J Therm Anal Calorim (2010) 102:261–267
DOI 10.1007/s10973-009-0445-1

DSC and EPR analysis of some radiation sterilized alkaloids

B. Marciniec · M. Kozak · M. Naskrent · M. Hofman · K. Dettlaff · M. Stawny

Received: 7 July 2009 / Accepted: 25 August 2009 / Published online: 12 September 2009

Abstract The effect of ionising radiation on the physico-chemical properties of salts of three alkaloids has been studied: codeine phosphate (COD), papaverine hydrochloride (PAP) and pilocarpine hydrochloride (PIL). These compounds in the solid state were irradiated with an e-beam of the energy of 9.96 MeV to achieve doses ranging from 25 to 400 kGy, and then they were subjected to organoleptic analysis, thermal analysis (differential scanning calorimetry, DSC), electron resonance (EPR) spectroscopy, scanning electron microscopy observations and X-ray diffraction study. The most informative were the results provided by the EPR and DSC methods. The EPR spectra revealed the presence of long-lived radicals whose concentration was directly proportional to the dose of irradiation for all the compounds studied. (PIL 2.14×10^{16} spin/g, COD 6.85×10^{15} spin/g, PAP 2.50×10^{14} spin/g—for the dose of 100 kGy). The DSC results revealed a decrease in the melting point by 5.9 °C for COD and by 0.8 °C for PIL after irradiation with 200 kGy, which is indicative of products of radiolysis, of which at least one is non-white, and changes the colour of the compounds. PAP, for which no decrease in the melting point and no colour change was observed and for which the concentration of free radicals was the lowest, was found to be most stable from among the compounds studied. It will probably be suitable for radiation sterilisation. The other two compounds COD and PIL show much lower radiochemical stability and should be subjected to more detailed examination to establish the mechanism of radiolysis and the possibility of radiation sterilisation. Our results have confirmed the earlier reports on high radiochemical stability of PAP, but do not confirm the resistance to ionising radiation of COD and PIL.

Keywords Codeine · Papaverine · Pilocarpine · DSC · EPR · XRD · SEM

B. Marciniec (✉) · K. Dettlaff · M. Stawny
Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Gruwaldzka 6, 60-780 Poznan, Poland
e-mail: bmarcin@amp.edu.pl

M. Kozak
Department of Macromolecular Physics, A. Mickiewicz University, Umultowska 85, 60-614 Poznan, Poland
e-mail: mkozak@amu.edu.pl

M. Naskrent
Department of Medical Physics, A. Mickiewicz University, Umultowska 85, 60-614 Poznan, Poland
e-mail: marekn@amu.edu.pl

M. Hofman
Department of Coordination Chemistry, A. Mickiewicz University, Gruwaldzka 6, 60-780 Poznan, Poland
e-mail: mhofman@amu.edu.pl

Introduction

Differential scanning calorimetry (DSC) is widely used in the examination of physico-chemical proprieties of drugs. The DSC curves provide information on the purity, poly- and pseudopolymorphism and polymerisation of the medical therapy substances [1–4] and permit estimation of interactions between their components (therapeutic substances, excipients, their solvates, salts, etc.) [5–8]. The information obtained from the DSC experiment allows us to draw conclusions regarding by bioavailability of drugs, their toxicity and the thermal or another type stability [9–14]. The DSC curves of the substances of low stability and undergoing degradation easily show a characteristic shift of the melting point towards the lower temperatures because degradation products act as contamination and decrease the

melting point of drugs. The other method applied, the electron resonance spectroscopy (EPR) provides information about the presence of free radicals, which are formed as a results of knocking an electron out of a molecule. EPR method is not required by the European Pharmacopoeia or national pharmacopoeias as a method of drug testing but it is a very useful way of assessing the effect of ionizing radiation of drugs [15–17].

The estimation of the number of free radicals appearing as a result of irradiation and their lifetime provide the information on the degree of degradation of the compound studied. Unfortunately, no norms have been established yet as to the content of free radicals in a unit of mass/volume of the drug, so the EPR results cannot be treated as indicative of whether a particular drug is suitable for sterilization by irradiation. However, the information on the number free radicals, their structure and lifetime can significantly facilitate identification of the mechanism of radiodegradation.

