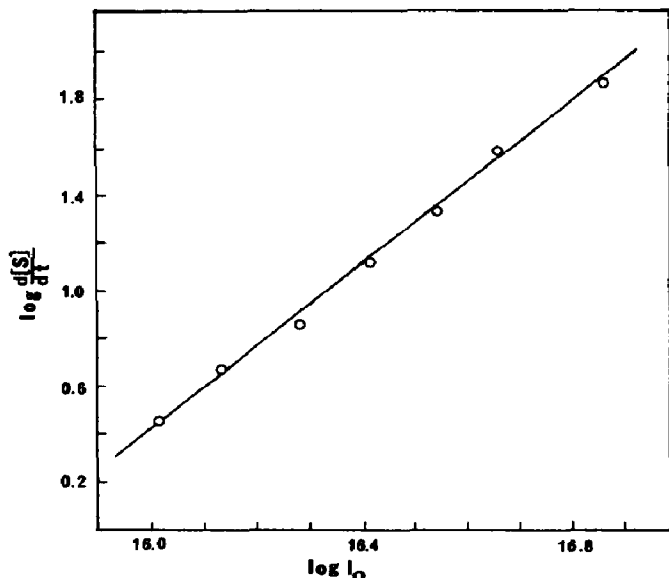


Fig. 3. Rate  $d[S]/dt$  of photoionization of purine in 8 M  $\text{NaClO}_4$  glass as a function of the incident light intensity ( $t_{\text{irr}} = 25$  s).

$[S]$  is the sum of the concentrations of the three radicals. From the slope of a plot of  $\log(d[S]/dt)$  as a function of  $\log I_0$  (Fig. 3), a value of  $n$  corresponding to  $1.73 \pm 0.04$  was obtained. This indicates that a stepwise two-photon absorption mechanism is occurring and that the process of photoionization for purine free base involves as an intermediate state the lowest triplet state of purine as was previously suggested by Helene *et al.* [6]. At low magnetic fields (1350 G) a broad singlet was observed [20]; this signal was assigned to the  $\Delta m_s = 2$  transition of the purine triplet state on the basis of its decay lifetime and r.m.s. zero-field splitting parameter. An intersystem crossing quantum yield of 0.39 was determined.

The calculated quantum yields for purine photoionization and purine radical cation are given in Table 1. The total electron concentration was obtained by summation of the ESR areas due to the  $\text{O}^-$  radical and the trapped electron and comparison with a stable radical standard of known concentration. The concentration of  $\text{O}^-$  radicals was included in the quantum yield calculation since the  $\text{O}^-$  precursor is the electron, according to reaction (1). The radical cation yield was calculated after the corresponding spectrum of an  $\text{O}^-$  radical generated independently had been subtracted from the combined ESR spectrum of the  $\text{O}^-$  and radical cation, obtained after a previously UV-irradiated sample had been photobleached. From Table 1, it can be noted that the average quantum yields for radical cation and electron production are, as expected, approximately the same. This fact is evidence that the only purine radical present in the matrix is the radical cation. The photodestruction yield of purine in this glass is 0.01 as determined from changes in the purine absorption maximum [16]. Since the yield of paramagnetic species is an order of magnitude less than the photodestruc-

TABLE 1

Quantum yields for trapped electron and purine radical production in 8 M NaClO<sub>4</sub> and 8 M NaOH glasses<sup>a</sup>

<i>Irradiation time (s)</i>	$[e^-] \times 10^5$ (M)	[purine radical] $\times 10^5$ (M)	$I_{\text{abs}} \times 10^{-17}$ (photons s <sup>-1</sup> ml <sup>-1</sup> )	$\phi(e^-)$	$\phi(\text{purine radical})$
8 M NaClO <sub>4</sub> glass					
25	1.7 <sup>b</sup>	1.6	1.82	0.0023	0.0021
8 M NaOH glass					
10	3.8	2.1 <sup>c</sup>	1.96	0.012	0.0065
30	5.5	2.5 <sup>c</sup>	1.88	0.0059	0.0027
60	5.4	4.2 <sup>c</sup>	2.22	0.0024	0.0019

<sup>a</sup>Values correspond to average values of at least four or five determinations.

