Journal of Thermal Analysis and Calorimetry, Vol. 68 (2002) 423–436

ANALYTICAL STUDY OF β-IRRADIATED ANTIBIOTICS IN THE SOLID STATE

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Abstract

This paper reports results of the study on the influence of β-irradiation on the physical and chemical properties of selected salts of β-lactam antibiotics in solid state (sodium salt: ampicillin, azlocillin, benzylpenicillin, carbenicillin and piperacillin; potassium salt of benzylpenicillin, ampicillin anhydricum, ampicillin trihydricum, amoxicillin trihydricum and bacampicillin hydrochloride). The source of irradiation was a linear accelerator of electrons, and the irradiation effects were checked on the basis of the following: determination of mass, melting point and water contrent, and spectrophotometric (UV, IR) chromatographic and thermal (DTG, DSC) studies.

Keywords: DSC, DTG, ionizing radiation, IR, penicillins, UV

Introduction

β-lactam antibiotics are very sensitive to the influence of external factors and quickly lose their bactericidal properties, especially when in water solutions. Therefore, they are usually used in medical therapy in the form of tablets, pills, capsules, ointments and dry injections dissolved *ex tempore*. Unfortunately, also in solid state these antibiotics undergo gradual decomposition especially under the effect of elevated temperature and moisture [1, 2]. Therefore, it is difficult to ensure microbiological purity of pharmaceutical preparations containing thermolabile compounds as classical methods of sterilisation cannot be applied. An alternative method is radiation sterilisation recently of increasing importance. Unfortunately, irradiation with γ, β or X-rays, even at small doses (10–25 kGy), can lead to changes in the structure and properties of the compounds [3–7].

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1418–2874/2002/ $ 5.00

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

Therefore, safe and successful application of this method of sterilisation of therapeutic substances requires comprehensive studies by diverse analytical methods. The paper reports results of preliminary studies of the effect of β-irradiation on physical and chemical properties of salts of selected β-lactam antibiotics, performed with the use of spectrophotometric, chromatographic and thermal methods. The substances studied were: sodium and potassium salts of bezylpenicilline and sodium salts of its α-amine derivatives and hydrochloride of its α-carboxy derivative. The irradiation with β-rays was applied at a dose 100 kGy, i.e. a dose four times higher than that admissible by the European Standards EN 552 for radiation sterilisation of medical substances, so that the destructive changes could be easier and faster detected. The results will be used for assessment of quality of the above-mentioned penicillins irradiated with smaller doses (10–25 kGy) and radiochemical stability of selected β-lactam antibiotics.

Experimental

Material for the study

The substances studied included sodium salts of benzylpenicillin (PEN-Na, *Benzylpenicillin Natric*), ampicillin (AMP-Na, *Ampicillin Natric*), azlocillin (AZLO-Na, *Azlocillin Natric*), piperacillin (PIPERA-Na, *Piperacillin Natric*) and carbenicillin (CARB-Na, *Carbenicillin Natric*), potassium salt of benzylpenicillin (PEN-K, *Benzylpenicillin Kalicum*) and hydrochloride of bacampicillin (BAKAMP, *Bacampicillin hydrochloride*), and ampicillin (AMP, *Ampicillin anhydrous*), and trihydrate ampicillin (AMP-H_2O, *Ampicillin trihydrate*) and amoxicillin (AMOX-H_2O, *Amoxicillin trihydrate*) provided by the Sigma-Aldrich Company Ltd.

Methodology

Exposure to β-irradiation

Carefully weighted portions of 0.3 g of the substances studied were placed in colourless glass vials of about 3 mL in capacity and closed with plastic corks. The samples in the vials were exposed to β-irradiation in a linear accelerator of electrons LAE 13/9 (electron beam 9.96 MeV, beam current 6.2 μA) till they absorbed a dose of 100 kGy.

Organoleptic analysis

Prior to and after the irradiation the compounds were subjected to organoleptic analysis, comparing their colour (against a white background), form, odour and clarity of solution (0.005 g of the substance was dissolved in 5 mL of a properly chosen solvent).