In this article, these two methods, EPR and DSC, as well as other methods recommended for investigation of solid state substances (not requiring phase transition) were applied for determination of the radiochemical stability of selected alkaloids: morphinane (codeine), isochinoline (papaverine) and imidazole (pilocarpine). The first and hitherto the only reports on the radiochemical stability of these alkaloids come from the 1970s [18, 19]. The authors of these articles on the basis of the UV and TLC results obtained for the above substances in solid phase have concluded that they are resistant to irradiation in doses up to 60 kGy.

The current study was undertaken to verify the literature results on radiostability of these alkaloids using a variety of instrumental methods such as scanning electron microscopy (SEM), X-ray diffraction (XRD), DSC and EPR. The DSC and EPR were found to be particularly useful in providing rapid and accurate information on the stability of these compounds.

Materials and methods

Materials

Characterisations of the investigated codeine phosphate COD (serial number 04-00995, producer: Pharma Cosmetic), papaverine hydrochloride PAP (serial number 055K0915, producer: Sigma–Aldrich) and pilocarpine hydrochloride PIL (serial number 04CJ04, producer: FarmImpex) are presented in Table 1. All investigated substances were in the form of white, loose powder and satisfied the pharmacopoeia requirements [20].

Methods

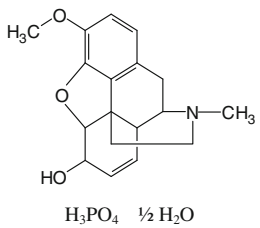
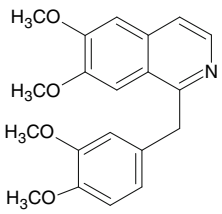
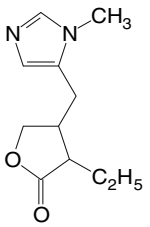
Exposure to irradiation

Approximately 1.0 g of COD, PAP and PIL was placed in a colourless glass vial of 5 mL in capacity and closed with a plastic stopper. The samples in the vials were exposed to irradiation in a linear electron accelerator LAE 13/9 (electron beam 9.96 MeV and current intensity 6.2 μA) till they had absorbed a dose of 25, 50, 100, 200 and 400 kGy.

Organoleptic analysis

Before and after irradiation compounds were subjected to organoleptic analysis comparing their colour against a white background, and observations of their form, odour,

Table 1 Chemical properties of investigated alkaloids [20–22]

	COD	PAP	PIL
Chemical name	7,8-Didehydro-4,5a-epoxy-3-methoxy-17-methylmorphinan-6a-ol phosphate hemihydrate	1-(3,4-Dimethoxybenzyl)-6,7-dimethoxyisoquinoline hydrochloride	(3S,4R)-3-Ethyl-4-[(1-methyl-1H-imidazol-5-yl)methyl]dihydrofuran-2(3H)-one hydrochloride
Chemical structure	 H_3PO_4 ½ H_2O	 HCl	 HCl
Molecular formula	$C_{18}H_{21}NO_3$, H_3PO_4, ½ H_2O	$C_{20}H_{21}NO_4$, HCl	$C_{11}H_{16}N_2O_2$, HCl
Molecular mass/g mol^{-1}	406.4	375.9	244.7
Melting point/°C	235	218–223	199–205

solubility and clarity of solution (0.005 g of the substance was dissolved in 5 mL of a properly chosen solvent) to those of the non-irradiated sample.

Scanning electron microscopy

SEM analysis was made using a SEM 515 (Philips) electron microscope with 14-mm working distance and 3–10-kV accelerating voltage.

X-ray diffraction

X-ray analysis was performed on a Philips powder diffractometer model PW 1070 controlled by an IBM PC unit, using CuK_{α} radiation (35 kV, 20 mA) and a nickel filter. The patterns were recorded for $5° < 2\theta < 50°$ for the gaps of 1°/1° at a counter step 0.02° s^{-1}. All XRD measurements were carried in an air atmosphere.

Electron paramagnetic resonance spectroscopy

The EPR experiments were carried out for non-irradiated, irradiated samples, in standard EPR quartz sample tubes from Wilmad. The measurements were performed with a Bruker EPR EMX-10 spectrometer working at 9.4 GHz (X-band) at room temperature (293 K). The sensitivity of the spectrometer is 1×10^{10} spins per gram. Induction of the magnetic field was measured to the accuracy of 0.001 mT. Microwave frequency was measured to the accuracy of 0.001 GHz. The spectra were double integrated over the magnetic field range 334–354 mT, which gives a figure proportional to the number of radicals in the sample.