<sup>b</sup> $[e^-] = [e_{\text{tr}}^-] + [O^-]$ .

<sup>c</sup>Purine radical cation and anion concentration.

tion yield this implies that most of the photodestruction processes proceed via non-paramagnetic intermediates. In MTHF glass [8] containing purine, a quantum yield of  $9.0 \times 10^{-3}$  for production of the solvent radical was reported. This was interpreted by assuming that the photoionization of purine in MTHF at 77 K is an inefficient relaxation process, at least a factor of 10 less efficient than that for radical production. The absence of an ESR signal due to the trapped electron was attributed to a prompt recombination of the electron-cation pair.

### 3.1.2. 12 M LiCl

UV irradiation of frozen 12 M LiCl solutions of purine produces a light blue coloration of the sample, characteristic of trapped electrons. In Fig. 4, curve A, the ESR spectrum recorded after 30 s of illumination with 254 nm light is shown. It consists of a complex signal with an unresolved singlet at the centre of the spectrum and a doublet about 500 G apart assigned to trapped hydrogen atoms. These are produced by the reaction of photoejected electrons with protons from the medium [21]. The singlet of higher intensity in the central part of the spectrum is considered to be the overlap of the spectra of several radicals.

Photobleaching of the sample with visible-IR light for about 5 min produces a reduction in the total intensity of the peak (a) at the center of Fig. 4, spectrum B, while the other two peaks (b, c) in the central part of the spectrum remain constant in intensity as shown in Fig. 4, curve B. The signals attributed to the hydrogen atoms also increased in intensity. The ESR spectrum recorded after UV photolysis and photobleaching of frozen 12 M LiCl solutions of purine closely resembles that obtained by Moan and Kaalhus [22] in X-irradiated 9 M HCl solutions. It is also similar to that obtained by Bennett *et al.* [23] in the photolysis of Cl<sub>2</sub> adsorbed on silica gel at 77 K. In both cases the seven-line ESR spectrum was attributed to Cl<sub>2</sub><sup>-</sup> radical



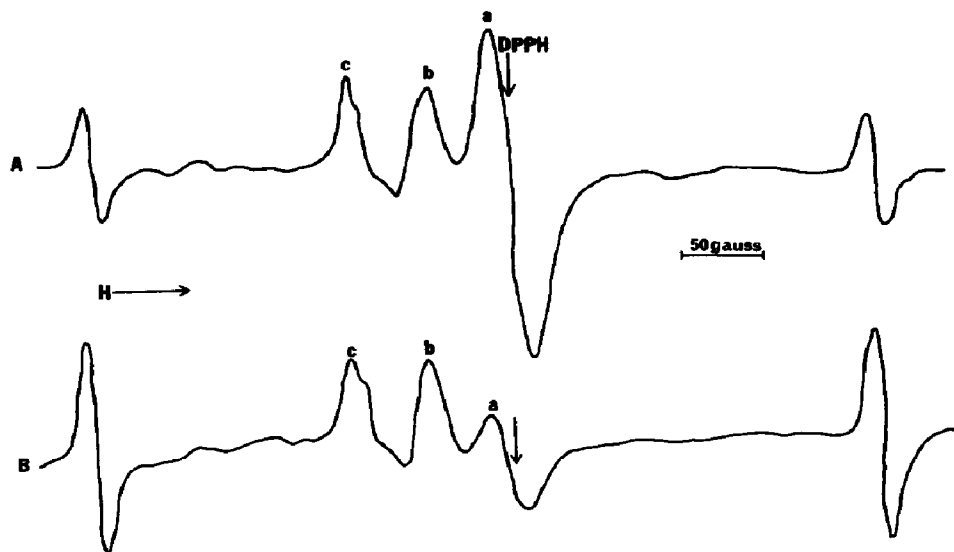


Fig. 4. ESR spectra of 12 M LiCl solution of purine at 77 K: spectrum A, illuminated for 30 s with UV light; spectrum B, photobleached with visible-IR light for 5 min.

ions [22, 23]. On Fig. 4 only the central part of the  $\text{Cl}_2^-$  spectrum is observed (Fig. 4, curve B), since the weaker lines on the wings of the seven-line spectrum (Fig. 4) could not be observed. The optical absorption spectrum of UV-irradiated frozen solutions of purine in 12 M LiCl shows an absorption band with a maximum at 340 nm, which is characteristic of  $\text{Cl}_2^-$  radical ions [22, 24]. On the basis of the ESR and UV absorption spectra, the asymmetric multiplet recorded after UV photolysis has been attributed to an overlap of spectra due to  $\text{Cl}_2^-$  radical ions, trapped electrons and purine radicals.

