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Electron spin resonance studies of some irradiated pharmaceuticals

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Abstract

Five antibiotics belonging to the cephalosporins and penicillins groups have been irradiated: anhydrous ampicilline acid, amoxicilline acid trihydrate, cefuroxime sodium salt, cloxacilline sodium salt monohydrate and ceftazidime pentahydrate. ESR studies have been carried out, showing the influence of irradiation and storage parameters on the nature and concentration of the free radicals trapped. These results may be used to detect an irradiation treatment on such pharmaceuticals.

Keywords: Gamma irradiation; Pharmaceuticals; Cephalosporin; Electron spin resonance

1. Introduction

The interest for using high-energy ionising radiation (gamma rays, electron beams) is increasing; both for sterilisation of medical devices or for improvement of the hygienic quality of foods (Saint-Lebe and Raffi, 1995). Its major advantage is due to its high penetrating power and the very small temperature rise induced. Micro-organisms are always radio-sensitive (Farkas, 1989; Whitby, 1993), even if more or less. The sterilisation dose (Sterility assurance level (SAL) of 10^{-6}) of pharmaceuticals both depends on the initial microbiological spoilage (bioburden) and on the microorganisms radiosensitivity (Jacobs, 1985). On account of the destructive nature of ionising radiation and the difficulty in predicting the radiolytic effects, the study of radio-induced radicals and chemical products in drugs is necessary, both to determine the feasibility of the radiation treatment and to control it. Moreover, the regulations vary from country to country: radiosterilisation is permitted in some countries and not in others (Pharmacopeia, 1992, 1993, 1995), which leads to the necessity for a method to differentiate between irradiated and non-irradiated samples.

Electron spin resonance (ESR), which is a very sensitive method for detection of free radicals, can be used for studying the radiolysis mechanism (Crucq, 1994) or for detection of irradiated drugs (Zeegers et al., 1993; Gibella et al., 1993; Basly et al., 1997) as already used elsewhere for foodstuffs irradiated at very low dose (100 Gy) (Raffi and Stocker, 1996). We describe here the results of experiments on five antibiotics (chemical