Water content determination using a Karl Fischer reagent

Water content was determined in the substance studied before and after the irradiation. The procedure was as follows: carefully weighted portions of 0.03 g of the penicillins studied were dissolved in anhydrous methanol and water was titrated by using Karl Fischer reagent in a titrator Mettler Toledo DL 38 Karl Fischer Titrator (Switzerland).

Iodometric method [1, 2, 8]

By the iodometric method the content of penicillin was determined in the studied preparation. For that purpose about $1\cdot10^{-8}$ FW dissolved in distilled water in a 100.0 mL measuring flask. For the determination 4.5 mL volumes were taken and handled as described previously [8].

UV spectrophotometry (UV method)

Carefully weighted portions of 0.003 g of substances PEN-Na, AZLO-Na, PIPERA-Na, CARB-Na, PEN-K, AMP, AMP-H_2O and AMOX-H_2O before and after irradiation were dissolved in 100 mL of water. The solutions of substances AMP-Na and BAKAMP were prepared by dissolving portions of 0.005 g in 10 mL of water. The absorption spectra were taken for 1 cm layers by a spectrometer Specord UV-VIS.

IR spectrophotometry (IR method)

IR study was performed for non-irradiated and irradiated samples weighted in 1 mg portions of the penicillins studied and for 200 mg of dry potassium bromide. IR spectra performed in the range 500 to 4 000 cm^{-1} against the reference sample, using apparatus FT-IR (Brüker).

Thin Layer Chromatography (TLC method)

In the TLC method aliquots of 5 μL~1% water solutions of the substances studied before and after irradiation were point wise deposited on a foil covered with silica gel film 60 F_{254} of 0.2 mm in thickness (Merck, Darmstadt, Germany). The process was conducted by the ascending technique to a height of about 12 cm in chambers previously saturated with mobile phase vapours [9].

Thermal methods

DSC

Measurements by differential scanning calorimetry (DSC) were performed using a system DSC-204 (Netzsch). The samples of about 3 mg of penicillins were sealed in aluminium cells with pierced lid. The measurement were performed in helium atmosphere in temperatures from 20 to 300°C at the scanning rate of 5°C min^{-1}. The results were processed using the program TAA (Netzsch). The peak area was evaluated using tangential-sigmoidal baseline correction.

TG/DTG

TG/DTG measurements were conducted with an instrument Setsys TG-DSC 15 (Setaram) on penicillins samples in portions of about 15 mg, in the air atmosphere.

The temperature range of the measurement was 20–300°C, the scanning rate 5°C min^{-1} and sensitivity TG 0.04 mg.

Results and discussion

Assessment of the appearance of the penicillins studied

No significant changes in the form of the compounds studied were noted after irradiation, although changes in colour were notable. Compounds PEN-Na, PIPERA-Na, CARB-Na, PEN-K, AMP, AMP-H_2O and AMOX-H_2O were white before irradiation and became light grey. Compounds AMP-Na and AZLO-Na became light-yellow in colour. The greatest changes were observed for compound CARB-Na white before irradiation and yellow-green after it, compound BACAMP became yellow (Table 1).

Compounds PEN-Na, PEN-K and BACAMP were characterised by a specific odour even before irradiation and this smell did not change. Compounds AMP-Na, AZLO-Na, PIPERA-Na and CARB-Na, odourless before irradiation, revealed a distinct odour after it (Table 1).

The observations of clarity of the solutions studied did not reveal changes before and after irradiation.

As a result of irradiation the mass of compounds CARB-Na, PEN-K and BACAMP increased (3.79%), (0.23%) and (0.27%), the mass of compounds PEN-Na, AZLO-Na and PIPERA-Na decreased (–0.10%), (–0.30%) and (–1.20%), and the mass of compound AMP-Na remained the same (Table 2).

All the initial preparations contained a certain amount of water, which was the highest for compounds AMP-H_2O (12.07%), AMOX-H_2O (11.91%), PIPERA-Na (9.96%), AZLO-Na (8.59%) and CARB-Na (6.05%), indicating that these compounds crystallised with the a water molecule. The other penicillins contained relatively low amount of water: PEN-Na (0.50%), AMP-Na (0.91%), PEN-K (0.11%) and BACAMP (0.65%). As a result of irradiation the amount of water insignificantly increased in the samples studied (0.01–0.85%) except compound CARB-Na in which it increased by 3.74% (Table 2).