Photolysis of 12 M LiCl solutions alone with 254 nm light for long irradiation times (15 min) at 77 K produces insignificant amounts of solvent radicals. Therefore, light absorption by purine molecules is necessary for the formation of  $\text{Cl}_2^-$  radical ions.

After 30 s of UV irradiation an absorption band in the visible region due to the trapped electron was observed [16]. A photoionization yield of 0.002 was calculated from the absorbance of the visible band. This yield is approximately half of the purine photodestruction yield. Although purine intersystem crossing quantum yields in both neutral glasses are almost the same [20], the purine photodestruction yield in 12 M LiCl is half of the value in 8 M  $\text{NaClO}_4$ , indicating that in 12 M LiCl glass a large fraction of the purine highly excited triplet states is deactivated by energy transfer to the surrounding medium and not by undergoing chemical reactions.

Purine solutions containing  $10^{-2}$  M  $\text{Mn}^{2+}$ , a triplet quencher, show no blue coloration and no  $\text{Cl}_2^-$  absorption band at 340 nm on UV illumination, and the photodestruction yield (0.005) is reduced by two orders of magnitude [20]. Therefore, these observations show that the triplet state of purine is involved in the formation of  $\text{Cl}_2^-$  radical ions as well as in the

photoionization of purine molecules in 12 M LiCl glasses. The  $\text{Cl}_2^-$  species is assumed to be formed from the reaction of a chlorine atom, formed by an energy transfer process from a purine highly excited triplet state to the surrounding chloride ions, and a chloride ion in the same solvent cage. Chlorine atoms could also be formed through an electron transfer from a  $\text{Cl}^-$  ion to a purine radical cation. The quantum yield for  $\text{Cl}_2^-$  radical ion production, as determined from the increase in the UV absorption band of  $\text{Cl}_2^-$ , is 0.003.

Sevilla and Mohan [19] have reported no ESR signals attributed to  $\text{Cl}_2^-$  radicals on UV irradiation of adenine or guanine in 12 M LiCl solutions at 77 K. However, Rodríguez and Arce [25] have found that prolonged irradiation times (about 15 min) of adenine solutions in 12 M LiCl produce  $\text{Cl}_2^-$  radical ions. The signal intensity of the recorded ESR spectrum is similar to that obtained after 30 s of UV irradiation of frozen 12 M LiCl solutions of purine. Thus, our studies show that the transfer of excess energy from the heterocyclic molecule to the solvent (12 M LiCl) is a more efficient process for purine than for adenine molecules. Quantum yields for triplet state production [20] for purine free base (0.37) in 12 M LiCl are also greater than triplet yields for adenine (0.17). This is additional evidence that the triplet state of purine is involved in the formation of  $\text{Cl}_2^-$  radical ions.

### 3.2. Intermediates in acid glasses

#### 3.2.1. 6 M $\text{H}_3\text{PO}_4$

In UV-irradiated glasses of 6 M  $\text{H}_3\text{PO}_4$  containing  $2.5 \times 10^{-4}$  M purine free base, no permanent blue coloration develops during 60 s irradiation and no ESR signal attributable to trapped electrons is observed. Instead, a signal from trapped hydrogen atoms characterized by a doublet splitting of about 500 G, not shown in Fig. 5, appears. Another doublet with a splitting of 33 G, centered at 3290 G and extending over 90 G, is also observed (Fig. 5). During the irradiation the samples emit a faint blue visible emission attributed to phosphorescence of purine triplet states for which an ESR

