

Effects of radiation on carbapenems: ESR identification and dosimetric features of gamma irradiated solid meropenem trihydrate

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Abstract

In the present work, effects of gamma radiation on solid meropenem trihydrate (MPT), which is the active ingredient of carbapenem antibiotics, were investigated by electron spin resonance (ESR)spectroscopy. Irradiated MPT presents an ESR spectrum consisting of many resonance peaks. Heights measured with respect to the spectrum baseline of these resonance peaks were used to explore the evolutions of the radicalic species responsible for the experimental spectrum under different conditions. Variations of the denoted 11 peak heights with microwave power, sample temperature and applied radiation doses and decay of the involved radicalic species at room and at high temperatures were studied. On the basis of the results derived from these studies, a molecular model consisting of the presence of four different radicalic species was proposed, and spectroscopic parameters of these species were calculated through spectrum simulation calculations. The dosimetric potential of MPT was also explored and it was concluded that MPT presents the characteristics of normal and accidental dosimetric materials.

Keywords: Dosimetry; ESR; Temperature; Kinetics; Carbapenem, Meropenem

1.Introduction

Meropenem belongs to carbapenem subgroup of beta-lactam antibiotics inhibiting cell wall biosynthesis. Its potency is expressed as mass of meropenem and it contains not less than 900 µg meropenem per mg . Meropenem trihydrate (here after MPT) is the active ingredient of meropenem antibiotics. MPT occurs as white to light yellow crystalline powder and has a molecular structure as given in Fig.1. It is sparingly soluble in water and practically insoluble in ethanol. Moisture content of MPT is 12.35 % and it is not hygroscopic [1]. Dehydrated MPT was observed to take up moisture quickly even under low humidity. Crystal water of MPT was found to stay bound and to be almost inert [1].

MPT is used in moderate to severe bacterial infections in patients who have been admitted to hospital. These infections include: pneumonia, abdominal infections, lung infections in cystic fibrosis patients, bacterial meningitis, skin and skin structure infections, bladder, kidney and womb infections etc. MPT is effective against most harmful bacteria that are clinically important and it exerts its bactericidal action by interfering with vital bacterial cell wall synthesis.

Radiation processing is a well-proven technique for achieving safe and effective sterilization of disposable medical devices, pharmaceuticals and pharmaceutical raw materials [2]. It is one of the three processes that can be used as a terminal sterilization method after the edition of the European Pharmacopia in 1997 under the ‘ Methods of preparation of sterile products’ as to the veracity of a particular supplier [2,3]. Therefore, methods providing ability of distinguishing irradiated pharmaceuticals from non-irradiated ones are needed. Electron spin resonance (ESR) spectroscopy appears to be well suited for the determination of free radicals produced in irradiated products and could permit distinguishing irradiated pharmaceuticals from non-irradiated ones [4-11].

In the present paper, we report experimental data on ESR identification of gamma irradiated meropenem trihydrate (a carbapenem antibiotics) and on characteristic features of the radical species produced after irradiation.

2.Experimental

2.1 Samples

Meropenem trihydrate was kindly supplied by Astra Zeneca drug company (İstanbul, Turkey) and it was used without any treatment except milling. Irradiated and non-irradiated MPTs

were kept in dark in tightly closed polycarbonate vials and samples of particle sizes smaller than 600 μm were used throughout the entire experiment.

2.2 Irradiation

Irradiations were performed at room temperature (290 K) using a ^{60}Co gamma cell supplying a dose rate 2.5 kGy/h as an ionizing radiation source at the Sarayköy Establishment of Turkish Atomic Energy Agency in Ankara. The dose rate at the sample sites was previously calibrated using Fricke dosimetry (ferrosulphate dosimetry). A non-irradiated sample was kept as reference.

2.3 Apparatus

ESR measurements were carried out using a Bruker EMX-131 X-band ESR spectrometer operating at 9.5 GHz and equipped with a cylindrical TE_{104} resonant cavity. The spectrometer operating conditions adopted during the experiment are given in Table 1. The spectra were recorded at room and different temperatures. Peak heights were calculated from the first derivative spectra and compared with peak heights obtained for a standard DPPH sample under the same spectrometer operating conditions. Sample temperature inside the microwave cavity was monitored with a digital temperature control system (Bruker ER 411-VT). The latter provided the opportunity of measuring the temperature with an accuracy of ± 0.5 K at the site of the sample. A cooling, heating and subsequent cooling cycle was adopted to monitor free radical signal evaluations. The temperature of the samples was first decreased to 125 K, starting from room temperature with an increment of 20 K, then increased to 400 K and finally decreased again to room temperature.

Annihilation experiments at high temperatures were also performed to explore kinetic behaviours of the radicals. To achieve this goal, the samples were heated to a predetermined temperature and kept at this temperature for a predetermined time, then they were cooled to room temperature and their spectra were recorded. Adopted predetermined temperatures and times were 360, 370, 380, 400 K and 3, 6, 10, 15, 25, 40, 60, 90 min, respectively, in the present study. The results were the average of six replicates for each radiation dose.

