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EVALUATION OF RADIOSTABILITY OF SOME STEROID DERIVATIVES

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Abstract

The effect of ionising radiation (15–100 kGy) on the physico-chemical properties of 6 steroid derivatives in solid state (Hydrocortisone, Hydrocortisone acetate, Prednisolone, Prednisolone acetate, Dexamethasone and Fludrocortisone acetate) was studied by differential scanning calorimetry (DSC), differential thermal analysis (DTA), scanning electron microscopy (SEM), UV spectrophotometry, high-performance liquid chromatography (HPLC), X-ray powder diffraction and polarimetry. DSC and DTA results revealed that the irradiated compounds undergo phase transitions at lower temperatures, show lower melting points and lower enthalpy of the melting process their non-irradiated analogues. The results of HPLC measurements proved a loss in the active substance content after irradiation ranging from 0.5 to 2.88%. No significant effect of irradiation was detected by the UV spectrophotometry or polarimetry in the course of the UV spectrum, absorbancy or optical rotation, as well as in the SEM photographs and X-ray patterns. For some compounds studied a correlation was found between the irradiation dose and the shifts in DSC curves and the loss of content determined by the HPLC method. Similar, but not so much pronounced relations were established in or earlier studies of the derivatives of nitroimidazole and 1,4-dihydropyridine. In general the results have shown relatively high radiochemical stability of the compounds studied and have proved that the DSC method is a sensitive detector of irradiation-caused changes in drugs in solid phase.

Keywords: drugs analysis, DSC, DTA, HPLC, radiation sterilization, radiolysis in the solid state, SEM, UV, X-ray and polarimetric methods

Introduction

Steroid derivatives belong to one of the most numerous groups of drugs. In the EP [1] they are represented by 40 drugs and in the FPV [2] by 27, of which 15 are glycocorticosteroids. They are widely used in medical therapy thanks to many beneficial properties such as anti-inflammatory, anti-allergic, antiexudative and others. Recently sterilization of these compounds is increasingly often performed by ionising radiation (β, γ, *X*) whose large doses can lead to the loss of content and appearance of decomposition products [3, 4]. Therefore, each compound should be tested as to the possibility of its radiation

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sterilization, i.e. it should be checked whether this type of sterilisation does not produce changes in its physico-chemical properties or does not cause a significant loss of content, which affect the therapeutic effect of the drug [5, 6]. The methods applied in the studies range from the classical ones (organoleptic analysis, melting point, optical rotation determination) to chromatography (GC, HPLC, TLC), spectrophotometry (UV, IR, EPR), mass spectrometry (MS) and others. Recent literature also reports the use of thermal methods in analysis of drugs [7–16], pharmaceutical excipients [17–19] and steroids to [20–22]. This study was undertaken to check if the thermal methods such as differential thermal analysis (DTA) or differential scanning calorimetry (DSC) are suitable for testing the effect of sterilisation radiation of steroids and to compare the results obtained by these methods with those obtained by polarimetry, UV spectrophotometry and liquid chromatography.

The study was performed for six steroid derivatives (Hydrocortisone, Hydrocortisone acetate, Prednisolone, Prednisolone acetate, Dexamethasone and Fludrocortisone acetate) irradiated with a dose of 100 kGy, which is much greater than that used for sterilization purposes, to facilitate determination of the products of radiolysis, even those forming in trace amounts.

Experimental

Exposure to irradiation

Approximately 0.1 g of each substance was placed in colourless glass jar of 3 mL volume and closed with a plastic stopper. The samples in the vials were exposed to beta irradiation in a linear electron accelerator LAE 13/9 (electron beam 9.96 MeV and current intensity 6.2 μA) till they absorbed a dose of 100 kGy. Prednisolone acetate sample was also irradiated with the doses of 15 and 25 kGy.

Differential scanning calorimetry (DSC)

Measurements were performed on an apparatus DSC-204 made by Netzsch. Samples of 3 mg ±5% were closed in aluminium crucibles with pierced lid. Prior to measurements the samples were isothermally incubated at T=20°C for 5 min, and the measurements were performed at the heating rate of 5°C min^{-1} in the helium atmosphere. For each sample three independent measurements were performed and the results were averaged. The data were analysed by a computer program TA (Netzsch). For the determination of the enthalpy values characterising phase transitions, the linear or tangent-sigmoidal base line was used.