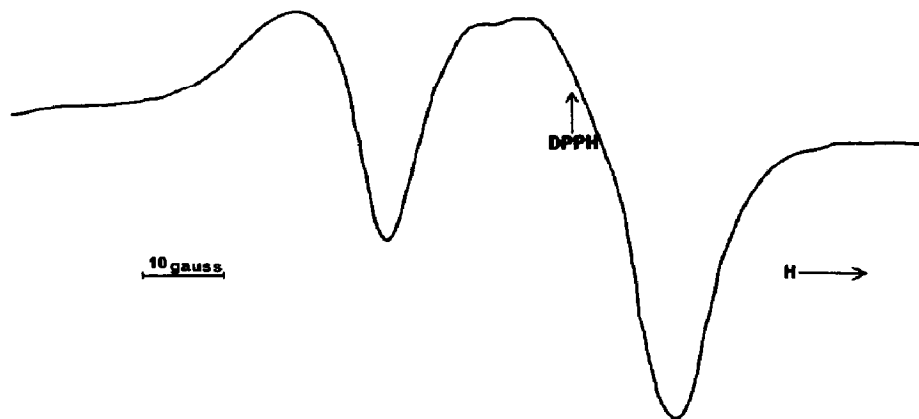
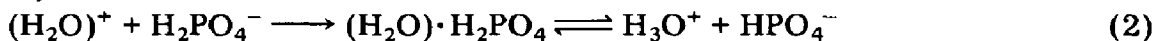


Fig. 5. ESR spectrum of frozen 6 M  $\text{H}_3\text{PO}_4$  solution of  $2.5 \times 10^{-4}$  M purine illuminated for 60 s with UV light.

signal is observed at a magnetic field of 1418 G. From this, an estimated intersystem crossing quantum yield of 0.1 was determined [20]. None of these signals is detected when pure 6 M  $\text{H}_3\text{PO}_4$  solutions are UV irradiated for much longer times. It seems, therefore, that absorption of light by purine molecules is necessary for the formation of the observed paramagnetic species.

The ESR spectrum recorded after UV photolysis of 6 M  $\text{H}_3\text{PO}_4$  solutions of purine closely resembles that obtained by Moorthy and Weiss [21] in the  $\gamma$  radiolysis of 6 M  $\text{H}_3\text{PO}_4$  and in UV-irradiated 6 M  $\text{H}_3\text{PO}_4$  solutions of  $\text{Ce}^{4+}$  ions. In the  $\gamma$  radiolysis of 6 M  $\text{H}_3\text{PO}_4$ , the ESR signal in the central part of the spectrum was attributed by these researchers to the  $\text{HPO}_4^-$  radical ion formed by reaction of the radiation-produced positive hole ( $\text{H}_2\text{O}^+$ ) with  $\text{H}_2\text{PO}_4^-$  according to



The hyperfine splitting arises presumably from interaction of the unpaired electron with  $^{31}\text{P}$  ( $I = 1/2$ ) nucleus [21]. In UV-irradiated frozen solutions of  $\text{Ce}^{4+}$  ions in 6 M  $\text{H}_3\text{PO}_4$ , the  $\text{HPO}_4^-$  radicals are postulated [21] to be formed through the reaction of  $\text{H}_2\text{O}^+$  with  $\text{H}_2\text{PO}_4^-$ . The  $\text{H}_2\text{O}^+$  ions are produced in the hydration shell of the excited  $\text{Ce}^{4+}$  ions via electron transfer from the water molecule to the ceric ions.

During the process of formation of  $\text{HPO}_4^-$  radical ions and hydrogen atoms in UV-irradiated purine solutions, the steady state purine triplet concentration remains constant over 60 s. Stopping the irradiation and re-exciting the solution produces the same maximum steady state triplet state concentration as that observed before the irradiation was discontinued. About 15% photodestruction occurs after 600 s of photolysis. In this acid glass a photodestruction yield of 0.001 was estimated [16]. Since the absorption of light by purine molecules is necessary for the formation of the  $\text{HPO}_4^-$  radical ions and hydrogen atoms and the purine molecules are unaffected during this short irradiation time, it is postulated that the observed solvent radicals result from energy transfer from the excited purine molecules to the surrounding solvent molecules, resulting in the breakage of one of the OH bonds in the  $\text{H}_2\text{PO}_4^-$  ions. The dissociation energy of the OH bond in water is 5.1 eV. On the basis of the phosphorescence of purines reported by Cohen and Goodman [26], the ground state triplet energy of purine is about 3.5 eV above the ground state. Thus such an energy alone is not enough to break the OH bond, but absorption of a second photon of wavelength 254 nm (4.9 eV) by a purine in a triplet state could produce a highly excited triplet level, with an energy of around 8.4 eV, which then transfers this energy to the solvent, resulting in solvent decomposition. The possibility exists that purine is photoionized and the resulting purine radical cation rapidly reacts with the solvent to produce  $\text{HPO}_4^-$  radicals.