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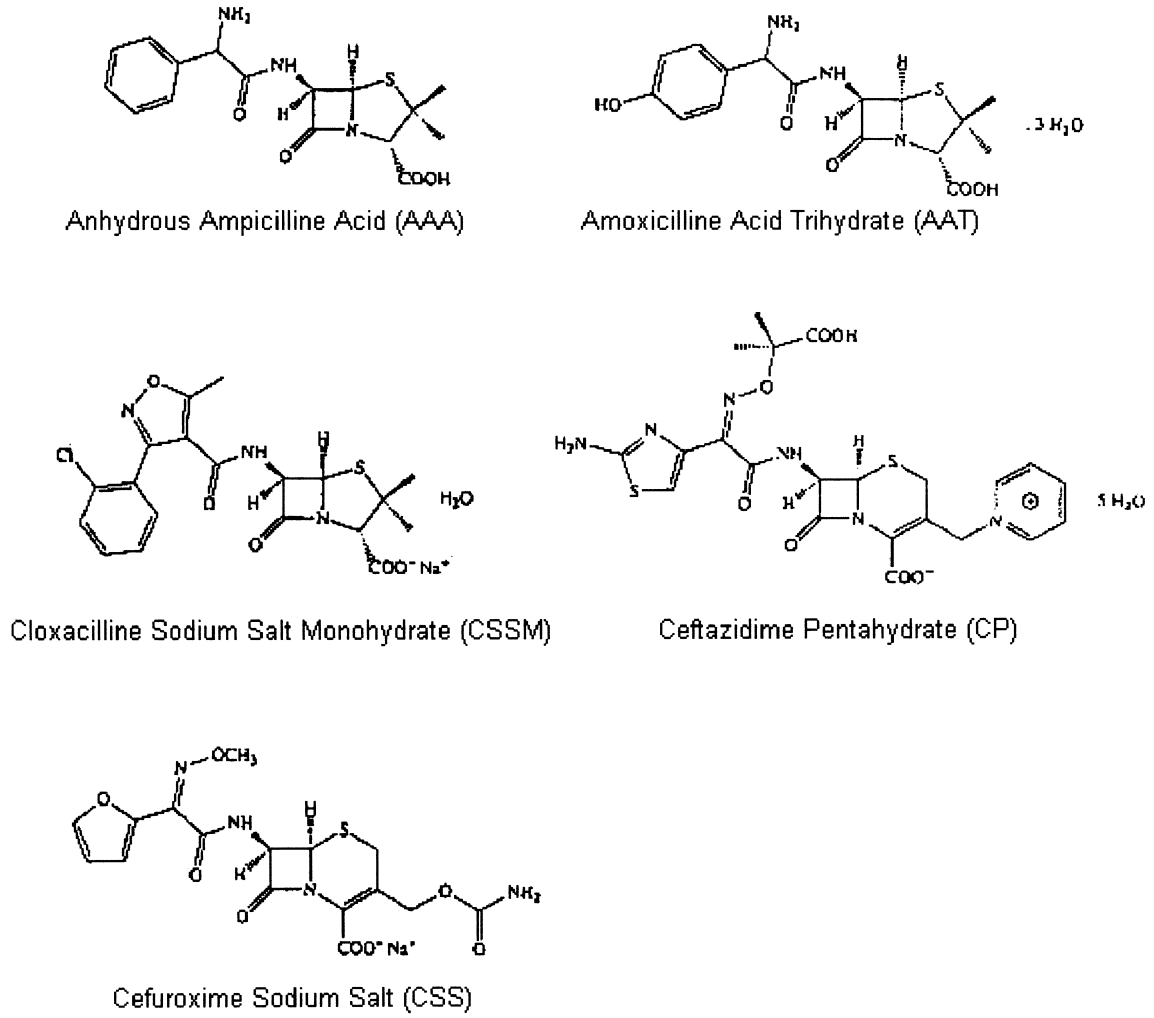

Fig. 1. Formula of the different studied antibiotics.

formula on Fig. 1) belonging to the cephalosporins and penicillins (Scorderet et al., 1988) groups: anhydrous ampicilline acid (AAA), amoxicilline acid trihydrate (AAT), cefuroxime sodium salt (CSS), cloxacilline sodium salt monohydrate (CSSM) and ceftazidime pentahydrate (CP) (Crucq and Tilquin, 1996; Barbarin et al., 1996; Miyazaki et al., 1994a). For the detection of radiolytic products at doses above 10 kGy, the chromatographic analysis with different detectors (Zeegers, 1997) of "unique radiolytic products" may be used.

There are two ways of following the radical evolution with respect to time after irradiation:

The radical concentration is proportional to the area of the integrated curve usually recorded by an ESR spectrometer. The areas of the individual peaks of the recorded spectrum are obtained by double integration of this curve and by subsequent deconvolution using e.g. Bruker Win-EPR software (can be obtained at: http://www.bruker.de/).

The radical concentration is to a first approximation proportional to the peak heights of the recorded spectrum.

The different ESR lines are numbered on the different spectra as independent lines. Their peak to peak intensity is measured directly and studied with regard to the different irradiation and storage conditions, as a first approximation of the spectra evolutions. In fact, it is also possible to make a double integration of the curves (for instance, we use the Bruker Win-EPR software), make a deconvolution of resulting curve and measure the relative area of each peak; if the values are different (we do not measure the same thing), the general evolution is the same and we do not carry out systematically these deconvolutions and do not present here them.