Differential thermal analysis (DTA)

DTA measurements were conducted, with an instrument Setsys TG-DSC 15 (Setaram) on selected steroid derivatives in portions of about 15 mg, in the air and helium atmosphere. The temperature range of the measurement was 20–300°C, the scanning rate 5°C min^{-1}.

X-ray diffractometry

The X-ray diffraction patterns in the range $2\theta = 2–60^{o}$ were obtained for powdered samples, using the CuK_{α} radiation and HZG-3 powder diffractometer, controlled by IBM PC unit.

Scanning electron microscopy (SEM)

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

Polarimetric method

The sample chirality was measured at 22°C on a Perkin Elmer 243 B polarimeter for the solutions of a standard, the initial compound and the compound after sterilization by irradiation. The solutions were prepared according to the relevant pharmaceutic monography [1, 2], using dioxane of analytical grade as a solvent.

High-performance liquid chromatography (HPLC)

HPLC analysis was performed according to the pharmacopoeia recommended method described in BP 99 [23] for dexamethasone, while for the other compounds the analysis was performed according to the requirements of USP 24 (conditions, standards etc.) [24]. The apparatus used was made by Waters and equipped with a photodiode array detector. The results obtained the method were charged with error ranging from 0.76 to 1.14% (SD).

UV spectrophotometry

The quantitative and qualitative UV spectrophotometric analysis was made on a spectrophotometer UV/VIS Perkin Elmer Lambda 20, using 1 cm thick quartz cells and the solvents recommended in relevant pharmaceutic monographs [23, 24]. If no specific solvent was given in the monograph, methanol of spectral purity was used as a solvent.

The initial solutions prepared in measuring flasks of 10 mL capacity, were dissolved using the solvent to obtain the solutions of the absorption value between 0.5–1.0 (at the absorption maximum, at the analytical wavelength).

The percent content of the compounds studied was determined by comparison with the standard. The final data are mean values for 3 independent portions of each compound from which 3 independent dilutions were prepared as measuring samples. The results were subjected to statistical analysis.

Results and discussion

DSC curves of the initial steroids before irradiation evidenced the occurrence of one phase transition corresponding to the melting process, often involving decomposition

of a given steroid. On the basis of the initial point of the transition (T_{onset}) the melting points of the steroids studied (non irradiated) were estimated and compared with literature values given in FP V [2] and EP [1] and the values obtained by the DTA method (Table 1). The observed small differences between the melting points (T_{onset}) reported in literature, obtained by DTA, Böetius apparatus and DSC. The observed small differences are insignificant and related to the differences in the measuring procedures e.g. in the heating rate, the sample atmosphere and inaccuracy of the visual discernment of the beginning of the melting process in the Böetius method.

Table 1 Melting points obtained by different methods

Compounds	Melting point/°C			
	References	Böetius apparatus	DSC method[3]	DTA method[3]
Prednisolone	~230[1]	227–232	238.9	234.0
Prednisolone acetate	~240[1] 230[2]	232–235	229.4	231.5
Hydrocortisone	~216[1]	213–217	223.3	219.7
Hydrocortisone acetate	~220[1]	220–222	222.1	221.0
Fludrocortisone acetate	~225[1]	225–227	229.4	227.1
Dexamethasone	~225[1] 255[2]	226–249	258.2	259.5

[1]Pharmacopoeia Polonica Editio V (FP V 1993)
[2]European Pharmacopoeia (E Ph 1997)
[3]evaluated from T_{onset}

DSC curves recorded for the steroids subjected to irradiation are shown in Figs 1–3, whereas the values of enthalpy and the temperature of the beginning of the phase transition (T_{onset}) and the maximum phase transition (T_{max}) are given in Tables 2 and 3. A comparison of DSC curves obtained for the irradiated and non-irradiated compounds revealed a general shift of the peaks corresponding to the melting points towards lower temperatures. The size of the shift varied from 1.7°C (fludrocortisone acetate) to 8.3°C (hydrocortisone). Moreover, the ranges of temperatures corresponding to the melting process were broadened, which was manifested by a significant shift of the temperature of the beginning of the phase transition (T_{onset}) and a considerable increase in the difference T_{max}–T_{onset}. The greatest difference between the values of (T_{onset}) for the irradiated and non-irradiated compounds was observed for hydrocortisone (ΔT_{onset}=9.6°C) and the smallest for fludrocortisone acetate (ΔT_{onset}=1.6ºC). The greatest change in the difference T_{max}–T_{onset} characterising the range of temperatures corresponding to the phase transition was observed for prednisolone (Table 3), while for fludrocortisone acetate no change in the difference T_{max}–T_{onset} was observed.