As follows from the above, no significant changes in water content or mass of the compounds studied were noted as a result of β-irradiation. The only exception was CARB-Na for which, after β-irradiation, a considerable mass increase (3.79%) correlating with a similar increase in the water content (3.74%) were observed.

Chromatographic analysis

The substance studied before and after β-irradiation were subjected to TLC analysis on silica gel and with a mixture of octane 2-butyl–glacial acid (1.05 kg L^{-1})–buffer phosphoric solution (pH 6)–2-butanol–methanol [150:75:45:27:10 (*v/v/v/v/v*)] [9], as mobile phases. The chromatograms taken before and after β-irradiation revealed no differences. The spots recorded were well-developed and well-visible, and no significant changes in the values of R_f were noted (Fig. 1).

Table 1 The changes in form, odour, colour and substances content of the compound studied as a result of β-irradiation (100 kGy)

Compound	Form	Odour		Colour		Content*/%	
		Starting	100 β	Starting	100 β	UV method	Iodometric method
Benzylpenicillin Na	Crystalline powder before and after irradiation	specific	no changes	white	light grey	96.36	97.2
Ampicillin Na		no	specific	white	light yellow	166.67**	98.6
Azlocillin Na		no	specific	white	light yellow	96.15	91.4
Piperacillin Na		no	specific	white	light grey	94.85	93.9
Carbenicillin Na		no	specific	white	yellow-green	97.89	93.6
Benzylpenicillin K		specific	no changes	white	light grey	95.45	96.9
Bacampicillin HCl		specific	no changes	white	yellow	125.00**	94.6
Ampicillin anhydrous		no	specific	white	light grey	140.91**	98.7
Ampicillin trihydrate		no	specific	white	light grey	131.58**	98.9
Amoxicillin trihydrate		no	specific	white	light grey	101.61	96.1

*The starting is assumed to be 100%
**λ_{max}=265 nm

Table 2 The changes in mass and water content of the compound studied as a result of β-irradiation (100 kGy)

Compound	Form	Mass/g			Water/%		
		Starting	100 β	Δ*m*/%	Starting	100 β	Δ*C*/%
Benzylpenicillin Na	Crystalline powder before and after irradiation	0.3002	0.2999	–0.10	0.50	0.51	+0.01
Ampicillin Na		0.3004	0.3004	0.00	0.91	1.11	+0.20
Azlocillin Na		0.3005	0.2996	–0.30	8.59	9.44	+0.85
Piperacillin Na		0.3010	0.2974	–1.20	9.96	10.01	+0.05
Carbenicillin Na		0.3006	0.3120	+3.79	6.05	9.79	+3.74
Benzylpenicillin K		0.3004	0.3011	+0.23	0.11	0.72	+0.61
Bacampicillin HCl		0.3001	0.3009	+0.27	0.65	1.33	+0.68
Ampicillin anhydrous		0.3002	0.3005	+0.10	0.24	1.16	+0.92
Ampicillin trihydrate		0.3000	0.3003	+0.12	12.07	13.20	+1.13
Amoxicillin trihydrate		0.3004	0.2995	–0.30	11.91	12.10	+0.19

*The starting is assumed to be 100%
**λ_{max}=265 nm

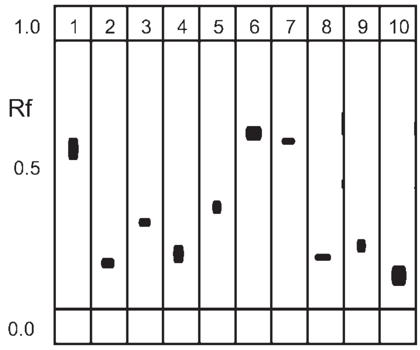

Fig. 1 The scheme of thin-layer chromatograms of the solutions of substance after β-irradiation (100 kGy): Solid phases: Silica gel 60 F_{254}; Mobile phases: octane 2-butyl–glacial acid (1.05 kg L^{-1})–buffer phosphate solution pH 6– 2-butanol–methanol [150:75:45:27:10 (*v/v/v/v/v*)]. 1 – benzylpenicillin sodium salt, 2 – ampicillin sodium salt, 3 – azlocillin sodium salt, 4 – piperacillin sodium salt, 5 – carbenicillin disodium salt, 6 – benzylpenicillin potassium salt, 7 – bacampicillin hydrochloride, 8 – ampicillin anhydrous, 9 – ampicillin trihydrate and 10 – amoxicillin trihydrate