Deuterated phosphoric acid (5.7 M  $\text{D}_3\text{PO}_4$ ) glasses containing thymine or 5-methylcytosine have been irradiated with UV light by Sevilla and coworkers [27, 28]. ESR studies have demonstrated that these pyrimidine

bases photoionize, producing radical cations and deuterium atoms. No ESR signals attributable to  $\text{DPO}_4^-$  radical ions have been described by Sevilla and coworkers [27, 28].

### 3.2.2. 5 M $\text{H}_2\text{SO}_4$

To check our conclusion that in the acid medium solvent decomposition results from an energy transfer of purine molecules to the surroundings, another acid glass 5 M  $\text{H}_2\text{SO}_4$  was examined. UV-irradiated purine solutions in this acid at 77 K produce the ESR spectrum presented in Fig. 6. This spectrum is due to two different paramagnetic species as determined from a microwave power saturation study. Signals from trapped hydrogen atoms were also observed (not shown in Fig. 6). These signals were not observed when irradiations were carried out in the absence of purine even for longer periods of illuminations.

The spectrum shown in Fig. 6 resembles that obtained by Moorthy and Weiss [21] in the  $\gamma$  radiolysis of 5 M  $\text{H}_2\text{SO}_4$  and in UV-irradiated 5 M  $\text{H}_2\text{SO}_4$  solutions of  $\text{Ce}^{4+}$  ions. The more intense singlet has previously been ascribed to the  $\text{SO}_4^-$  radical ion formed by reaction of the  $\gamma$ -radiation-produced positive hole  $(\text{H}_2\text{O})^+$  with the  $\text{HSO}_4^-$  ion. The much weaker outer line actually forms part of a more complex pattern due to  $\text{SO}_3^-$  radical ion, partly obscured by the more intense  $\text{SO}_4^-$  line.

Besides the formation of the solvent radicals ( $\text{SO}_4^-$ ,  $\text{SO}_3^-$  and hydrogen atoms) and purine triplets, no other radical that could be associated with purine was detected by ESR techniques. It seems that in acid medium (6 M  $\text{H}_3\text{PO}_4$  or 5 M  $\text{H}_2\text{SO}_4$ ) solvent radicals are produced as a result of an energy transfer from the excited triplet purine molecules to the solvent molecules.

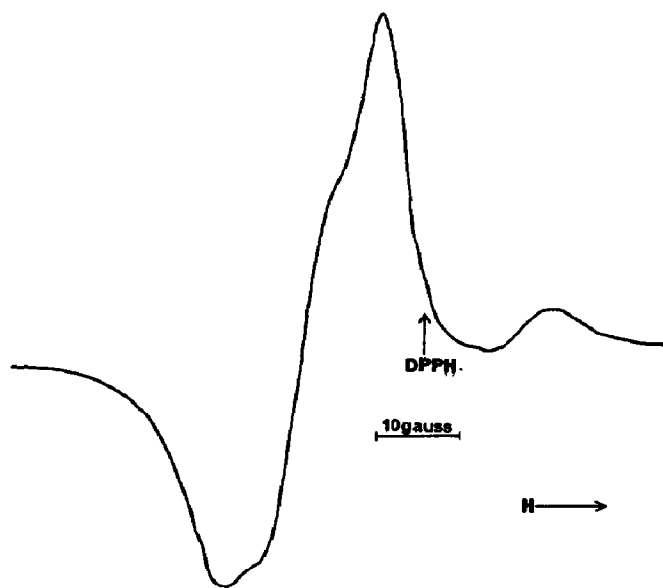


Fig. 6. ESR spectrum of 5 M  $\text{H}_2\text{SO}_4$  glass containing  $2.5 \times 10^{-4}$  M purine illuminated for 60 s with UV light.