3. Results and discussion

3.1 Room temperature results

Although unirradiated (control) MPT exhibited no ESR signal, irradiated MPT showed a nonresolved ESR spectrum consisting of many resonance peaks spread over a magnetic field range of 8.7 mT as shown in Figure 2a. Numbers were assigned to the observed resonance peaks and the following g factors, which fall into the free radical g factor range were calculated for easily distinguishable peaks numbered as 1 ($g_1=2.0203$); 2 ($g_2=2.0145$); 4 ($g_4=2.0091$) and 5 ($g_5=2.0016$). The positions and heights of the observed resonance peaks were found not depending on the orientation of external magnetic field.

Variations of the peak heights measured with respect to the spectrum base line of the numbered resonance peaks with applied microwave power were studied first in the range of 0.2-20 mW. The results are given in Fig.3. The measured peak heights exhibited the characteristics of inhomogeneously broadened resonance lines arising from more than one radical species whose g-values depend on the orientation of external magnetic field with respect to the molecular axes of these species. Although peaks 3 and 5 continued to increase in the studied microwave power range (0.2-20 mW), peaks 1, 10 and 11 saturated at about 5 mW, while peaks 2 and 4 reached to saturation at about 10 mW. This result was considered as an manifestation of the presence of more than one radical species in gamma irradiated MPT, likely four.

3.2 Variations of peak heights with temperature

Variations of the measured peak heights with temperature were also studied in the temperature range of 100-380 K to monitor thermal behaviours of the involved radical species. The results obtained for a sample irradiated at a dose of 15 kGy is given in Figure 4. While peaks 1, 2 (Fig.4), 10 and 8 (not given in figure 4) exhibited saturation behaviours at low temperatures at 1 mW microwave power, the others continued to increase even at the lowest temperature (100 K) implying the presence of the species having different saturation characteristics in irradiated MPT. However, peaks exhibiting saturation behaviours did not saturate at the same temperature. While peaks 1 and 8 begin to saturate at about 200 K, saturation of peaks 2 and 10 was found to occur at about 140 K. Measured changes in the heights of the studied peaks in cooling the sample from 290 K to 100 K were irreversible. This result was considered as a revelation of the fact that the radical species responsible from experimental spectrum and not undergo any irreversible structural changes during the cooling

period, but the changes in the peak heights were rather due to Curie effect and/or to saturation.

Heating samples up to 380 K caused significant irreversible decreases in the measured heights of the peaks 2, 3, 4, 5, 9 and 10, but created almost no changes in the heights of peaks 1, 6, 8 and 10. Above 380 K, the spectrum begun to transform into a broad single resonance line centered at about the position of peaks 5. Merging of the assigned peaks in this broad line created difficulty in reliable measurement of the heights of the assigned peaks above this temperature. Observation of an ESR signal at high temperatures was considered as an indication of the presence of radical species of high thermal stabilities even above 380 K in irradiated MPT.

3.3 Long term decays of the peak heights at normal conditions

Room temperature (290 K) long term stability studies have been carried out using a sample irradiated at a dose of 6 kGy. The sample was sealed and kept at dark throughout long term decay experiment. Peak heights data derived for assigned peaks from the spectra recorded in regular time interval during days after stopping of irradiation were employed to monitor room temperature stability characteristics of the radical species contributing to the formation of experimental spectrum. The results are given in Figure 5 for some characteristic peaks. Peaks heights presented in Figure 5 were measured with respect to the spectra base lines and heights of the peaks above and below base lines were represented as positive and negative numbers, respectively, to show more clearly the variations. The decays in the peak heights were minor and they occurred in about first fifteen days after termination of irradiation. Peaks 2 and 10 were the peaks undergoing the most severe decays among others.

Theoretical functions compatible with a model based on the presence of four different radical species explaining best the experimental data derived from microwave saturation, long term stability, high temperature stability studies and room temperature ESR spectrum intensities were used to describe experimental decay data presented in Figure 5. A function such as

$$I = \sum_{i=1}^4 (I_{0i} e^{-k_i t})$$

which consists of the sum of four different exponential functions each

representing different contributing radical species exhibiting first order decay kinetics was found to fit best experimental long term decay data given in Figure 5. In this function I_{0i} s and k_i s are the weights and decay constants, respectively, of the involved radical species and t is the time elapsed after stopping irradiation. Reliably measurable peaks height values of peaks

1, 2, 3, 4, 5, 10 and 11 were used in curve fitting process and decay parameters given in Table 2 were obtained for adopted tentative radical species. It is seen that fast decays observed at the beginning of storage period is governed in large extent, by species B and C. Although the decay constant of species D is very small, it dominates, heavily, the intensities of all resonance peaks.

3.4 Annealing study results

Increase in the sample temperature is expected to cause increases in the decay rates of the radicalic species induced in gamma irradiated MPT. The decay rates of the species were low up to 350 K, but significant increases in the decay rates were observed to occur above 350 K. Therefore, annealing studies were performed at six different temperatures above 350 K (360 K; 370 K; 380 K; 385 K; 390 K and 400 K) using six different samples. To do that samples were kept at a given temperature for predetermined times then cooled to room temperature and the spectra were recorded. Measured peak height values were normalized utilizing the values obtained for the same samples before any heat treatment. Above 380 K the decay rates of the peak heights were so fast that even for very short annealing times, assigned peaks were observed to merge into a broad resonance line avoiding reliable measurements of the heights of the peak assigned at room temperature. Hence, the decay constants of the involved species at 360 K, 370 K and 380 K were calculated using the peak height values measured at the same temperatures for all assigned peaks by fitting experimental data to a mathematical function consisting of the sum of four different exponentially decaying functions similar to those used in the calculation of room temperature decay constants. The results are given in Table 3. Variations of the experimental and theoretical peak heights calculated using decay constants given in Table 3 with annealing times and annealing temperatures were represented together on the same graphs for comparison. To save space, one of these graphs relevant to peak 4 is given in Figure 6. As is seen from this figure, experimental and theoretical data are in close agreement for all annealing times and annealing temperatures. That was also the case for other assigned peaks.