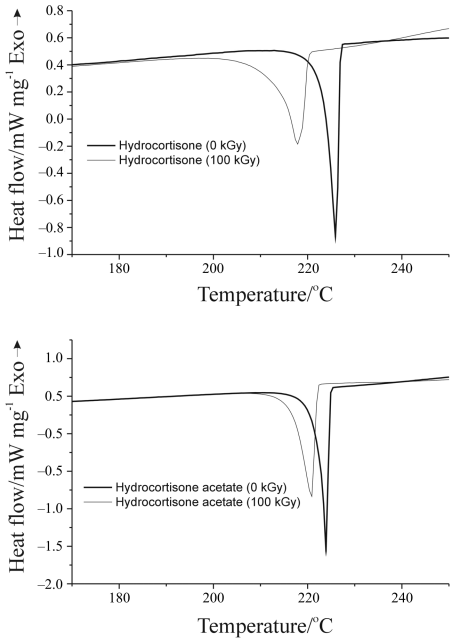

Fig. 1 DSC curves obtained for hydrocortisone and hydrocortisone acetate before and after irradiation

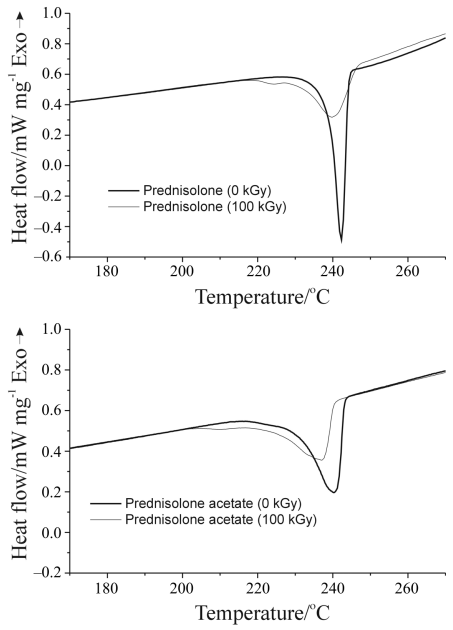

Fig. 2 DSC curves obtained for prednisolone and prednisolone acetate before and after irradiation

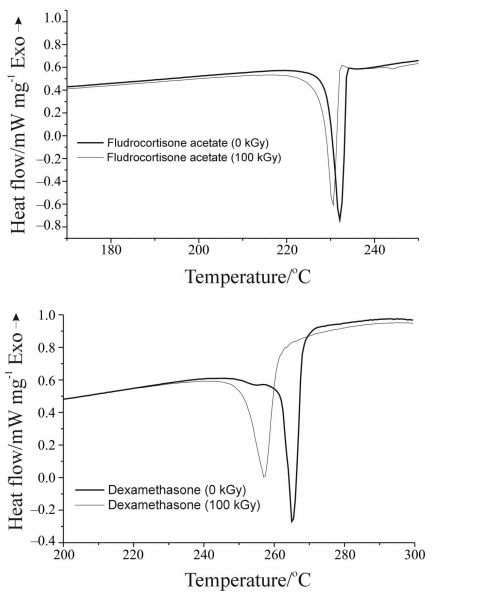

Fig. 3 DSC curves obtained for fludrocortisone acetate and dexamethasone acetate before and after irradiation

Another result of irradiation was a decrease in the energetic effect accompanying the phase transition. This decrease varied from 5.13% for fludrocortisone acetate to 13.79% for prednisolone. The values for all compounds studied are given in Table 2.