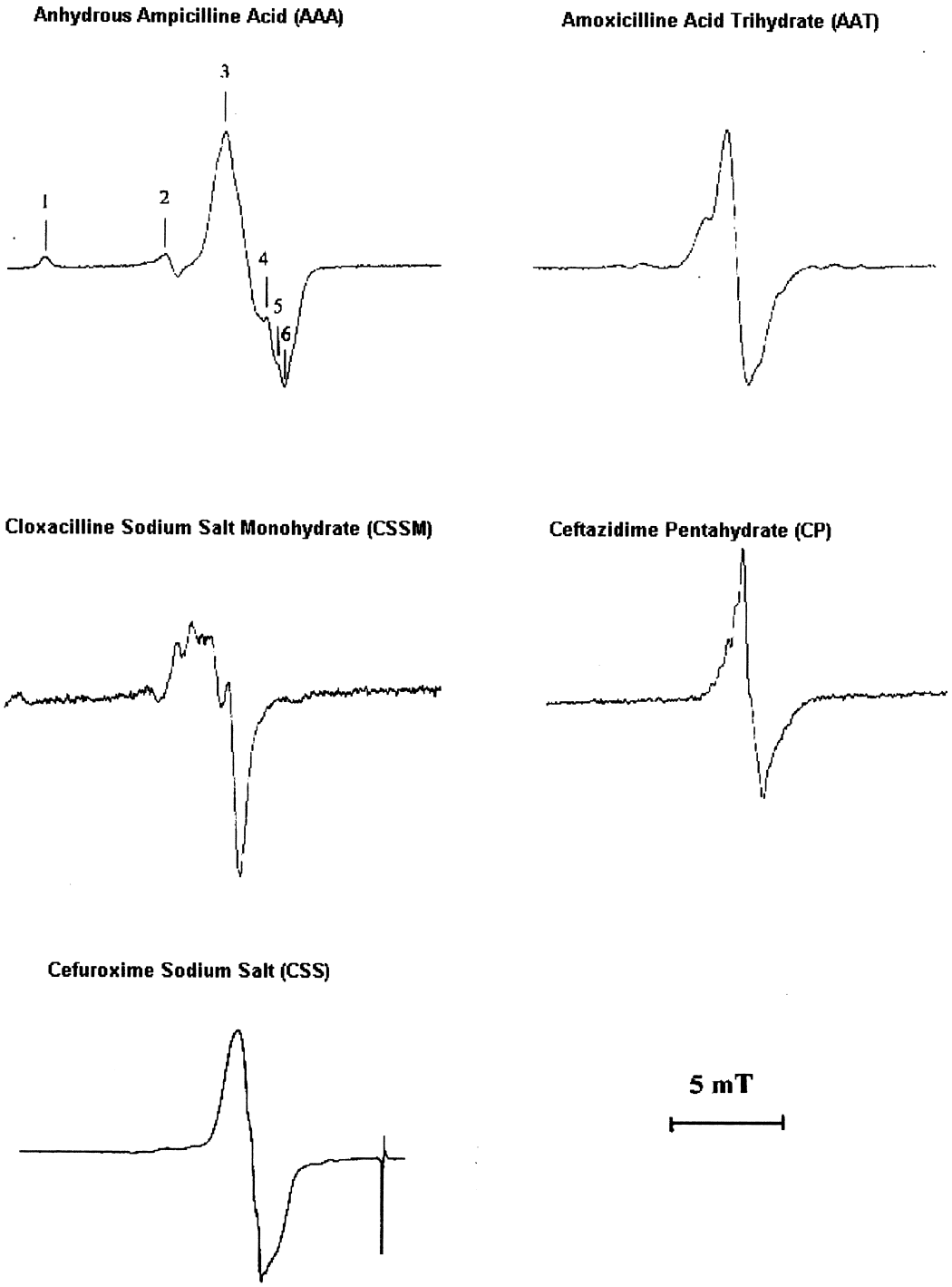

Fig. 2. ESR spectra of the different studied antibiotics irradiated at 25 kGy.

