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THERMOGRAVIMETRIC ANALYSIS OF CALCIUM AND DISODIUM FOSFOMYCIN. ANALYTICAL APPLICATION FOR PURITY CONTROL

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ABSTRACT

Thermogravimetric analyses in oxygen, nitrogen and static air between 20 and 1000°C and DSC calorimetric analyses in static air between 20 and 500°C of the sodium and calcium salts of fosfomycin were performed. The main decomposition processes were examined and the thermogravimetric residues analysed by IR and X-ray powder spectra, and were found to be constituted by the respective pyrophosphates, which are thermally very stable. On these residues the evaluation of the purity of the initial compound was performed, based on the comparison between the theoretical and the experimental calcium and sodium percentages found in the final residue. Satisfying values of precision (SD $<1\%$) and accuracy ($\Delta <1\%$) were obtained. In the case of disodium fosfomycin, succinic acid had to be added, in order to obtain reliable results.

INTRODUCTION

Thermo-analysis is going to become a routine method for the analysis of drugs and substances of pharmacological interest [1]. We applied this technique to commercial products and the openings to be explored seem promising. We continued to employ DSC calorimetry [2,3] and thermogravimetry [4,5]. The information obtained generally concerned the thermal stability of these compounds, the characterization of the decomposition processes in different atmospheres (oxygen, nitrogen, air), the values of parameters such as melting temperature, decomposition temperature, heat of fusion, heat capacities; also we often obtained information about the purity and percent humidity of the compounds examined. In some cases the percent by weight of a certain compound in an examined commercial product was also determined, operating directly on the solid matter, without the need for a chemical dissolving attack or of a separating operation [3]. The most recently

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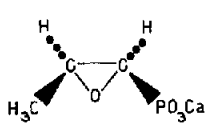
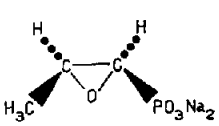
published research concerns the thermal analysis of the sodium and potassium salts of some antibiotics of significant therapeutical interest, such as penicillins and cephalosporins [4–6], and of compounds usefully employed against biliar cholesterol gallstones, such as cholic acids [7]. In these studies the chance of controlling the purity of these compounds on the basis of a comparison between the theoretical and the experimental sodium and potassium % concentration in residues of defined chemical composition, obtained at well-established temperatures, was investigated. In particular the different accuracy of these controls was found to depend on whether the residue comprised sodium or potassium sulphate or carbonate [7], due to the different thermal stability of these salts. Actually we are studying some organic compounds of pharmaceutical interest containing phosphorus atoms, such as the sodium and calcium salts of fosfomycin (see Table 1), a broad-spectrum antibiotic of large diffusion [8]. The thermogravimetric curves of these compounds were constructed and the analysis of the obtained residue was performed to allow the evaluation of the purity and of the percent by weight of one of these compounds in some commercial drugs.

MATERIALS AND METHODS

Fosfomycin calcium or disodium salt and a drug containing disodium fosfomycin with 8.33% (w/w) of succinic acid associated, were supplied by Crinos S.p.A. Succinic, glutaric, oxalic and malonic acids were from Merck, all analytical grade. The TG and DTG curves of the examined compounds were obtained with a DuPont model 951 thermobalance. The heating rate was 10°C min^{-1} and the furnace atmosphere was oxygen or nitrogen at a

TABLE 1

Compounds examined

Antibiotic salt	Structural formula	Empirical formula	Mol. wt.
Calcium fosfomycin		$C_3H_5CaO_4P$	176.12
Disodium fosfomycin		$C_3H_5Na_2O_4P$	182.02

flow rate of 100 ml min^{-1}, or static air. The DSC curves were obtained with a DuPont model 990 DSC cell and console, in static air, with a heating rate of 10°C min^{-1}. The IR spectra were obtained with a Perkin-Elmer 177 IR spectrophotometer and nujol as solvent. The X-ray powder spectra were recorded by an Isodebyeflex III A Seifert apparatus employing Cu$K\alpha$ radiation ($\lambda = 1.5405$ Å). The samples were analysed under vacuum, for 10 h, by the Debye–Scherrer method.