### 3.3. Intermediates in basic glass (8 M NaOH)

UV irradiation of  $5 \times 10^{-4}$  M solutions of purine in 8 M NaOH glass produces an intense blue coloration of the sample, characteristic of trapped electrons. On examination by ESR, an unresolved singlet at  $g = 2.004$  is observed with a linewidth (14 G) greater than that of the ESR signal of the trapped electron (13.5 G) and extending over 80 G compared with the 55 G extension of the singlet due to the trapped electron (Fig. 7). Thus, the long tails of the spectrum in UV-irradiated purine solutions are due to purine radicals and not to trapped electrons. Photobleaching of the sample with visible-IR light produces a 70% - 75% reduction in the total intensity of the ESR signal and leaves a broader singlet (linewidth, 25 G) at  $g = 2.007$ . These changes in  $g$  value and linewidth are evidence of different paramagnetic intermediates before and after photobleaching of the sample. The photobleached electrons do not totally recombine with their geminate cations since this would result in the total disappearance of the ESR singlet. Although after photobleaching the visible absorption band of the trapped electron disappears completely, only partial regeneration of the purine UV absorption band is observed [16].

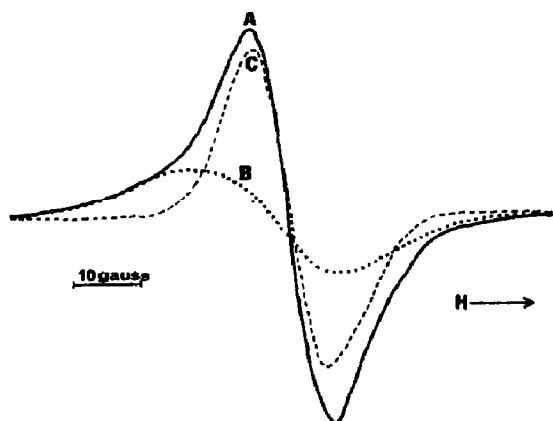


Fig. 7. ESR spectra of  $2 \times 10^{-4}$  M purine in 8 M NaOH glass UV irradiated for 10 s (spectrum A), purine radicals obtained after photobleaching for 5 min at a suitable receiver gain of the instrument (spectrum B) and the result of subtraction of spectrum B from spectrum A (spectrum C).

During the UV irradiation of the deprotonated purine a broad singlet at 1438 G is observed. After the photolysis is stopped this singlet disappears with a decay time of 3.4 s, which is similar to the phosphorescence lifetime of deprotonated purine; thus the singlet was attributed to the purine triplet state, produced with a quantum yield of 0.62 [20].

In 8 M NaOH solutions, the deprotonated form of purine  $\text{Pu}^-$  is present and it, as well as the neutral form of purine, is an efficient electron scavenger [17]. Nevertheless, photobleached electrons are not totally scavenged by purine molecules to form radical anions since in such a case the total

paramagnetic species concentration would remain constant during and after photobleaching and this is not observed. Therefore, the broader singlet observed after photobleaching (Fig. 7, spectrum B) is probably due to the overlap of the ESR spectra of purine radical cations and anions.

The purine radical anion was produced by photoionization of  $K_4[Fe(CN)_6]$  followed by the subsequent reaction of the detrapped electrons with purine molecules. In this way, the spectrum of the purine radical anion was recorded without interference from an overlap spectrum of the radical cation. It consists of an unresolved singlet at  $g = 2.004$  with a linewidth of 25 G.

To produce the radical cation without interference from the radical anion or the trapped electron, inorganic solutes ( $N_2O$ ,  $KNO_3$ ,  $K_3[Fe(CN)_6]$ ,  $KMnO_4$ ,  $K_2CrO_4$ ) which act as electron scavengers were added. The rate constants [18] for the reaction of the hydrated electrons with these solutes are all of the order of  $10^9 - 10^{10} M^{-1} s^{-1}$ . In irradiated solutions into which  $N_2O$  was bubbled for more than the 1 h the ESR signal of the trapped electron or the blue coloration did not disappear and no appreciable reduction in intensity of the trapped electron singlet was observed. This can be attributed to the low solubility of  $N_2O$  in the glass at 77 K. The presence of  $KNO_3$ ,  $K_2CrO_4$  or  $KMnO_4$  ( $10^{-2} M$ ) during the irradiation removed the blue color due to the trapped electron. Furthermore, the ESR spectrum no longer consisted of the characteristic singlet and instead other signals resulting from the electron scavenger reaction were observed which overlap the radical cation spectrum. The scavenger which produced least interference was  $K_3[Fe(CN)_6]$  at a concentration of  $2.5 \times 10^{-2} M$ . In its presence and after 60 s of UV irradiation and subsequent photobleaching, purine solutions ( $2.5 \times 10^{-3} M$ ) showed an unresolved singlet with  $g = 2.003$  and a linewidth of 32 G extending over 110 G. A poorly resolved hyperfine structure (quintet) could be observed superimposed on this singlet.