Differential scanning calorimetry

The measurements were performed using a DSC-204 Netzsch instrument. The samples of about 10 mg were sealed in aluminium cells with pierced lids. The measurements were performed in helium atmosphere in temperatures from 20 to 300 °C at a scanning rate of 5 °C min^{-1}. The results were processed using TA (Netzsch) program. For the determination of the enthalpy values of the representative phase transitions, linear or tangent-sigmoidal baseline was used.

Results and discussion

According to the European Norm EN 552 [23], radiation sterilisation of medical therapy substances is performed with a dose of 25 kGy. The higher doses used in our study were intended to detect even the smallest changes that take place in a given drug upon irradiation. For reference, the initial non-irradiated drugs were subjected to the same examinations on the same equipment, in the same conditions and using the same reagents. Organoleptic analysis performed according to the requirements of Pharmacopoeia has shown that the ionizing radiation applied in the doses from 25 to 400 kGy does not cause changes in the form and smell. Only one compound, PAP, even when subjected to a dose of 400 kGy, remained a white, crystalline powder. The other two drugs, PIL and COD, showed some degree of discolouration when subjected even to the lowest dose of 25 kGy.

The COD colour changed from white to dirty-white, and that of PIL from white to pale yellow. As the irradiation dose increased, the colour changes intensified.

The results of the organoleptic analysis showed that these three compounds studied have different sensitivity to irradiation. The colour changes of PIL and COD can be related either to formation of the product of radiolysis that are coloured or be a result of damage to the crystal lattice [24, 25]. The SEM images (Fig. 1) proved that all the drugs studied were still in the form of fine crystalline state. The largest well-developed crystallites were observed for PIL, while the smallest for COD. The ionising irradiation in the doses applied did not change the microscopic image,

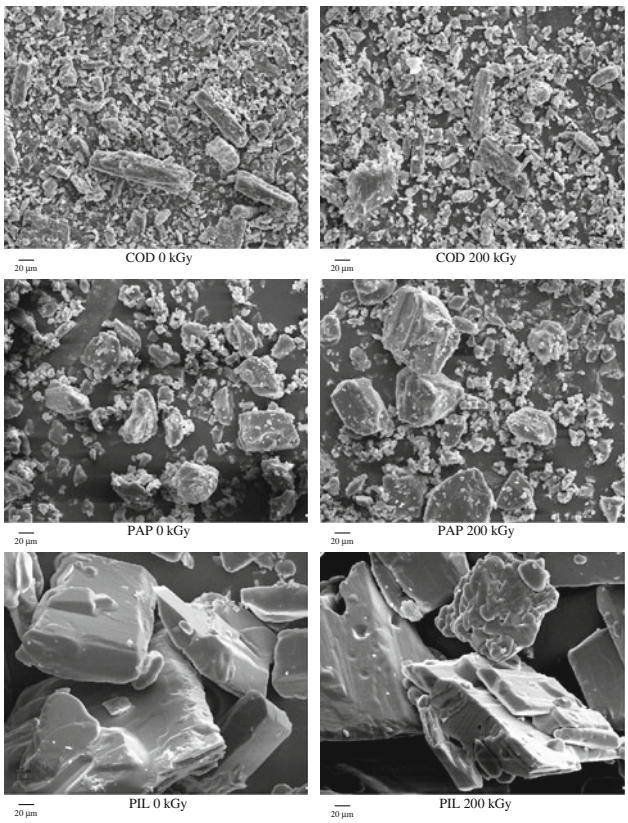


Fig. 1 SEM microphotogaphs of the alkaloids investigated before and after irradiation