RESULTS

The TG and DTG curves in nitrogen, oxygen and static air, of calcium and disodium fosfomycin salts, were recorded and are reported in Figs. 1 and 2, respectively, while the corresponding TG data are summarized in Table 2. The behaviour is very different in the two cases, while the influence of the different atmospheres is not really marked. For calcium fosfomycin a loss of about one water molecule is observed at about 200°C (Fig. 1); the fact that this loss is very well defined and reproducible in the different atmospheres and that it occurs at a relatively high temperature induces one to think that the water is of crystallization nature, rather than due to the humidity of the compound. This is well confirmed by the marked endothermal transition which can be revealed by the DSC calorimetric curve reported below. The thermal decomposition of the calcium salt of the antibiotic proceeds slowly and gradually, with increasing temperature up to about 1000°C. In oxygen and static air two decomposition processes can be seen: the first very slowly begins at about 250°C and ends at 720–750°C; the full oxidative decomposition of the antibiotic is performed here, and a residue of carbon and calcium pyrophosphate is produced. The X-ray analysis of this residue has been shown that it constitutes the α form of calcium pyrophosphate; in Table 3 the experimental d_{hkl} values are compared with the standard values by ASTM [9]; a good agreement is generally observed. The second process occurring at higher temperatures (800–950°C) in oxygen or static air, is the substantial loss of carbon, obviously faster in oxygen than in static air. In nitrogen, on the contrary, carbon is only lost at temperatures higher than 950°C and this process is complete probably at > 1000°C. The residues, in oxygen and static air, appear (white coloured) after this second process, without any carbon; they completely constitute calcium pyrophosphate. Nevertheless, both the X-ray powder analysis (Table 3) and the IR spectrum of this residue (Fig. 3) have shown that its chemical nature is clearly the β form of calcium pyrophosphate [10], it is therefore hypothesized that on the second process not only the loss of carbon occurs, but also the transformation from the α into the β form of pyrophosphate. The thermal decomposition of disodium fosfomycin behaves quite differently (Fig. 2): after a slight loss of humidity, at about 40°C, a single decomposition process

is observed, beginning in the range 290–300°C, according to the atmosphere employed, and ending at about 400°C. By the thermogravimetric curves (Fig. 2) the rapidity and exothermicity of this process are well evidenced; particularly in oxygen, where it produces a very fast and strong increase in temperature to which the typical behaviour of TG and DTG curves is related. The white residue obtained at the end of this process, analysed by the X-ray powder spectrum (Table 3) [9] and IR spectrophotometry (Fig. 3) [10], was found to constitute only sodium pyrophosphate. The DSC calorimetric curves of these compounds between 20 and 500°C (Fig. 4) precisely