3.5 Spectrum simulation calculations

Spectroscopic parameters of the radical species responsible from experimental spectrum were also calculated. Signal intensity data gathered from room temperature experimental spectrum were used as input for simulation calculations. Four tentative radical species of different concentration and of different linewidth and exhibiting different hyperfine structures and spectroscopic splitting factors were tried throughout the calculation. Parameter values calculated for the proposed tentative radical species are given in Table 4. As is seen, calculated concentrations of the involved species are very different. While species D contributes heavily to the formation of the central peaks such as 2, 3, 4, 5 and 6, A and B were the species contributing to the high field peaks. However, the contribution of species A was very weak due to its very low concentration. Theoretical spectrum calculated using parameter values given in Table 4 is represented with its experimental counterpart in Figure 7. Agreement between these spectra are reasonably well. Theoretical spectra due to contributing species are also represented in Figure 7 to show clearly the contribution weights of each radical species to the assigned peaks. While unpaired electrons of A, C and D species interact with three protons of neighbouring hydrogen atoms, species B experiences hyperfine splittings due to one nitrogen and two hydrogen atoms giving rise to the formation of resonance lines with narrow linewidths. g factors of the species were found to be isotropic except species A which exhibited a weak axial g anisotropy.

3.6 Activation energies of proposed radical species

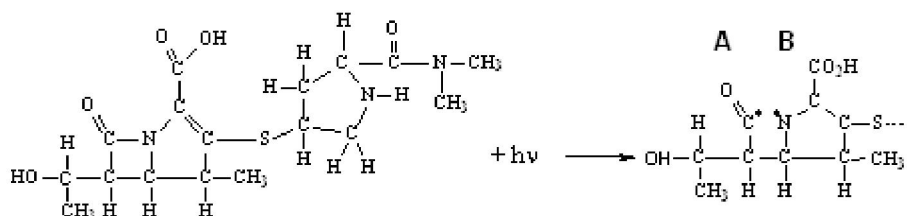
Decay activation energies (E_{ac} 's) of the contributing radical species were also calculated using decay constants (k 's) derived from annealing studies at high temperatures (Table 3). k is related to E_{ac} through Arrhenius equation ($k=k_0e^{-(\Delta E/RT)}$) where R , T and E_{ac} are the gas constant, absolute temperature and activation energy, respectively. The slopes of the straight lines best fitting to the data of $\ln(k)-1/T$ graphs constructed for each radical species were used to calculate E_{ac} 's. The results are summarized in Table 5. It is seen that, in gamma irradiated MPT, the species with the highest concentration, that is species D, has also the highest activation energy (152.0 ± 13.5 kJ/mol) and that the least stable species is B with its smallest activation energy (53.2 ± 4.0 kJ/mol). However, relatively high activation energies calculated for involved species were taken as a manifestation of the high stabilities of the latter at room and around room temperature.

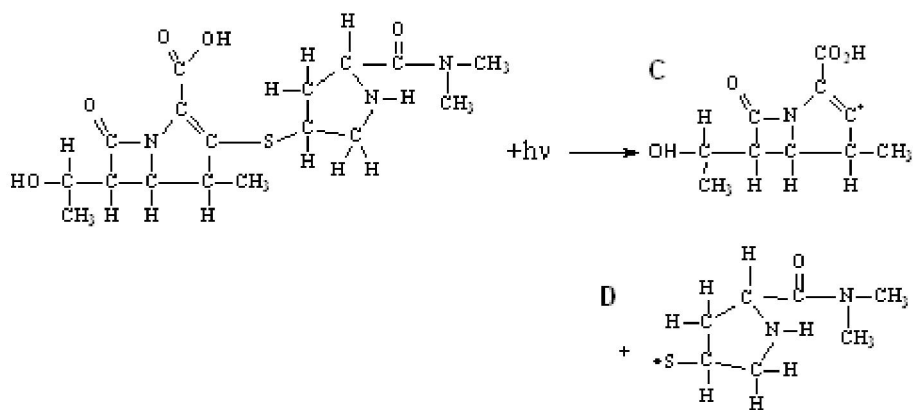
3.7 Dose-response curves

Dosimetric potential of MPT in the dose range of 3-15 kGy was also explored. Samples irradiated at 3, 6, 10 and 15 kGy were used to achieve this goal. Peak-height-applied dose graphs were constructed for easily measurable peaks such as 1, 2, 4 and 5. The results are presented in Figure 8. An important step in investigating the dosimetric features of pharmaceuticals has been the choice of mathematical functions to fit data. Linear, quadratic, power and exponential functions of applied dose have been tried to describe experimental data. The results are summarized in Table 6. It should be noted that no attempt has been made to force the regression through zero. Although tried functions described dose-response data relevant to peak 1 with high correlation coefficients, it was not the case for other investigated peaks. However, it should be noted that linear and quadratic function correlated reasonably well with experimental dose-response data of the studied peaks. Peaks 2 and 5 are the most sensitive peaks to the applied dose. In order to verify the utility of the mathematical functions used, back-projected doses were calculated by entering the measured heights of peak 5 in the quadratic equation described in Table 6. It was found that applied dose could be determined with an accuracy of 8 % in the dose range of 3-15 kGy.