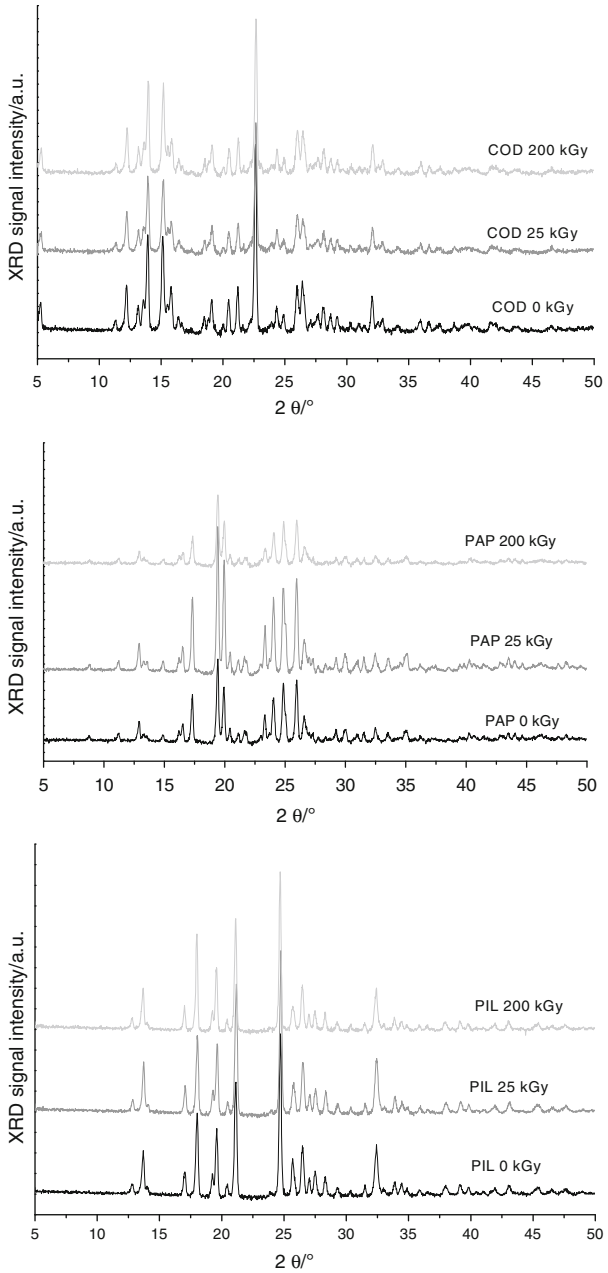


Fig. 2 XRD spectra of the alkaloids investigated before and after irradiation

neither in shape nor in the size of the crystallites. The XRD powder method did not provide any evidence of changes in the crystalline structure of the compounds studied as a result of irradiation, except for a decrease in the intensity of the peaks recorded for PAP at the highest irradiation dose (Fig. 2).

The EPR results evidenced the appearance of free radicals in the compounds studied even after irradiation with

Table 2 EPR results for irradiated alkaloids

Compound	Dose/kGy	EPR/spin $g^{-1} \times 10^{15}$	
		After 5 days	After 428 days
COD	0	0	0
	25	2.74	1.68
	50	4.33	2.60
	100	6.85	3.31
PAP	0	0	0
	25	0.15	0
	50	0.18	0
	100	0.25	0
PIL	0	0	0
	25	6.56	2.87
	50	9.89	7.54
	100	21.39	17.01

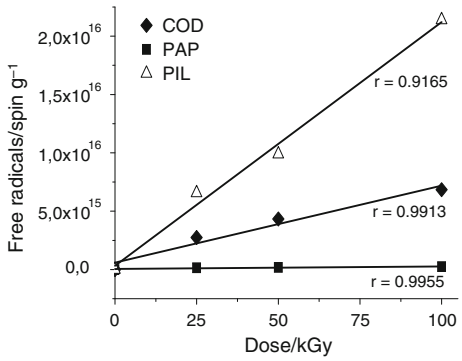


Fig. 4 Free radicals concentration versus irradiation dose

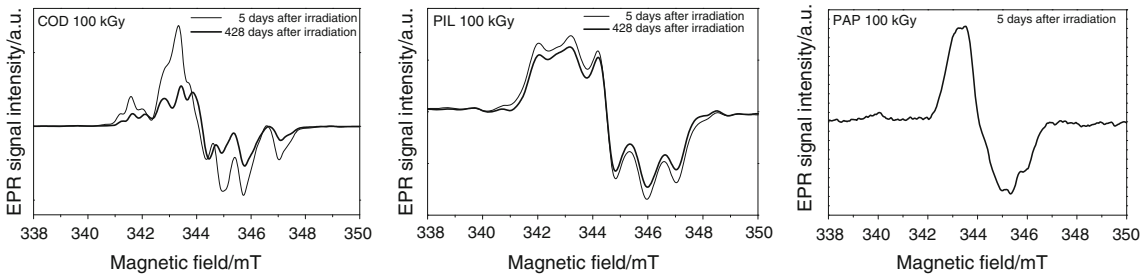


Fig. 3 EPR spectra of COD, PAP and PIL after irradiation (100 kGy)