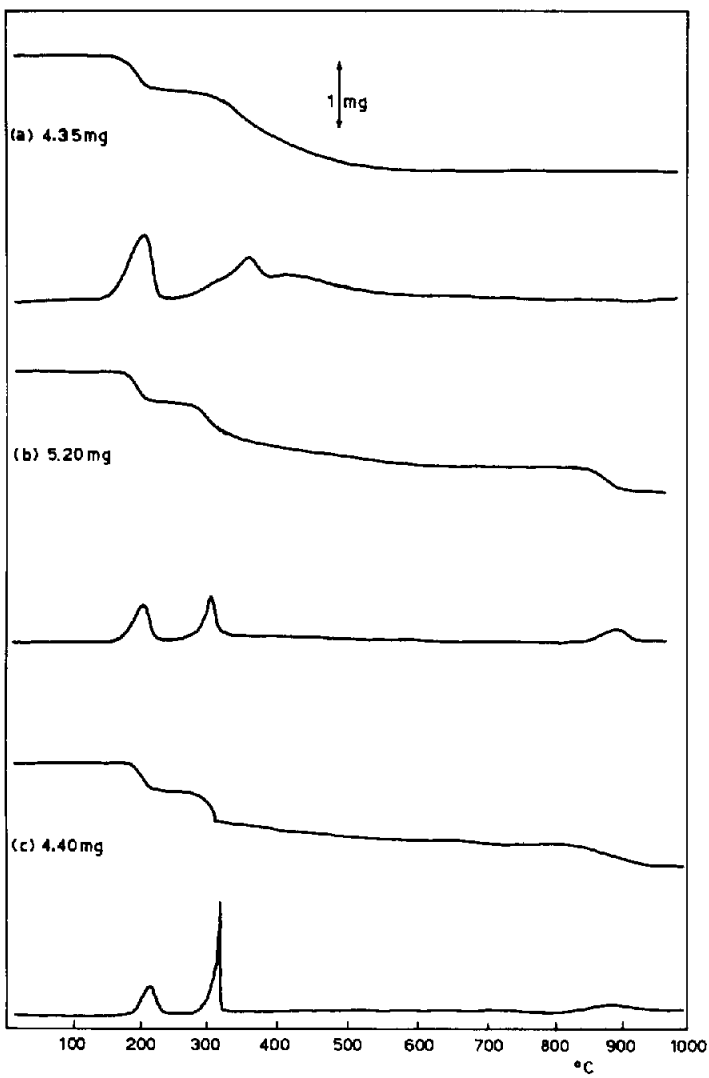


Fig. 1. TG and DTG curves of calcium fosfomycin in different atmospheres: nitrogen (a) or oxygen (b) at a flow rate of 100 ml min^{-1}, or static air (c). Heating rate 10°C min^{-1}.

confirm the above observations about the thermogravimetric curves. The curve of calcium fosfomycin shows a strong endothermal transition concerning the crystallization-water evaporation, at about 200°C, and a slow exothermal transition, relative to the first decomposition process. On the contrary, the DSC curve of disodium fosfomycin shows an initial, very slightly pronounced endothermal transition, corresponding to humidity loss, followed at about 300°C by a very acute exothermal peak, corresponding to the violent decomposition process evidenced by the thermogravimetric curves.

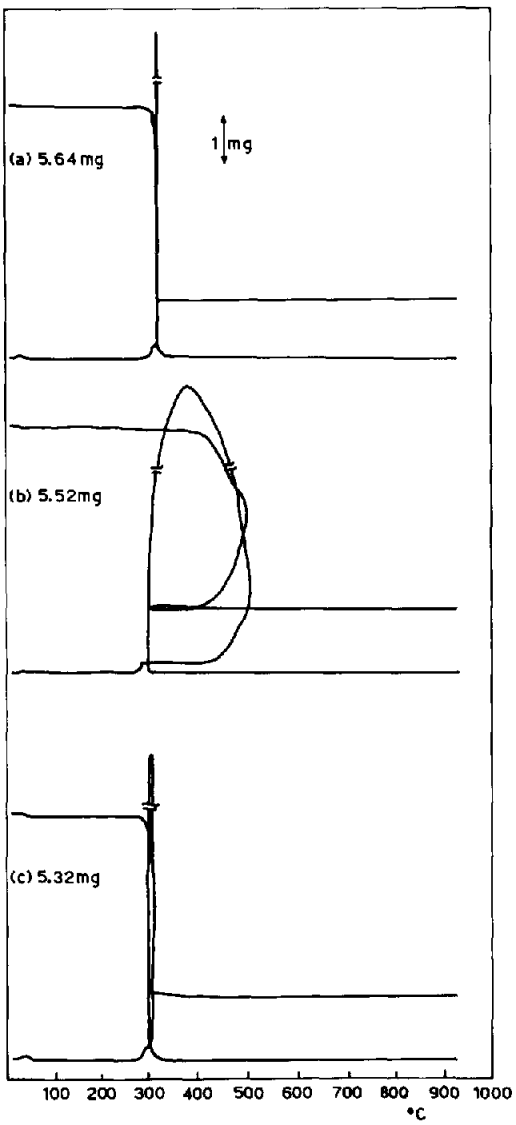


Fig. 2. TG and DTG curves of disodium fosfomycin in different atmospheres: nitrogen (a) or oxygen (b) at a flow rate of 100 ml min^{-1}, or static air (c). Heating rate 10°C min^{-1}.