3.8 Proposed tentative radical species

Following radical species were believed to be produced upon gamma by irradiation of MPT by homolytic dissociations of C-N and C-C bonds of beta-lactam ring giving rise to the production of CO gas and one of the C-S bonds connecting five member rings as show below:





Dissociations of C-C, C-N and C-S bonds are preferential and the number of the dissociated C-S bonds is much higher than the number of the dissociated C-C and C-N bonds for a given applied dose. On the other hand, the stabilities of produced species are very different. Species produced upon localisation of unpaired electron on the beta-lactam ring carbon atom (species A) is less stable compared to the species whose unpaired electron is localised on the nitrogen atom (species B) of the same ring. While unpaired electron of species A interacts with three neighboring unequivalent hydrogen atoms, species B exhibits hyperfine splittings originating from nitrogen atom itself and two neighboring hydrogen atoms. Due to its high stability ($E_{ac}=152.0\pm13.5$ kJ/mol) and high preferential dissociation of C-S bonds concentration of species D is much higher compared to the others.

4. Conclusion

Unirradiated MPT exhibited no ESR signal, but gamma irradiated MPT showed a non resolved ESR spectrum consisting of many resonance peaks. A model based on the presence of four radical species designed as A, B, C and D of different spectroscopic and decay features was found to describe well the experimental results derived in the present work. Radical species taking part in the formation of experimental spectrum appeared to be relatively stable at room temperature but highly unstable at high temperatures. The decays of eleven assigned peaks over a storage period of two months at room temperature indicated that radical species responsible from multi-peaks ESR spectrum of irradiated MPT could be detected even after a storage period of 60 days and that ESR spectroscopy could be used for discrimination of irradiated MPT from unirradiated one.

The decays of the assigned peak heights above 350 K were relatively fast. From the decays of the studied peak heights at high temperatures, it was concluded that involved radical species followed first order kinetic at these temperatures and that the decay activation energies of the species were relatively high. The species D dominated experimental spectrum with its highest relative concentration (Table 4) and highest activation energy (Table 5). Four radical species produced after dissociation of C-N and C-C bonds adjacent to the C=O group of beta-lactam ring and one of the C-S bonds connecting five membra rings were proposed to be as the tentative species responsible from the formation of experimental ESR spectrum of gamma irradiated MPT. On the basis of high stabilities of the involved radical species at room temperature and relatively high concentration of species D, it was concluded that MPT presents the characteristics of a normal and accidental dosimetric material and that it could be used for this purpose.

Acknowledgement

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Captions for tables

Table 1. ESR spectrometer operating conditions adopted throughout the experiment.

Table 2. Decay constants calculated for radical species responsible from room temperature experimental spectrum.

Table 3. Decay constants calculated for contributing radical species from high temperature annealing studies.

Table 4. Spectroscopic parameters and percent concentrations obtained from spectrum simulation calculations.

Table 5. Activation energies calculated for contributing radical species.

Table 6. Mathematical functions used and parameter values calculated from curve fitting. Figures in the brackets are the correlation coefficients.

Table 1

Central field	330 mT
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Speed width	20 mT
Microwave frequency	9.25 GHz
Microwave power	1 mW
Modulation frequency	100 kHz
Modulation amplitude	0.1 mT
Receiver gain	5×10^2 - 5×10^3
Scan time	240 s
Time constants	1 s

Table 2

Radical	Decay constant
species	$k \times 10^5$ (hour ⁻¹)
A	0.26
B	503.00
C	369.00
D	0.00

Table 3

Radical	Temperature	Decay constant
species	(K)	$k \times 10^4$ (dak ⁻¹)
A	360	424
	370	1297
	380	2300
B	360	605
	370	1073
	380	1577
C	360	100
	370	177
	380	350
D	360	13
	370	69
	380	200

Table 4

Radical species	Percent concentrations	g factors	Hyperfine splittings (mT)	Linewidths (mT)
A	0.37	$g_{\perp}=2.0001$	$A(H_1)=1.917$	0.246
		$g_{\parallel}=2.0015$	$A(H_2)=0.990$	
			$A(H_3)=0.485$	
B	20.34	2.0035	$A(N)=1.491$	0.541
			$A(H_1)=1.346$	
			$A(H_2)=1.341$	
C	34.48	2.0047	$A(H_1)=1.734$	0.405
			$A(H_2)=1.190$	
			$A(H_3)=0.899$	
D	44.81	2.0031	$A(H_1)=0.828$	0.322
			$A(H_2)=0.785$	
			$A(H_3)=0.484$	

Table 5

Radical species	Activation energies (kJ/mol)
A	94,3 ± 13,8
B	53,2 ± 4,0
C	69,2 ± 6,2
D	152,0 ± 13,5