Iodometric method

Iodometric method of the penicillin studied before and after irradiation minimal differences in amount of penicillin were observed AZLO-Na (91.4%), CARB-Na (93.6%), PIPERA-Na (93.9%), BACAMP (94.6%), AMOX-H_2O (96.1%), PEN-K (96.9%), PEN-Na (97.2%), AMP-Na (98.6%), AMP (98.7%), AMP-H_2O (98.9%) (Table 1).

Spectrophotometric analysis

The character of UV spectra of the penicillins studied before and after irradiation did not change, although some changes in absorption intensity were noted. The intensity of absorption increased for compound AMP-Na (67% at 258 nm, 88% at 265 nm and 45% at 270 nm), AMP (41% at 258 nm, 44% at 265 nm, 46% at 270 nm), AMP-H_2O (32% at 258 nm, 30% at 256 nm, 39% at 270 nm) (Fig. 2) and for compound BAKAMP (25% at 259 nm, 24% at 264 nm and 35% at 269 nm). For the other penicillins absorption decreased at λ_{max} by 2.11% (CARB-Na) to –5.15% (PIPERA-Na) (Table 1).

Most probably the reason for such a significant increase of absorption at the above mentioned wavelength were changes in the chromophore systems in the molecules of the compounds studied.

Similarly, no significant changes were observed in the IR spectra of compounds PEN-Na, PIPERA-Na, AMOX-H_2O and PEN-K taken before and after irradiation. However, notable changes appeared in the IR spectra of compounds AMP-Na, AMP, AMP-H_2O and CARB-Na in the shape, position and disappearance of some bands. In the spectrum of compound AMP-Na, AMP, AMP-H_2O (Fig. 3) the changes appeared in the range 2500–3600 cm^{-1} and the absorption band assigned to CO carboxylic group was shifted from 1588 to 1602 cm^{-1}. In the spectrum of compound CARB-Na the band corresponding to the amide group I ($\nu_{c=o}$) before irradiation at 1671 cm^{-1} disappeared, the absorption band of a carboxylic group ($\nu_{as\ c=o}$) was shifted from 1586 to 1603 cm^{-1} and the shape of the absorption band changed in the range 3300–3600 cm^{-1}. In the spectrum of compound AZLO-Na the band assigned to the amide group II (ν_{NH}) was shifted from 1525 to 1538 cm^{-1} and the band originally at 3237 cm^{-1} (ν_{NH}) was shifted by 60 to 3297 cm^{-1}.