TABLE 2

Thermal analysis of the compounds in nitrogen or oxygen stream and in static air. The % by weight of the residues, at the end of each process, are referred to the anhydrous compound (water is expressed as a fraction of the anhydrous compound)

Atmosphere	H_2O less			First process			Second process		
	Calcd. (%)	Found (%)	pdt	Calcd. (%)	Found (%)	pdt	Calcd. (%)	Found (%)	pdt
Calcium fosfomycin									
Nitrogen	10.2 [a]	9.6	140	–	77.1	250	72.1	–	> 990
			200			360			
			225			720			
Oxygen	10.2 [a]	9.6	160	–	80.0	240	72.1	72.8	820
			205			305			885
			235			720			940
Static air	10.2 [a]	9.6	180	–	80.9	250	72.1	72.2	820
			205			320			880
			230			750			970
Static air	–	–	–	–	76.4	150	72.1	72.2	600
with SA						225			640
added						600			700
Disodium fosfomycin									
Nitrogen	–	1.1	20	73.0	24.8	300			
			30			320			
			50			400			
Oxygen	–	1.0	20	73.0	27.5	270			
			30			380			
			50			400			
Static air	–	1.0	25	73.0	24.8	280			
			45			300			
			60			400			
Static air	–	–	–	–	77.4	150	73.0	73.0	350
with SA						230			405
added						350			500

SA = succinic acid.

[a] Based on a theoretical fraction of water corresponding to a compound formula containing exactly one molecule of crystallization water.

Also, since the absence of fusion peaks can be observed, the employment of DSC to perform eventual purity controls of the two salts of this antibiotic can be excluded. From the analytical point of view thermogravimetry seems in this case to be more useful than DSC, firstly allowing a rapid and reliable determination of the percentage and of the nature of water present in the compound; more the observation that the final residue in oxygen and static air is constituted by only disodium or calcium pyrophosphate, respectively, both also thermally stable above 1000°C. This has induced us to think that a purity control can be performed analogously to that previously possible for other compounds [4–7], the decomposition of which produces residues which are stoichiometrically well defined and thermally stable. A rapid analysis of

TABLE 3

X-ray powder spectra of some TG residues of calcium and disodium fosfomycin in static air

d_{hkl} [a]	d_{hkl} [b]	d_{hkl} [c]	d_{hkl} [d]	d_{hkl} [e]	d_{hkl} [f]
4.90	4.91	4.72	4.73	4.67	4.67
4.26	4.27	4.38	4.40	4.41	4.40
3.78	3.80	3.38	3.36	3.84	3.84
3.53	3.55	3.22	3.23	3.37	3.37
3.32	3.32	3.09	3.10	3.04	3.02
3.21	3.21	3.02	3.02	2.73	2.72
3.08	3.08	2.93	2.92	2.65	2.64
2.77	2.79	2.81	2.80	2.49	2.51
2.66	2.67	2.75	2.75	2.42	2.43
2.61	2.61	2.68	2.69	2.34	2.33
2.46	2.47	2.54	2.55	2.25	2.25
2.42	2.43	2.40	2.40	2.12	2.12
2.35	2.36	2.34	2.34	2.07	2.07
2.11	2.11	2.24	2.24	2.03	2.02
2.05	2.05	2.15	2.16	1.92	1.92
1.99	1.99	2.11	2.10	1.87	1.87
1.89	1.90	2.02	2.01	1.81	1.81
1.83	1.84	1.96	1.96	1.77	1.76
1.79	1.79	1.86	1.86		
1.71	1.71	1.84	1.84		
1.57	1.57	1.77	1.78		
1.50	1.50	1.73	1.73		
		1.69	1.69		

[a] Reported in the literature [9] for calcium pyrophosphate, α form.

[b] Experimentally found for the TG residue of the calcium fosfomycin at the end of the first process (~ 750°C).

[c] Reported in the literature [9] for calcium pyrophosphate, β form.

[d] Experimentally found for the TG residue of the calcium fosfomycin at the end of the second process (~ 970°C).

[e] Reported in literature [9] for sodium pyrophosphate.