the standard sterilisation dose of 25 kGy. The concentrations of the free radicals in PAP, COD and PIL were 0.15×10^{14} spin g^{-1}, 2.74×10^{14} spin g^{-1} and 6.56×10^{14} spin g^{-1}, respectively (Fig. 3, Table 2). As the dose increased, a proportional increase in the concentration of free radicals was observed (Fig. 4) and their lifetime for PIL and COD exceeded 428 days. In PAP, no free radicals were detected after 428 days (Fig. 5).

DSC curves revealed a single endothermic peak for PAP and PIL, appearing at the melting points of the compounds, ~226 °C for PAP and ~205 °C for PIL (Fig. 6). The DSC curve recorded for COD showed two endothermic peaks (Fig. 7) with the maxima at ~180 and ~235 °C. The first one was interpreted as related to the polymorphous change [26], while the other one (ca. 235 °C) as corresponding to the melting process. The shape of DSC curves for irradiated

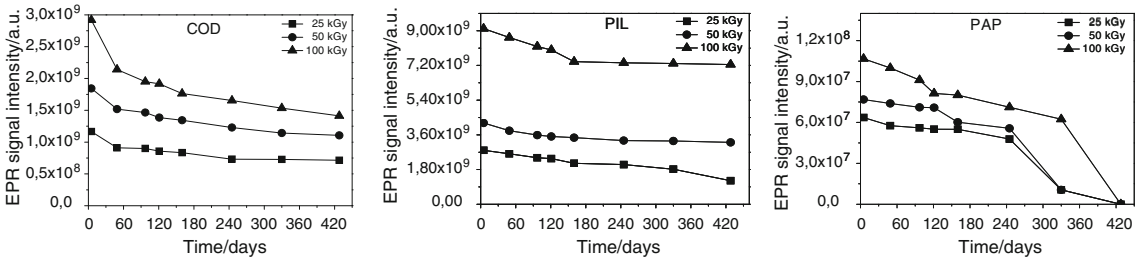


Fig. 5 The decay of EPR signal intensities for COD, PIL and PAP irradiated with a dose 25, 50 and 100 kGy stored at room temperature