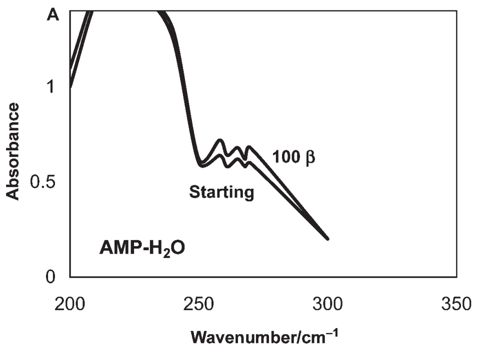

Fig. 2 UV spectra for ampicillin trihydrate

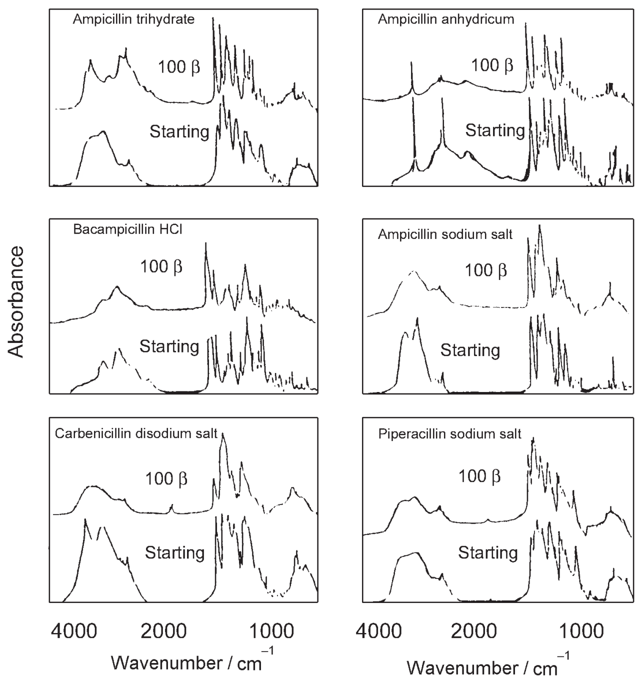

Fig. 3 IR spectra of the studied antibiotics

In the spectrum of compound BACAMP only the shape of the absorption band assigned to the carbonyl group in the β-lactam ring changed (1765 cm^{-1}) (Table 3).

Table 3 The changes in the IR spectra after exposure to β-radiation

Compound	Wavenumber/cm^{-1}		
	4000–2000	2000–1300	1300–500
Benzylpenicillin Na	–	1775 → 1620 ≈ 1614 ↑	–
Ampicillin Na	3440 ↓ 3330 ↓ 3300 ↑	1766 ≈ 1602 ↑ 1403 →	1262 ↓ 892 → 762 → ≈
Azlocillin Na	3298 ↑ 3238 ↓	–	–
Piperacillin Na	3307 → ≈	1720 ≈ 1612 →	–
Carbenicillin Na	3550 ↓ 3350 ↓ 3407 ↑	1672 ↓ 1603 ↑ 1382 → ≈	–
Benzylpenicillin K	–	1602 → ≈	–
Bacampicillin HCl	3205 →	1764 → ≈ 1742 ↓	–
Ampicillin anhydrous	–	1775 ≈ 1510 ↓ 1314 ↓	–
Ampicillin trihydrate	3510 ↑ 3447 ↑ 3311 ↓ 3207 ↑ 3040 ↑	1713 ↓ 1575 ↑ 1396 → ≈ 1368 → ≈ 1327 ≈	1258 → ≈ 1187 ↓ 1170 ↑ 730 ↓ 590 ↑ 534 ↓
Amoxicillin trihydrate	3565 → ≈ 3464 ↑ 3170 ↑ 3040 ↑	1780 → ≈ 1586 → ≈	–

↓ disappearance of a band, → shift in the position of a band,
↑ appearance of a new band, ≈ changes in the shape of a band

Thermal analysis

Results of DSC measurements have shown that after irradiation the melting point of the preparations studied decreased by a few degrees.

Since ampicillin and its derivatives are the most representative of the whole group of penicillins studied, the results of DSC, TG and DTG measurements are given only for these compounds. Typical DSC curves recorded for ampicillin and its derivatives are shown in Fig. 4.

For ampicilin (Fig. 4a) the difference in the melting points before and after irradiation was 4.0°C , while the difference in enthalpy only 0.65 J g^{-1} (Table 4). For sodium salt of ampicillin (Fig. 4b) the analogous difference in the melting points was smaller of 2.2°C, however, the difference in the thermal effects was much higher –5.98 J g^{-1} (Table 4), which means that the enthalpy of melting of the irradiated substance decreased by 8.94% relative to that of the enthalpy of non-irradiated substance.

Table 4 The changes in enthalpy and melting point of the substances studied as a result of β-irradiation (100 kGy)

Compound	Melting point T_{max}/°C			ΔH/J g^{-1}		$\frac{\Delta H_s - \Delta H_{100\beta}}{\Delta H_s}$
	Starting	100 β	ΔT	Starting	100 β	%
Benzylpenicillin Na	225.3	222.4	-2.9	–16.45	–13.15	20.06
Ampicillin Na	223.2	221.1	-2.1	–66.87	–60.89	8.94
Azlocillin Na	218.1	214.5	-3.6	–60.12	–52.50	12.67
Piperacillin Na	184.6	183.4	-1.2	–40.62	–33.17	18.34
Carbenicillin Na	195.1	194.1	-1.0	–24.91	–16.19	35.01
Benzylpenicillin K	243.5	239.6	-3.9	–6.46	–4.71	27.09
Bacampicillin HCl	132.2	130.0	-2.2	–33.20	–27.16	18.19
Ampicillin anhydrous	211.5	207.5	-4.0	33.85	33.20	1.92
Ampicillin trihydrate	199.6	197.6	-2.0	94.33	92.10	2.36
Amoxicillin trihydrate	190.1	189.1	-1.0	39.19	37.53	4.24