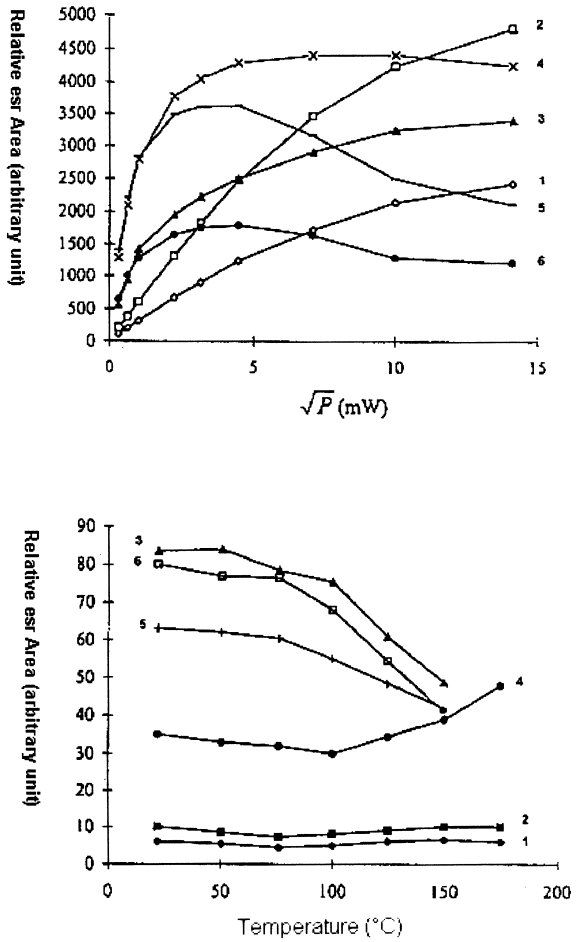

Fig. 3. Influence of microwave power (top) and of recording temperature (bottom) on relative intensities of ESR spectra (arbitrary units) of irradiated (25 kGy) AAA (the lines numbers are defined on the AAA spectrum of Fig. 2). We use a microwave power of 400 μW to avoid saturation.

2. Experimental section

The antibiotics (current in Belgium) were purchased from Federa Belgium Ltd (ampicilline and amoxicilline), Beecham Ltd (cloxacilline) and Glaxo Belgium Ltd (ceftazidime); they were irradiated, at 77 K or at room temperature, in two ^{60}Co irradiators: one delivering a dose rate of approximately 640 Gy/h (Unité de Chimie Inorganique et Nucléaire, Louvain la Neuve, Belgium), and the second one delivering 10 kGy/h ("CIGAL", CEA Cadarache, France). The doses were 5, 15, 25 and 50 kGy.

The samples irradiated at 77 K (liquid nitrogen) were sealed in air, before treatment, in a quartz suprasil tube. A good quality of quartz reduces the number of trapped ionic species; for room temperature irradiation, the tubes were sealed after treatment, we have to avoid the water condensation in the tube specially at low temperature.

ESR spectra were always recorded at room temperature on a JEOL JES-RE2X spectrometer (Louvain la Neuve, Unité de Chimie des Interfaces, Belgium) or on a BRUCKER EMS 104 spectrometer (LARQUA, France). Microwave power 400 μW, sweep width 20 mT, modulation 0.402 mT. A microwave power of 100 μW is used at 77 K (Miyazaki et al., 1994a).

3. Results

3.1. Samples irradiated at room temperature

Unirradiated samples generally present no signal; however, in case of anhydrous ampicilin acid (AAA), a very small singlet may be observed in some cases, due to sunlight-UV irradiation.