Table 2 Melting enthalpies of irradiated (ΔH_i) and reference (ΔH_s) samples evaluated from DSC data

Compounds	ΔH_s/J g^{-1}	ΔH_i/J g^{-1}	$[\Delta H_s - \Delta H_i]/\Delta H_s$/%
Prednisolone	–96.70	–83.36	–13.79
Prednisolone acetate	–101.58	–90.66	–10.75
Hydrocortisone	–91.99	–86.56	–5.91
Hydrocortisone acetate	–101.44	–95.20	–6.15
Fludrocortisone acetate	–92.20	–87.47	–5.13
Dexamethasone	–106.98	–94.34	–11.82

The results of the parallel study of the irradiated and non-irradiated steroids by the DTA method confirmed the results obtained by DSC. The DTA results also indicated a single-phase melting process, the temperature of which for the irradiated compounds was shifted towards lower temperatures. The results of DTA study are collected in Table 4 and illustrated in Fig. 4.

For prednisolone acetate additional DSC measurements were performed for the samples irradiated with lower doses (15 and 25 kGy), the results are also given in Fig. 5. For these two samples the character of DSC curve changes was similar as for

Table 3 Characteristic temperatures (in °C) evaluated from DSC data

Compounds	0 kGy			100 kGy			ΔT_{max} [1]	ΔT_{onset} [2]	$(T_{max}-T_{onset})^{100}/(T_{max}-T_{onset})^{0}$
	T_{max}	T_{onset}	$T_{max}-T_{onset}$	T_{max}	T_{onset}	$T_{max}-T_{onset}$			
Prednisolone	242.2	238.9	3.3	240.0	230.8	9.2	2.2	8.1	2.79
Prednisolone acetate	239.9	229.4	10.5	237.0	221.7	15.3	2.9	7.7	1.46
Hydrocortisone	226.0	223.3	2.7	217.7	213.7	4.1	8.3	9.6	1.52
Hydrocortisone acetate	224.0	222.1	1.9	220.9	216.7	4.2	3.1	5.4	2.21
Fludrocortisone acetate	232.1	229.4	2.7	230.3	227.8	2.7	1.7	1.6	1
Dexamethasone	263.1	258.2	4.9	256.5	251.0	5.5	6.6	7.2	1.12

[1] $\Delta T_{max}=T_{max}^{0kGy}-T_{max}^{100\,kGy}$

[2] $\Delta T_{onset}=T_{onset}^{0kGy}-T_{onset}^{100\,kGy}$

Table 4 Characteristic temperature evaluated from DTA data

Compounds	0 kGy T_{max}/°C	100 kGy T_{max}/°C	ΔT_{max}[1]/°C
Prednisolone	241.0	239.2	1.8
Prednisolone acetate	238.8	235.8	3.0
Hydrocortisone	223.3	216.3	7.0
Hydrocortisone acetate	222.1	220.3	1.8
Fludrocortisone acetate	231.2	230.6	0.6
Dexamethasone	263.5	260.5	3.0

$\Delta T_{max}=T_{max}^{0kGy}-T_{max}^{100kGy}$

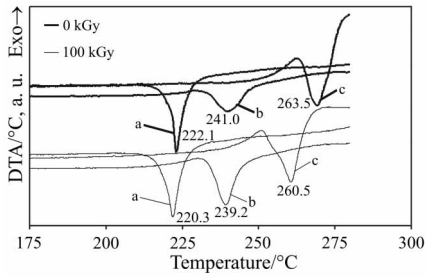

Fig. 4 DTA curves obtained for selected steroids; a – hydrocortisone acetate, b – prednisolone and c – dexamethasone, before and after irradiation

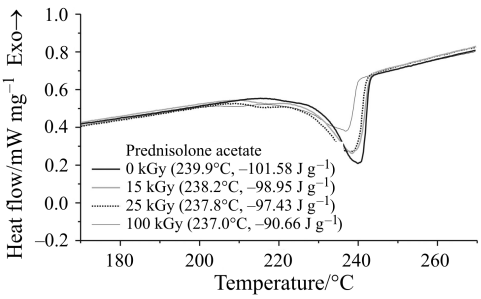

Fig. 5 DSC curves obtained for prednisolone acetate before and after irradiation with the different doses

the sample irradiated with 100 kGy , i.e. the melting point (T_{max}) and enthalpy corresponding to the phase transition decreased, but these changes were smaller and equal to 238.2 and 237.8ºC (T_{max}), and 98.95 and 97.43 J g^{-1} for the samples irradiated with doses 15 and 25 kGy, respectively.