Table 6

Functions	Resonance peaks				
		1	2	4	5
I=k+lD	k	119,963	5,506	39,407	93,222
	ℓ	56,942 (0,999)	205,234 (0,987)	174,481 (0,989)	208,944 (0,989)
I=m+nD+pD ²	m	94,845	443,154	421,659	521,207
	n	64,301	77,021	62,497	83,562
	p	-0,404 (0,999)	7,050 (0,988)	6,158 (0,993)	6,895 (0,991)
I=rD ^g	r	116,681	187,067	172,692	228,865
	g	0,779 (0,999)	1,038 (0,975)	1,012 (0,977)	0,980 (0,976)
I=s(1-e ^{-dD})	s	1794,43	59858,000	212562,000	32476,640
	d	0,06 (0,995)	0,003 (0,973)	0,001 (0,974)	0,007 (0,974)
I=I _A (1-e ^{-d_AD}) + I _B (1-e ^{-d_BD})	I _A	1196,100	26551,970	14346,580	16622,840
	d _A	0,025	0,004	0,006	0,007
	I _B	905,56	26622,310	14346,210	16619,730
	d _B	0,068 (0,995)	0,004 (0,972)	0,006 (0,975)	0,007 (0,974)

Captions for figures

Fig.1. Molecular structure of MPT

Fig.2. Room temperature ESR spectrum of MPT sample irradiated at 15 kGy. Resonance peaks assigned with numbers were used to follow the evolution of the spectrum under different conditions.

Fig.3. Variations of the measured peak heights with applied microwave power for a sample irradiated at a dose of 15 kGy. [! (1); 6 (2); ζ (3); ; (4); # (5); $\perp$ (6); $\square$ (7); % (8); ξ (9); π (10); Σ (11)]

Fig.4. Variations of the heights of some important peaks (1, 2, 3, 4 and 5) with temperature for a sample irradiated at a dose of 15 kGy. Cooling [! (1); $\square$ (2); Ω (3); ξ (4); $\bullet$ (5)]; heating; [% (1); $\square$ (2); Ψ (3); ζ (4); $\circ$ (5)].

Fig.5. Decays of the peak heights at normal conditions with time elapsed after irradiation cessation. Symbols (experimental) [! (1); 6 (2); Δ (3); 9 (4); N (5); α (10); $\square$ (11); continuous line (theoretical)]

Fig.6. Variations of the heights of peak 4 with annealing time and annealing temperature. Symbols (experimental) $\oplus$ (360 K); $\blacksquare$ (370 K); 9 (380 K) continuous lines (theoretical)]

Fig.7. Experimental and calculated spectra. Continuous line (experimental), dashed lines (theoretical). Top dashed: total; bottom dashed contributing species: 1-) A; 2-) B; 3-) C; 4-) D

Fig.8. Variations of peak-heights with applied radiation dose. Symbols (experimental): ! (1); 6 (2); 9 (4); N (5). Continuous lines (theoretical obtained from $I=k + I*D$ linear function)

Fig.1.

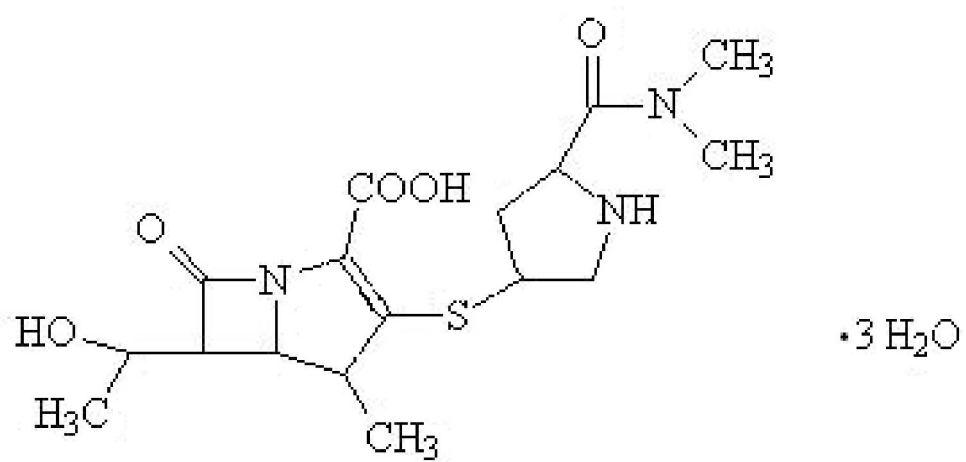


Fig.2.