The samples irradiated at room temperature show complex spectra (Fig. 2), not very well resolved: mainly singlet or doublet (AAT, CP), doublet (AAA, CSS), doublet or more (CSSM); moreover the spectra depend on irradiation dose, but also on recording conditions (temperature, microwave power) and on storage time. The influence of these different factors vary from one line to another, showing that we always have mixtures of radicals in each irradiated pharmaceutical (Figs. 3 and 4):

- When the microwave power is increased, the different ESR lines saturate at different values (Fig. 3) showing the presence of different radicals;
- When the irradiation dose is increased, the different ESR lines do not vary in the same way in case of AAT (Fig. 4), which is an additional proof of the presence of different radicals;
- During the storage time, there is a decrease of the signal intensity (ranging from 40 to 60% in 2 years), following a composite first order rate law for all studied samples; but this decrease in signal intensity (measured via the integrated value of peaks) is generally not associated with any change of the ESR signal shape; however, in case of AAA (Fig. 5) or CP, the ratio between the relative intensities of lines can be followed (Fig. 5).

3.2. Samples irradiated at low temperature

- The spectrum, recorded at room temperature, of AAA irradiated at 77 K is quite identical to the case of irradiation at room temperature.
- For AAT irradiated at 77 K the spectrum shows a line with a shoulder at the centre of 6 small lines, in comparison with the apparently unique line of the room temperature spectrum.

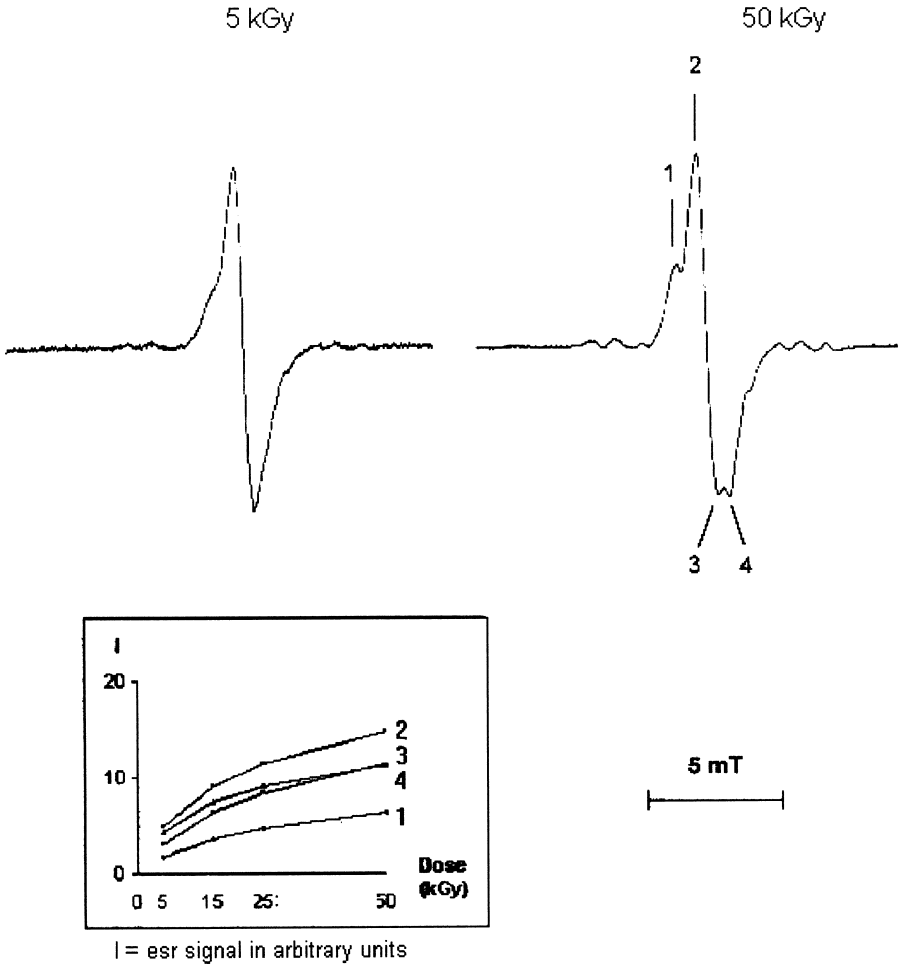

Fig. 4. Influence of irradiation dose on AAT ESR spectra. The lines numbers of the curves put in the scroll correspond to the ones of the right spectrum. The doses are of 5 and 50 kGy respectively; without irradiation, ESR spectrum is not registered. Relative ESR area are given for each peak as a function of dose.