The DSC curve recorded for AMP-H_2O well revealed the occurrence of dehydration and irradiation induced changes. For the non-irradiated substance the temperature corresponding to the maximum of dehydration was 113.7°C, while for the irradiated sample 106°C. The same effect was observed for the melting process.

The melting point of pure non-irradiated AMP-H_2O (199.6°C) was higher than that for the irradiated substance (197.6°C) (Fig. 4c). Similar effects were indicated by TG and DTG results.

The TG curve recorded for AMP-H_2O (Fig. 5) indicates two processes corresponding to mass loss – the first by about 10% is assigned to dehydration process and the second – to the melting and decomposition.

As established from the DSC curve, the maximum of the process of dehydration corresponds to the temperatures 121.0 and 115.1°C (Table 4) for non-irradiated and irradiated AMP-H_2O. The temperatures corresponding to the maxima of the dehydration process are 199.5 and 197.4°C for non-irradiated and irradiated samples, respectively, are almost the same as obtained from DSC data.

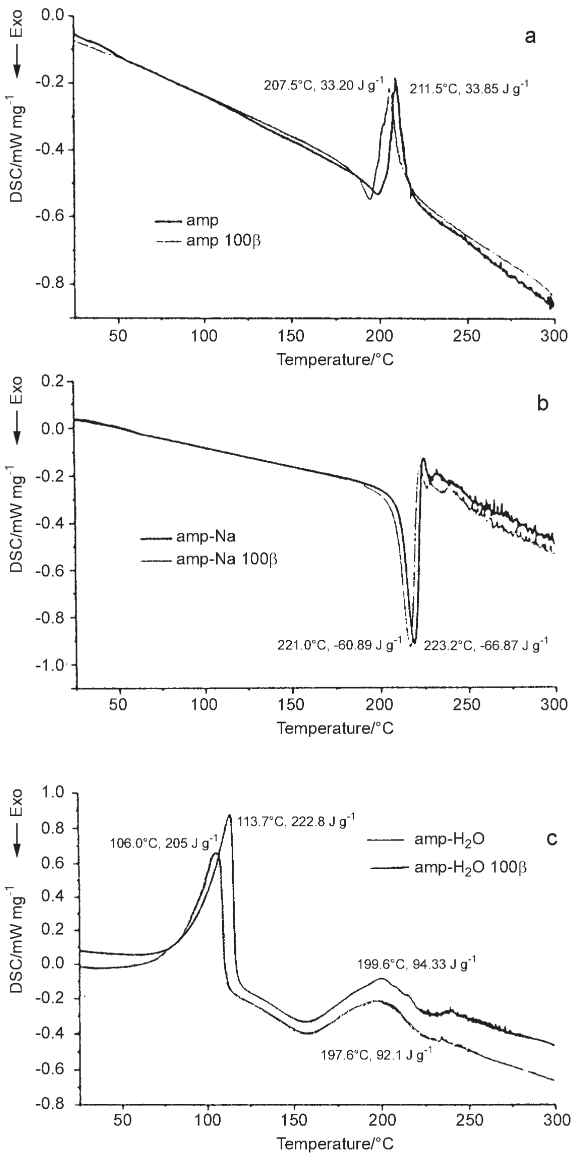

Fig. 4 DSC curves for ampicillin, sodium salt of ampicillin and ampicillin trihydrate

All the effects observed including a decrease in the melting point and the values of the enthalpies of melting and dehydration processes of penicillins after irradiation can be interpreted as a result of the appearing defects of the crystal lattice.

It is known that the energy of ionizing radiation is high enough to cause a break of covalent bonds, so also to disturb weaker interactions stabilising the crystal lattice.