Looking for a correlation between DSC parameters and the dose of irradiation, a linear relation, characterised by a high value of the correlation coefficient r=0.9896 was found between the enthalpy of the melting process and the irradiation dose for prednisolone acetate (Figs 6, 7). The effect of decreasing values of enthalpy charac-

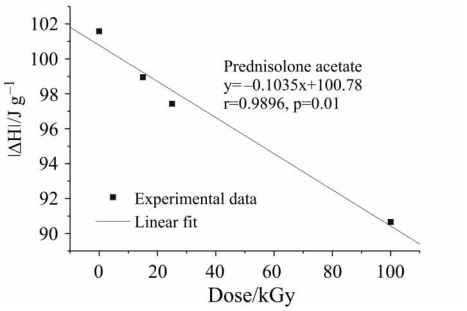

Fig. 6 Enthalpy of melting *vs*. the dose of irradiation for prednisolone acetate

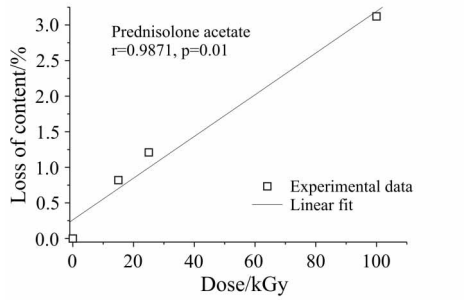

Fig. 7 The loss of content *vs*. the dose of irradiation for prednisolone acetate

terising phase transitions as a result of irradiation has already been signalled in literature [25]. For example, it has been observed for the phase transition from the micellar to the lamellar phase in the system lysophosphatidylocholine–water as a result of its gamma-irradiation with different doses [25]. Similar effect as a result of irradiation has already been observed for some antibiotics [10].

The significant changes in the temperatures of the melting processes and the energetic effects corresponding to them observed as a result of irradiation could suggest a decrease in the content of the active substances relative to the initial values, changes in the crystal structure and appearance of the products of their decomposition. The decomposition as a result of radiolysis could affect changes in the physico-chemical properties of the compounds studied. The possible occurrence of such changes was verified by the polarimetric and spectrophotometric methods [2].

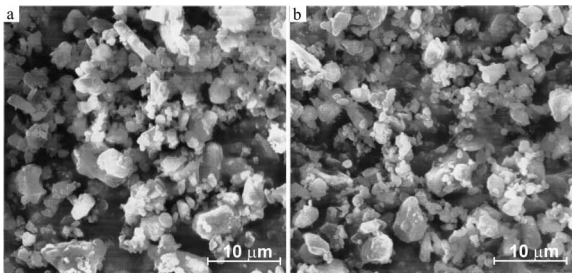

Fig. 8 SEM photographs of dexamethasone before a – and after b – irradiation

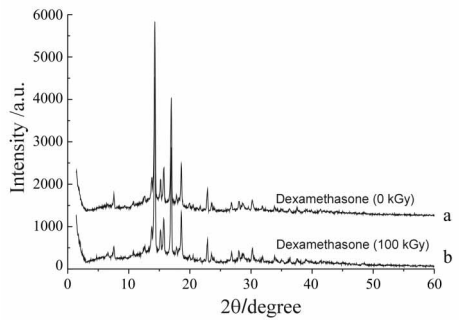

Fig. 9 X-ray powder diffraction patterns of dexamethasone before a – and after b – irradiation

The SEM photographs and X-ray diffraction patterns obtained for the compounds studied before and after irradiation, did not show significant differences (Figs 8, 9).

Hence, it is reasonable to conclude that the changes in the melting point and in enthalpy, measured by DSC and DTG methods, were not a consequence of polymorphous changes or an increase in the content of the amorphous phase.

The results of the polarimetric studies collected in Table 5 did not reveal significant differences in the chirality of the steroids studied before and after the irradiation. Only for hydrocortisone after the irradiation the chirality decreased by 6.58ºC, but it was still within the limits required by FP V, being from 150 to 156ºC for hydrocortisone.