To demonstrate that both the radical cation and the radical anion were produced during the UV irradiation of purine solutions at 77 K, microwave power saturation studies were carried out on  $1 \times 10^{-4}$ ,  $2 \times 10^{-4}$  and  $5 \times 10^{-4} M$  purine samples and in solutions in which the radical anion was generated by the photoionization of  $K_4[Fe(CN)_6]$  in purine-containing solutions. Power saturation curves of the ESR signal height (peak to peak) as a function of the square root of the applied microwave power were traced for the UV-photolyzed and subsequently bleached solutions (Fig. 8). Linear behavior up to a power of 3 mW was found only for the most diluted solutions, indicating no power saturation in this concentration range. Increasing the purine concentration caused a deviation from linearity due to saturation, e.g. the  $5 \times 10^{-4} M$  purine solution saturated at a power of 0.16 mW. This change in saturation behavior is evidence that at high purine concentrations the paramagnetic species present after irradiation are not the same as those at dilute concentrations. However, these results do not demonstrate that the intermediate species present at the higher concentration is the purine radical anion. It was also observed that the saturation curves for the purine radical anion produced by the photoionization of  $K_4[Fe(CN)_6]$  in purine solutions

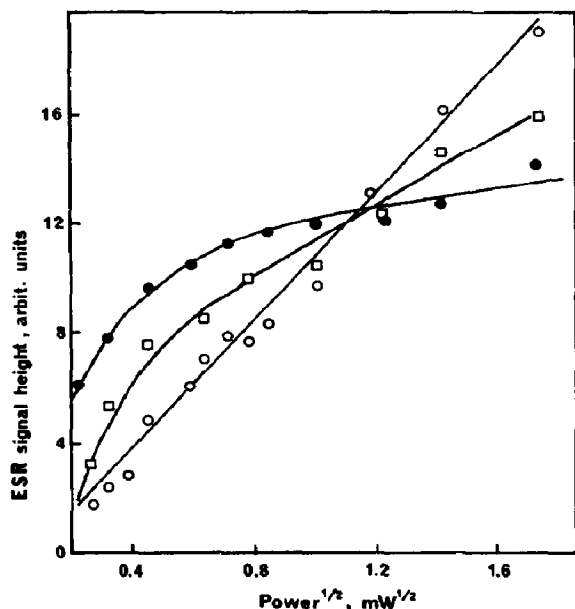


Fig. 8. Microwave power saturation curves for paramagnetic species produced by UV irradiation (10 s) and subsequently photobleached (5 min) in 8 M NaOH glasses containing purine at various concentrations:  $\circ$ ,  $1 \times 10^{-4}$  M;  $\square$ ,  $2 \times 10^{-4}$  M;  $\bullet$ ,  $5 \times 10^{-4}$  M.

and the saturation curve corresponding to the paramagnetic species produced in UV-irradiated  $5 \times 10^{-4}$  M purine solutions were quite similar. It seems, therefore, that a considerable amount of radical anion is produced during the UV irradiation of 8 M NaOH glass containing  $5 \times 10^{-4}$  M purine. From the observed power saturation curve of dilute purine solutions ( $1 \times 10^{-4}$  M), it can be concluded that at this concentration the greater percentage of paramagnetic species corresponds to purine radical cations.