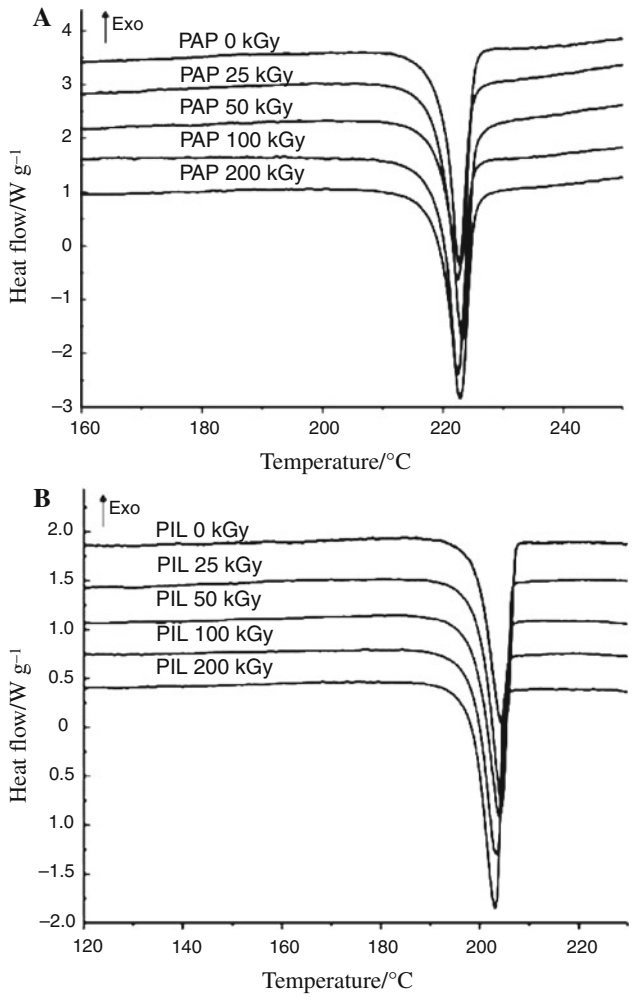


Fig. 6 DSC curves of PAP (**a**) and PIL (**b**) before and after irradiation

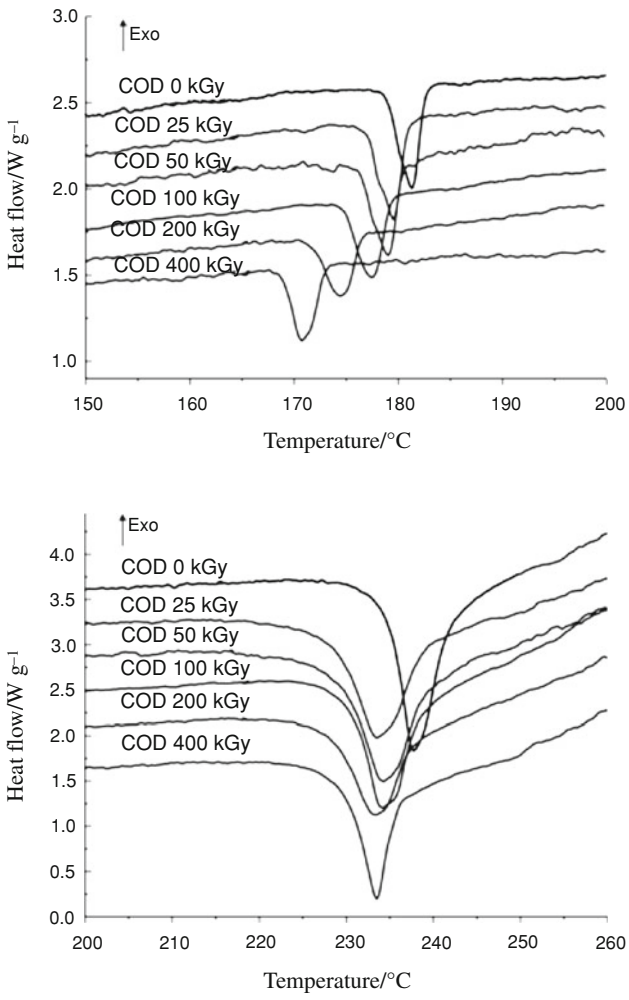


Fig. 7 DSC curve of COD before and after irradiation

Table 3 DSC results for irradiated alkaloids

	T_{onset}/ °C	ΔT_{onset}/ °C	T_{peak}/ °C	ΔT_{peak}/ °C	H/ J g^{-1}	ΔH/ J g^{-1}
COD first peak						
0 kGy	178.9	–	178.9	–	17.7	–
25 kGy	177.2	−1.7	177.2	−1.7	17.1	−0.6
50 kGy	176.5	−2.4	176.5	−2.4	16.1	−1.6
100 kGy	174.9	−4.0	174.9	−4.0	14.8	−2.9
200 kGy	172.0	−6.9	172.0	−6.9	13.4	−4.3
400 kGy	169.2	−9.7	169.2	−9.7	13.6	−4.1
COD second peak						
0 kGy	234.8	–	237.7	–	136.6	–
25 kGy	229.3	−5.5	233.8	−3.9	128.9	−7.7
50 kGy	229.4	−5.4	234.0	−3.7	128.8	−7.8
100 kGy	230.0	−4.8	234.1	−3.6	126.6	−10
200 kGy	228.7	−6.1	233.4	−4.3	111.7	−24.9
400 kGy	228.9	−5.9	233.3	−4.4	108.5	−28.1
PAP						
0 kGy	219.7	–	222.9	–	198.8	–
25 kGy	219.0	−0.7	222.4	−0.5	195.3	−3.5
50 kGy	220.4	+0.7	223.2	+0.3	195.4	−3.4
100 kGy	219.8	+0.1	222.6	−0.3	195.0	−3.8
200 kGy	219.5	−0.2	222.8	−0.1	198.2	−0.6
PIL						
0 kGy	199.3	–	204.7	–	−125.6	–
25 kGy	199.9	+0.6	204.4	−0.3	−124.2	−1.4
50 kGy	199.0	−0.3	204.0	−0.7	−122.1	−3.5
100 kGy	198.8	−0.5	203.4	−1.3	−120.2	−5.4
200 kGy	198.5	−0.8	203.0	−1.7	−116.9	−8.7