Table 5 Results of steroids analysis by polarimetric method before and after irradiation (100 kGy β-rays)

Compounds	Optical specific rotation/$[\alpha]^0$			Difference
	Required acc. FP V	Obtained		$\Delta[\alpha_{20}]^{\circ}/\%$
		0 kGy	100 kGy	
Hydrocortisone	+150–156	+156.75	+150.17	–4.20
Hydrocortisone acetate	+157–168	+163.44	+164.00	+0.39
Prednisolone	+96–103	+96.46	+96.15	–0.31
Prednisolone acetate	+112–119	+114.59	+115.56	+0.85
Dexamethasone	+75–80	+77.89	+76.19	–1.31
Fludrocortisone acetate	+148–156	+150.46	+149.15	–0.87

The spectrophotometric analysis also gave little discerning information (Table 6). No changes in the course of the spectra were observed in the range 200–350 nm, the results revealed only small changes in the absorbancy at λ_{max}. The difference in the content of the active substance between the initial value and the value after the irradiation did not

exceed 1.72%, and for the majority of the compounds studied the difference was smaller than the error of the method (RSD 1.6–2.2%).

Table 6 Results of steroids determination by UV and HPLC methods

Compounds	Content/%			
	UV method		HPLC method	
	0 kGy	100 kGy	0 kGy	100 kGy
Hydrocortisone	100.89	102.17	99.14	98.25
Hydrocortisone acetate	102.56	103.41	100.63	99.04
Prednisolone	100.69	102.41	99.07	97.86
Prednisolone acetate	99.18	98.38	98.93	96.05
Dexamethasone	100.14	100.23	99.64	99.14
Fludrocortisone acetate	98.45	97.81	98.45	96.92

In these circumstances it should be supposed that the spectra of the radiolysis products are very similar to those of the compounds studied and the UV method detects the sum of the parent compound and the products of its radiolysis, which would explain the observed increase of the content observed in some cases, e.g. for hydrocortisone and prednisolone.

As follows from the above the true content of active substance in the irradiated steroids can be determined only after they are separated from the products of radiolysis. The most suitable for the purpose seemed the HPLC method [1, 23, 24]. The results collected in Table 6 and compared with those obtained by the UV method confirm the earlier suppositions about determination of a sum of the parent compounds and the products of their radiolysis by the UV technique. The content of the active substance in the irradiated samples of the steroids studied was always lower than that obtained by the UV method, however, the decrease did not exceed 2.88%.

A linear relationship was found between the loss of content as a result of irradiation and the irradiation dose, characterised by the correlation coefficient r=0.9871 (Fig. 7).

This value is similar to that established earlier for the relationship between the enthalpy and the irradiation dose (Fig. 6), which additionally confirms the suitability of DSC method for detection of changes in the physico-chemical properties in the steroids studied subjected to radiation sterilization.

Conclusions

The above discussed results have proved that the most suitable methods for investigation of the effect of ionising radiation on the steroids studied are DSC, DTA and HPLC, while the least informative proved the SEM, X-ray, UV spectrophotometry and the polarimetric method.

The reasons for the above are probably very small changes taking place under the effect of irradiation, undetectable by the methods applied (SEM, X-ray) or falling within the error limits of the methods (UV, polarimetric methods).

It could be explained by a similarity of the physico-chemical properties of the parent compounds and the products of their radiolysis [26, 27], which means that their absorption coefficients and chirality are similar and no differences in the content of the active substance are revealed by the UV and polarimetric methods. The results obtained by the HPLC method reveal differences in the content of the active substance in the samples before and after irradiation because this method enables a separation of the parent compounds from the products of their radiolysis.

In this context relatively great differences in the melting points observed by DSC method suggest that the thermal properties of the radiolysis products are considerably different than those of the parent compounds, which makes DSC method the most sensitive and reliable tool to study changes taking place in steroids subjected to ionising radiation.

On the other hand, the changes detected by the DSC method correspond to small differences indicated by the HPLC method, so small mass loss of the active substance, which means that the steroids studied are resistant to the effect of ionising radiation and are characterised by great radiochemical stability and thus can be successfully and safely subjected to sterilization by irradiation.

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