The rate of photoionization represented by  $d[S]/dt$ , where  $[S]$  is the sum of the concentrations of trapped electrons, purine radical cations and a small contribution of radical anions produced after 10 s of UV irradiation, was found to depend on the  $1.61 \pm 0.05$  power of the incident light intensity, consistent with a biphotonic mechanism. However, when irradiation times of 60 s were used, a value of  $1.19 \pm 0.05$  is obtained, which corresponds to a monophotonic mechanism. Moan [29] has shown that this apparent discrepancy is due to an increase in the probability of spontaneous recombination of the photoejected electrons with their geminate ions, which increases with irradiation time. This was confirmed by plotting the relative total number of trapped radicals as a function of the irradiation time and by observing that, for irradiation times exceeding 15 s, the total radical concentration starts to deviate from linearity. For irradiation times of 60 s, the relative number of radicals has considerably diminished being about 40% of that for its linear behavior. This produces a marked decrease in  $d[S]/dt$  as well as in the slope of a plot of  $\log(d[S]/dt)$  versus  $\log I_0$ .

The quantum yields for trapped electron and for purine radical cation and anion production were determined at different irradiation times and for convenience at a microwave power of 1 mW. At this power, the signal corresponding to trapped electrons is partially saturated (32%). Thus proper corrections were made to take this into consideration. No signal decay was observed during the time elapsed to record the ESR spectrum after the UV irradiation. This spectrum has been attributed to an overlap of the signals of the trapped electron, the purine radical cation and the purine radical anion. The long tails in Fig. 7, spectrum A, are due exclusively to absorption by purine radicals and not to trapped electrons as was determined by comparing the ESR parameters of the spectrum of the trapped electron generated by photoionization of  $K_4[Fe(CN)_6]$  in 8 M NaOH with Fig. 7, spectrum A. Thus, to obtain the contribution of trapped electrons to Fig. 7, spectrum A, the spectrum of purine radicals obtained after photobleaching (Fig. 7, spectrum B) and recorded at a suitable receiver gain was subtracted from Fig. 7, spectrum A. The spectrum resulting from this subtraction (Fig. 7, spectrum C) shows the same linewidth, total extension and  $g$  value of the ESR signal, characteristic of trapped electrons. The doubly integrated area associated with Fig. 7, spectrum B, is almost the same as that of Fig. 7, spectrum C. Thus, to a first approximation, half of the doubly integrated area of the ESR spectrum obtained after UV irradiation (Fig. 7, spectrum A) corresponds to contributions from trapped electrons. This assumption is not totally valid since a fraction of the photobleached electrons is scavenged by purine molecules to produce paramagnetic species. Nevertheless, using this approximation and with a correction for power saturation of the electron signal, trapped electron yields of 0.012, 0.006 and 0.002 were calculated at irradiation times of 10 s, 30 s and 60 s respectively (Table 1). This variation in yield with irradiation time is due to partial bleaching of the electron and to a decrease in the light absorption by the sample due to purine photodestruction. From the visible absorption band of the trapped electron and its extinction coefficient at 585 nm a yield of 0.02 [16] was determined after 10 s of irradiation. This higher yield compared with the yield determined by ESR techniques confirmed that the original assumption that 50% of the area of Fig. 7, spectrum A, corresponds to trapped electrons is not valid. This implies that, of the total initial concentration of paramagnetic species, the trapped electron fraction corresponds to a fraction greater than 0.5. In such a case the geminate cation population has decreased through rapid deprotonation reactions or through reactions with species present in the medium, yielding non-paramagnetic species. The quantum yields for purine radical cation and anion production obtained after photobleaching of the previously UV-irradiated sample are of the order of 0.0064.

From the absorption changes at 271 nm, a purine photodestruction yield of 0.042 was determined in 8 M NaOH; thus photoionization of purine constitutes one of the principal photodestruction routes for the deprotonated species.



## 4. Conclusions

Biphotonic ionization of purine free base through a triplet intermediate is observed in neutral and basic glasses. This process is one of the major paths for purine photodestruction in aqueous glasses. Photoejected electrons react readily with species in the environment and/or with other purine molecules to form radical anions. The deprotonated form of purine is the most photo-reactive of the different ionic forms in terms of its photodestruction, photoionization and intersystem crossing quantum yields. Protonated excited purine undergoes mainly energy transfer reactions to the surrounding solvent molecules, leading to the formation of paramagnetic species.

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