- In the case of CSS, the 77 K spectrum is very strong relative to the room temperature spectrum and probably more complex.
- The CP spectrum, at low temperature, is complex, deriving from at least two signals: one singlet ($g = 2.009$), a sextuplet ($g = 2.011$) attributed to the radical $^{\cdot}C(CH_3)_2COOH$ and a triplet assigned to iminoxyl radicals (Miyazaki et al., 1994a). On the other hand, the room temperature spectrum apparently presents a unique line.

4. Discussion

4.1. Comments on mechanisms of radicals formation

Excited molecules are produced both directly and by neutralisation of radical-cations (Dusaucy and Tilquin, 1991).

$$RH \xrightarrow{\textit{radiolyse}} RH^{+\bullet} + e \rightarrow RH^{*}$$

$$RH \xrightarrow{\textit{radiolyse}} RH^{*}$$

Excited molecules may decompose to radicals by rupture of chemical bonds.

$$RH^{*} \rightarrow R'^{\bullet} + R''^{\bullet}$$

However, fragment radicals do not diffuse in solid matrices (“cage effect”) and immediate geminate termination reactions are possible (Tilquin, 1985). If the

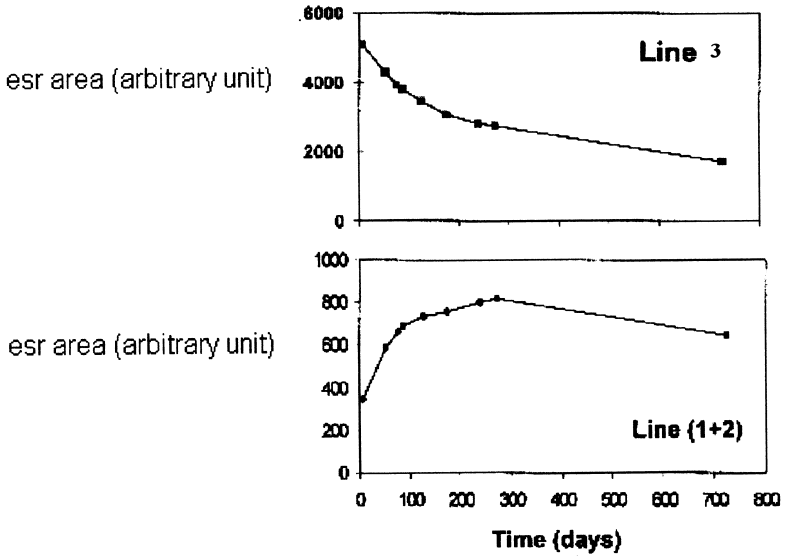

Fig. 5. Influence of storage time at room temperature on two lines of the irradiated AAA ESR spectrum (the different lines are defined on Fig. 2) at 25 kGy.

original radical cation escapes immediate neutralisation, a very important ion molecule reaction may give radicals.

$$RH^{+\bullet} + RH \rightarrow R^{\bullet} + RH_2^+$$

$$RH^{+\bullet} + RH \rightarrow r^{\bullet} + r^+$$

This mechanism is very important with polar molecules like H_2O where electron is trapped far from the geminate cation.

The cation is neutralised ($RH_2^+ + e$) when electrons are ejected from their physical chemical traps; some luminescence occur and are recorded by increasing the temperature of the irradiated samples (thermoluminescence) (Gibella, 1997).

$$RH_2^+ + e \rightarrow RH_2^*$$

$$RH_2^* \rightarrow h\nu + RH_2$$

Excited molecules and radical cations are produced by the direct interaction with the ionizing radiation and are localised along the track in regions of high local concentration (spurs). So the radicals are also formed in spurs and the kinetic must take the effects of non homogeneous formation of the species into consideration.