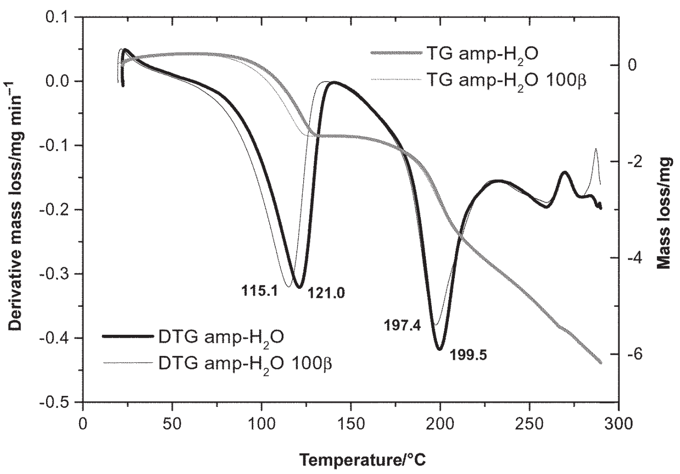

Fig. 5 TG and DTG curves for ampicillin trihydrate

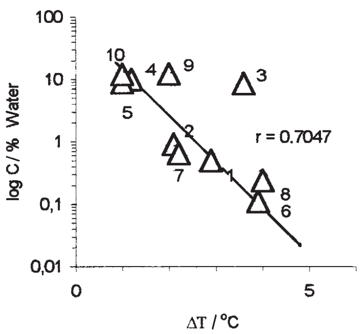

Fig. 6 The difference in melting points of the preparations studied *vs*. the water content in these preparations. 1 – benzylpenicillin sodium salt, 2 – ampicillin sodium salt, 3 – azlocillin sodium salt, 4 – piperacillin sodium salt, 5 – carbenicillin disodium salt, 6 – benzylpenicillin potassium salt, 7 – bacampicillin hydrochloride, 8 – ampicillin anhydrous, 9 – ampicillin trihydrate and 10 – amoxicillin trihydrate

This effect is particularly well evidenced by a decrease of the temperature of dehydration of hydrated penicillins.

Water content

A relatively large content of water (0.1–12%) in the initial compounds studied and an increase in this content after irradiation (0.05–3.74%), Table 2, prompted an attempt at finding a relationship between the content of water and the melting point value. As a result of this attempt, a relationship has been found between the content of water and the difference in the melting points before and after the irradiation. The relationship can be expressed as a linear function $\lg c_{H_2O}=f(\Delta T)$, characterised by the correlation coefficient r=0.7047 (Fig. 6). In general, the results of the studies performed have confirmed the earlier literature data indicating relatively great resistance of penicillins [3, 10] and cefalosporins [5] to the effect of irradiation.

In doses smaller than 25 kGy, the irradiation does not cause detectable qualitative and quantitative changes in the majority of β-lactam antibiotics, while after irradiation in doses 50–200 kGy the products of radiolysis [3, 5] and a decrease of the therapeutic substance content [10] have been detected.

The results of our study have shown that a dose of 100 kGy of β-irradiation leads to a decrease in the content of the therapeutic substances (from 11.1 to 8.6%) and in the melting points (from 1.0 to 4.0°C) observed for all compounds studied and for some also a decrease in mass (from 0.10 to 1.2%). This decrease is small and (can be comparable) comparable with the error of the method, but it can also indicate the formation of volatile products of decomposition CO, CO_2 and H_2 , whose appearance has been suggested earlier [3].

On the other hand, for all compounds studied an increase in the content of water has been noted (by 0.01–3.74%), and for some compounds also an increase in mass (by 0.10–3.79%), which could suggest that one of the products of decomposition is water.

Conclusions

The results of the study on the effect of the β-irradiation with a dose of 100 kGy on the physical and chemical properties of penicillins in solid state, have provided evidence for significant destructive changes detectable by the organoleptic methods, titration, in spectrophotometric (UV and IR) and thermal (TG, DTG, DSC) studies.

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This work was supported by the state Committee for Scientific Research (KBN) in Poland, Project no. 4PO5F 007 15

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