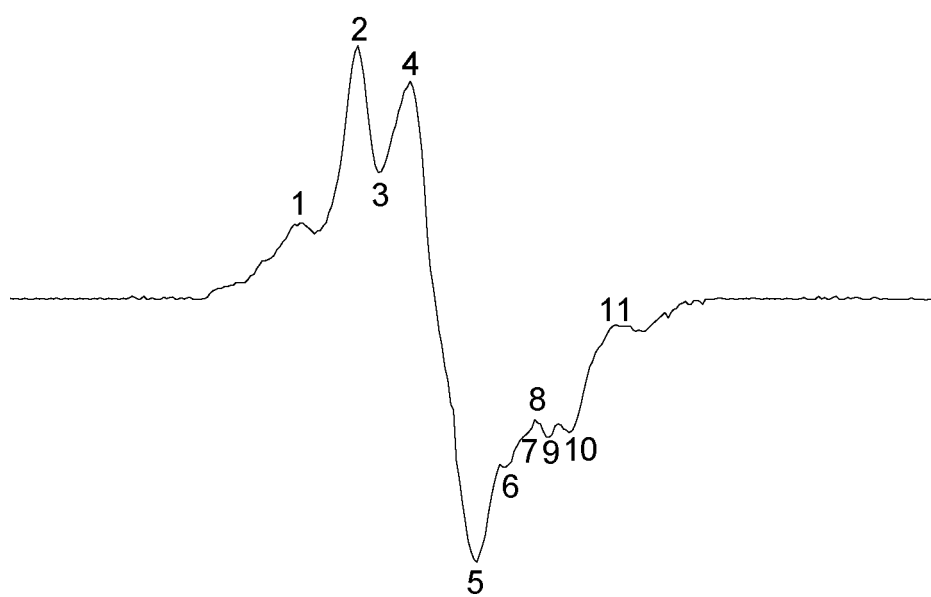


Fig.3.

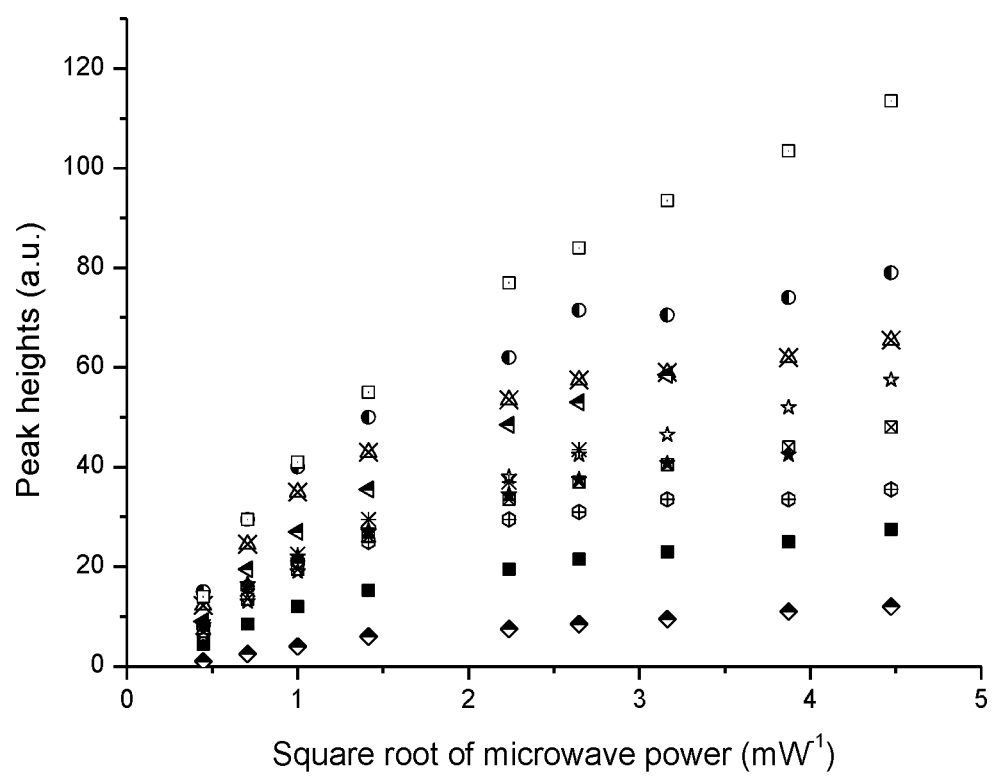


Fig.4.

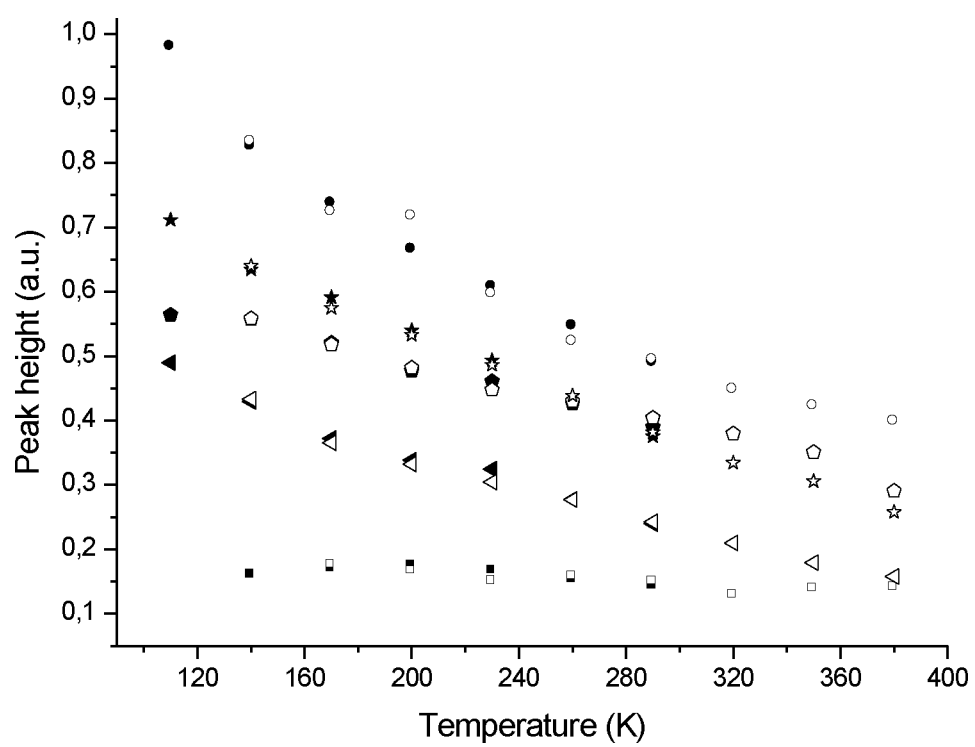


Fig.5.