4.2. Radical decay

The most important reactions are radical–radical combination and disproportionation. These reactions do not follow the second order kinetics that would result from random radical encounters throughout the medium (Basly et al., 1997).

Radicals are produced in spurs. The radicals that escape intraspur combination or disproportionation decay by combination with radicals from other spurs encountered after random diffusion (second order). All diffusion processes are related to minor changes in the physical properties of the irradiated solids. The rates of radical decay depend on the nature of the solid matrix (Tilquin, 1985), annealing is a constant process with local diffusion of radicals and molecules in some softening of defects or irregularities.

At room temperature, for some irradiated solid pharmaceuticals, particularly antibiotic molecules, the radical decay is very slow and many radical-molecule reactions observed in liquid state are not possible. The activation energy (E_A) (2–5 kcal) for radical mechanisms is not compatible with the trapping of radicals during several years (Miyazaki et al., 1994a,b). So, the radical mechanism is not the same in solid as in liquid. This matrix effect is well known and enhanced by the ESR observations on radicals in solid pharmaceuticals.

Typically, radical decay in irradiated solid pharmaceuticals becomes fast enough for observation at approximately the melting point (Dusaucy et al., 1991; Miyazaki et al., 1994b). The radical concentration decreases rapidly as the diffusion of molecular species is possible.

4.3. The dose effect

The concentration of radicals grows linearly at first and then at a progressively lower rate until a steady-state plateau is reached (Fig. 4). Plateau concentrations of radicals are very low (10^{-6} M) which excludes the possibility of saturating all the traps. It appears that during irradiation, radicals are removed by rapid processes other than those which occur in the absence of radiation (after the radiolysis). Mechanisms that may contribute to the dose effect include the following:

1. For the trapping of the moving electron, we have physical and chemical scavengers. During the radiolysis, more and more radicals are trapped. These radicals present a better electron affinity than the original molecule and are in competition to pick up the electron.

   $R^{\bullet} + e \rightarrow R^{-}$

   Tunneling electron transfers at long distance are physically possible in solid.
2. Part of the energy used to produce the initial radicals ($R^{\cdot}$) now favors the activation of solute radiolytic products. So the result is a deactivation of primary species producing the $R^{\cdot}$ radical by energy transfer.

4.4. The numerous trapped radicals

All experiments shows that the shape of the spectrum changes with different conditions (microwave power Fig. 3, temperature Fig. 3, dose Fig. 4, elapsed time Fig. 5). For the ampicilline (AAA), lines 1+2 increase with time of measurements and are attributed to radicals centred on S atom, more stable than carbone centered ($C^{\cdot}$) structure. So a first hypothesis is that radicals may be converted to more stable radicals during and after the radiolysis.

5. Conclusions

For the present study, we have been unable, up to now, to assign a chemical structure to each radical; in order to do this, we have first to separate the different lines, by mathematical deconvolutions. But as it is quite impossible to make interpretations of ESR spectra in solid state, even if they are very stable, we will also have to carry out spin-trapping (Thiéry et al., 1983) experiments.

It is also important to note that, in case of ampicilline, some ESR line intensities begin to grow with storage time (Fig. 5), which is relatively exceptional in powder state (De Laet and Tilquin, 1991).

However, even without any chemical interpretation of the ESR spectra, it is more often easy to distinguish the irradiated samples from the unirradiated ones; this method can lead very easily to detection protocol (for each group of pharmaceuticals), as has been set up elsewhere for identification of irradiated foodstuffs (Raffi et al., 1996).

On the other hand, it will be probably impossible to determine, by examining the sample, the value of the dose delivered to the sample, except if we are given both the irradiation temperature and its date, as the concentration of induced radicals both depends on the irradiation temperature and storage time.

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