PAP and PIL remained the same as for unirradiated drugs, with just a small deviation from the original values of T_{peak}; for PAP in the range of +0.3 and +0.5 °C, for PIL between −0.3 and −1.7 °C (Table 3).

The DSC curves recorded for COD showed changes in the course of the first and the second peak, which meant that the polymorphous transition in irradiated COD took place at lower temperatures than in the nonirradiated COD (180 °C for the doses 25 and 50 kGy; 175 °C for 200 kGy), similarly as the phase transition solid → liquid.

The decrease in the melting point of the alkaloids studied was not proportional to the dose of irradiation (Fig. 8). Only the temperature of the polymorphous transition taking place in COD decreased in proportion to the dose applied ($r = 0.9731$). The greatest difference in T_{onset} ($\Delta = 5.9$ °C) for COD was observed after irradiation with 400 kGy. Changes in the DSC curves observed for PIL and COD suggest the presence of the products of radiolysis that can act as impurities and decrease the melting point relative to that of the initial compound [12–14].

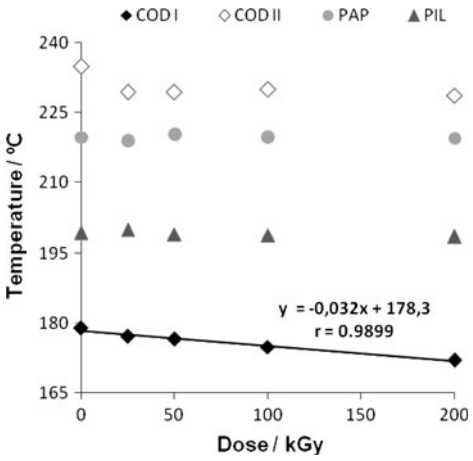


Fig. 8 T_{onset} versus irradiation dose

Conclusions

On the basis of the results presented above, we have concluded that the alkaloids studied show different sensitivity to ionising radiation. In all three of them, there were no changes in the crystalline form, as indicated by SEM and XRD methods. For two of them COD and PIL, a change in colour and the presence of free radicals was observed (2.74×10^{14} spin g^{-1} for COD and 6.56×10^{14} spin g^{-1} for PIL). For PAP no change in colour was noted and the lowest concentration of free radicals was detected (0.15×10^{14} spin/g). For PAP, no decrease in the melting point was apparent, but it was observed for COD and PIL, which was indirect indication of the presence of radiolysis products in the latter two compounds. It should be noted that the decrease in the melting point of PIL was much smaller than in that of COD, which can suggest a greater radiochemical stability of PIL. To sum up, the results obtained for PAP (low concentration of free radicals, no change in the melting point, no change in colour, form and crystalline lattice) indicate a high radiochemical stability of this compound. In contrast COD and PIL show low resistance to irradiation and they will have to be subjected to more detail investigation to discern if they could be sterilised by irradiation.

The results of the study have confirmed once again [12–14] the suitability of the DSC and EPR methods for preliminary evaluation of radiochemical stability of medical therapeutic substances in solid state.

Acknowledgements The authors gratefully acknowledge and thank Dr. Ewa Jaroszkiewicz for her contributions and assistance in manuscript editing.