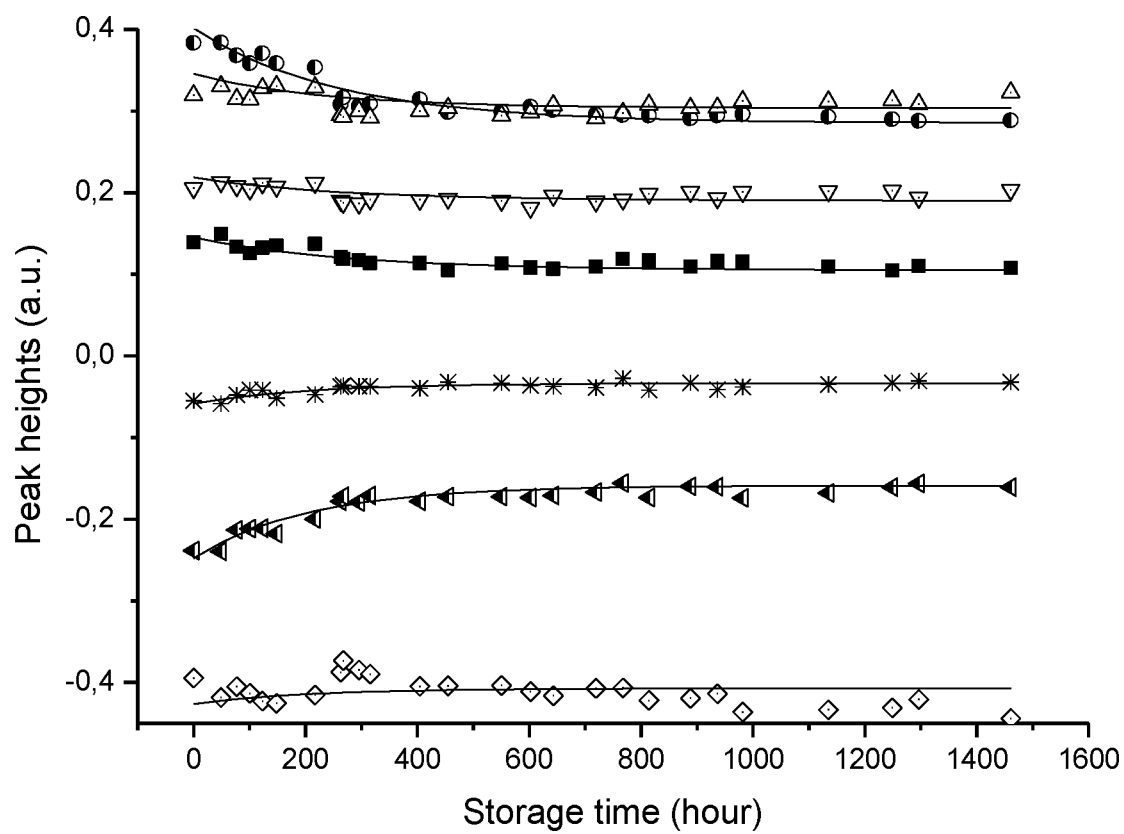


Fig.6

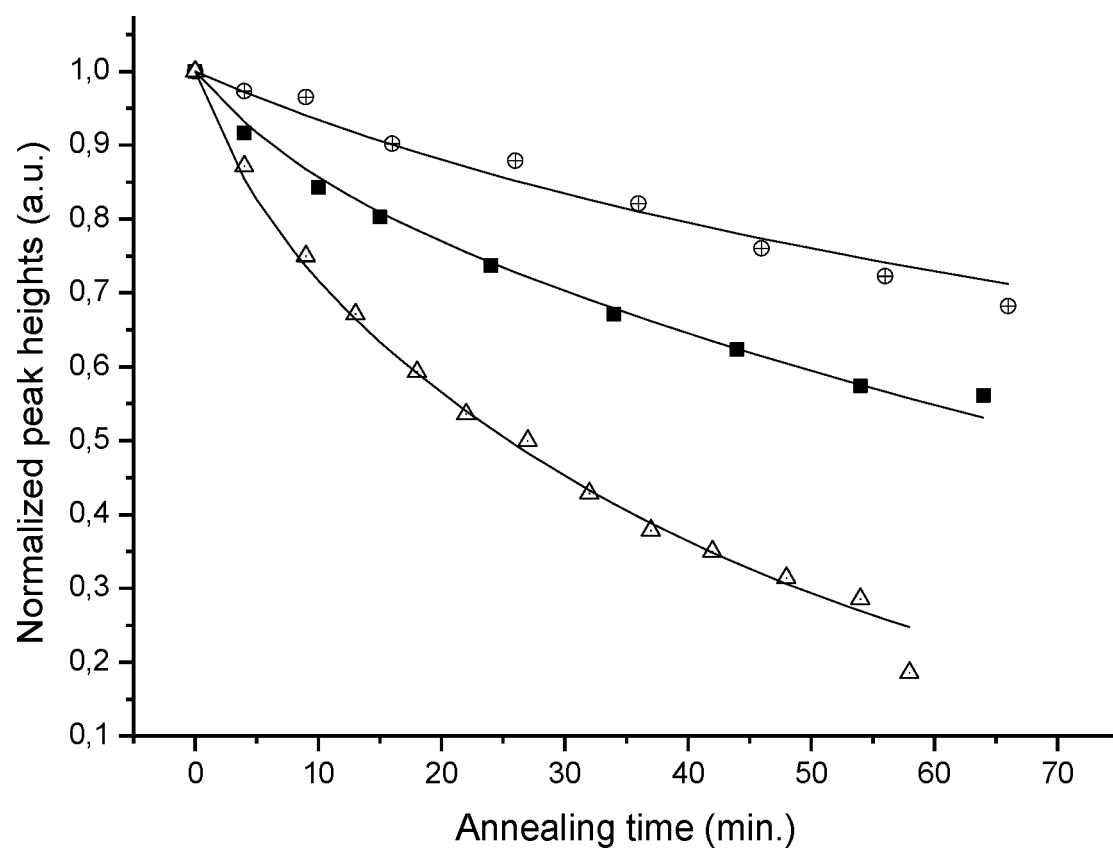