References

1. Giron D. Thermal analysis and calorimetric methods in the characterisation of polymorphs and solvates. Thermochim Acta. 1995;248:1–59.
2. Bartolomei M. Solid-state studies on the hemihydrate and the anhydrous forms of flunisolide. J Pharm Biomed Anal. 2000;24:81–93.
3. Rustichelli C, Gamberini G, Ferioli V, Gamberini MC, Ficarra R, Tommasini S. Solid-state of polymorphic drug: carbamazepine. J Pharm Biomed Anal. 2000;23:41–54.
4. Karabas I, Orkoula MG, Kontoyannis CG. Analysis and stability of polymorphs in tablets: the case of risperidone. Talanta. 2007;71:382–6.
5. Fernandes NS, Carvalho Filho MAS, Mendes RA, Ionashiro M. Thermal decomposition of some chemiotherapic substances. J Braz Chem Soc. 1999;10:459–62.
6. Verma RK, Garg S. Selection of excipients for extended release formulations of glipizide through drug–excipient compatibility testing. J Pharm Biomed Anal. 2005;38:633–44.
7. Ceschel GC, Badiello R, Ronchi C, Maffei P. Degradation of components in drug formulations: a comparison between HPLC and DSC methods. J Pharm Biomed Anal. 2003;32:1067–72.
8. Mura P, Maestrelli F, Cirri M, Furlanetto S, Pinzauti S. Differential scanning calorimetry as an analytical tool in the study of drug-cyclodextrin interactions. J Therm Anal Calorim. 2003;73:635–46.
9. de Souza NAB, Medeiros ACD, Santos AFO, Macêdo RO. Thermal stability of metronidazole drug and tablets. J Therm Anal Calorim. 2003;72:535.
10. Cieśla K, Eliasso AC. Influence of gamma radiation on potato starch gelatinization studied by differential scanning calorimetry. Radiat Phys Chem. 2002;64:137–48.
11. Katusin-Razem B, Hamitouche K, Maltar-Strmecki N, Kos K, Pucic I, Britvic-Budicin S, et al. Radiation sterilization of ketoprofen. Radiat Phys Chem. 2005;73:111–6.
12. Marciniec B, Kozak M, Naskrent M, Dettlaff K, Ogrodowczyk M, Stawny M, et al. Thermal study of four irradiated imidazoline derivatives in solid state. J Therm Anal Calorim. 2007;88:337–42.
13. Marciniec B, Kozak M, Ogrodowczyk M. Thermal analysis in evaluation of the radiostability of some 1, 4-dihydropyridine derivatives. J Therm Anal Calorim. 2004;77:581–96.
14. Marciniec B, Stawny M, Kozak M, Naskrent N. The influence of radiation sterilization on thiamphenicol. Spectrochim Acta A. 2008;6:865–70.
15. Basly JP, Basly I, Bernard M. ESR spectroscopy applied to the study of pharmaceuticals radiosterilization: cefoperazone. J Pharm Biomed Anal. 1998;17:871–5.
16. Basly JP, Longy I, Bernard M. Radiation sterilization of formoterol. Pharm Res. 1997;14:810–4.
17. Colak S, Korkmaz M. Investigation of radiosterilization and dosimetric features of sulfacetamide sodium. J Pharm Biomed Anal. 2004;19:791–8.
18. Schulte KE, Henke G. Einwirkung von Gamma- und Neutronen-Strahlung auf Arzneistoffe [Effect of gamma and neutron radiation on drugs]. Arch Pharm. 1973;306:182–97.
19. Boess C, Bögl KW. Influence of radiation treatment on pharmaceuticals—a review: alkaloids, morphine derivatives, and antibiotics. Drug Dev Ind Pharm. 1996;22:495–529.
20. Pharmacopeia Polonica VI. Warszawa: PTFarm; 2002.
21. European Pharmacopoeia, 5th ed. Strasbourg: Council of Europe; 2005.
22. Pharma Cosmetics. Material Safety Data Sheet of Codeine Phosphate Hemihydrate http://www.pharma-cosmetic.com.pl/PDF/Codeini_phosphas.pdf. Accessed 15 Jan 2007.
23. EN 552. Sterilization of medical devices—validation and routine control of sterilization by irradiation. European Committee for Standardization, Rue de Strassart 36, B-1050, Belgium. CEN; 1994.
24. Varshney L, Dodke PB. Radiation effect studies on anticancer drugs, cyclophosphamide and doxorubicin for radiation sterilization. Radiat Phys Chem. 2004;71:1103–11.
25. Crucq AS, Deridder V, Tilquin B. Radiostability of pharmaceuticals under different irradiation conditions. Radiat Phys Chem. 2005;72:355–61.
26. Yang L, Yin H, Zhu W, Niu S. Determination of codeine phosphate and codeine in thermal characterization by differential scanning calorimetry. J Thermal Anal. 1995;45:207–10.