Fig.7

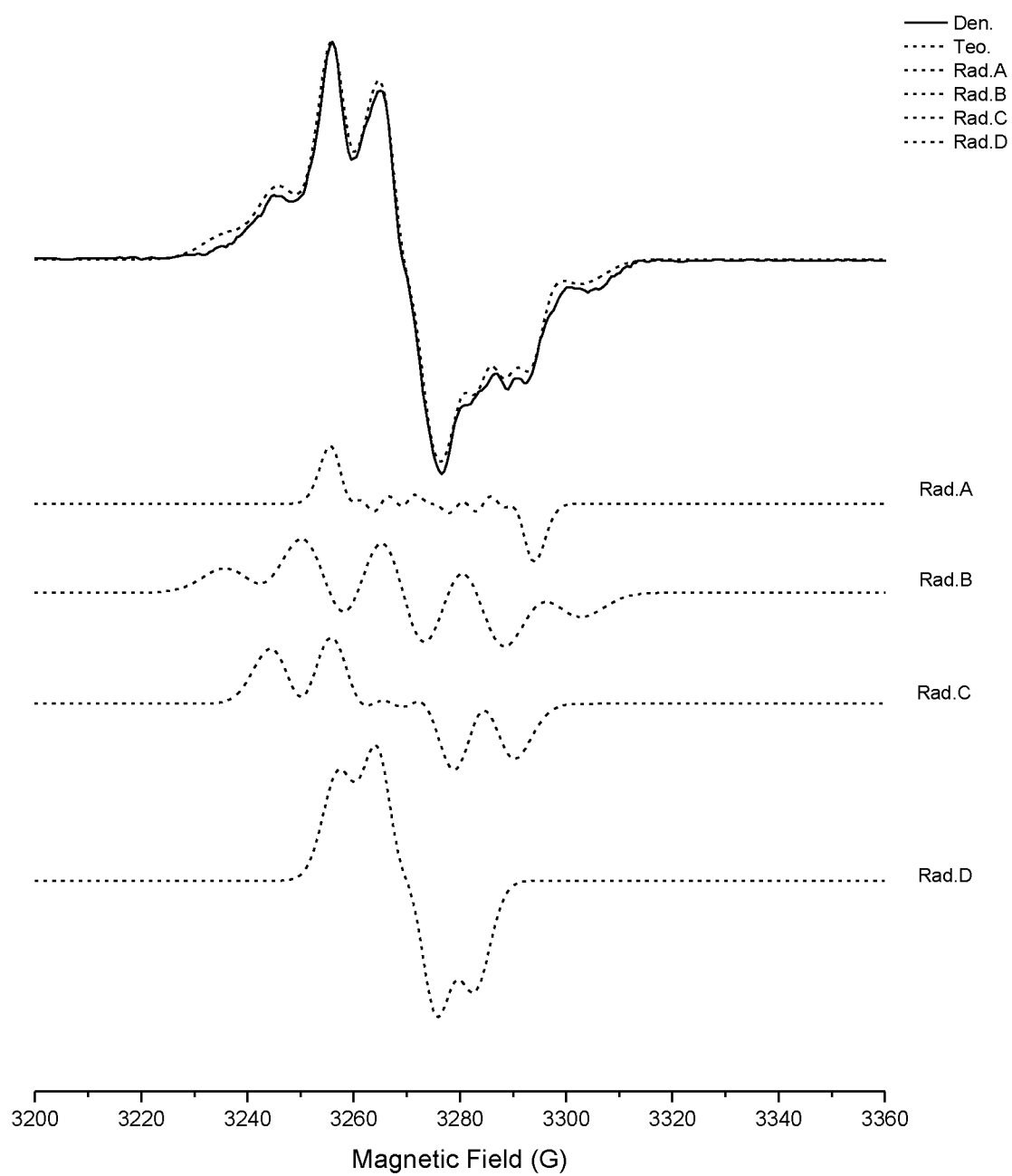


Fig.8