[f] Experimentally found for the TG residue of the disodium fosfomycin after the unique decomposition process; at ~ 500°C.

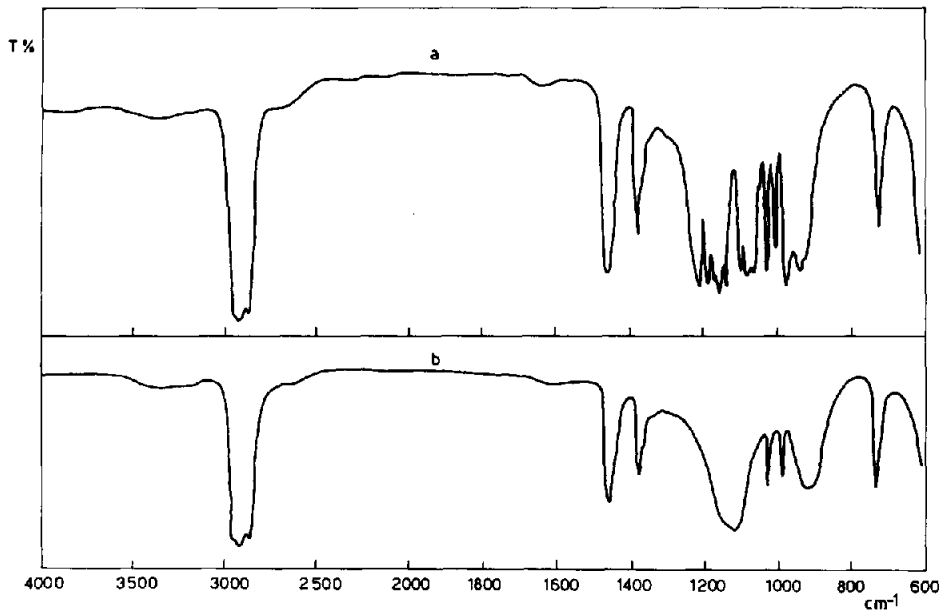


Fig. 3. IR spectra of the final TG residues of calcium fosfomycin and disodium fosfomycin in static air; nujol used as solvent. (a) spectrum of the residue (~ 970°C) of calcium fosfomycin; (b) spectrum of the residue (~ 500°C) of disodium fosfomycin. The IR spectra of the same residues in an oxygen stream are not significantly different. It is possible to compare these spectra with those of β-calcium pyrophosphate and sodium pyrophosphate, respectively, reported in the literature [10].

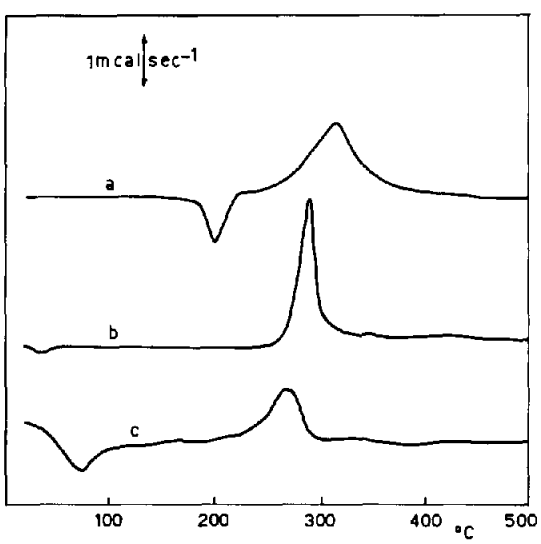


Fig. 4. DSC, in static air, of calcium fosfomycin (a), disodium fosfomycin (b) and of a commercial drug containing disodium fosfomycin with 8.33% (w/w) succinic acid associated (c). Heating rate 10°C min^{-1}.

Table 2 shows that, under the described operating conditions, only in the case of calcium fosfomycin do the calculated and experimental values of the percentage of calcium pyrophosphate in the final residue agree well, while the same agreement is not observed for sodium pyrophosphate after the single decomposition process. Initially, therefore, under the experimental conditions described, an analytical control of the method only on calcium fosfomycin was attempted. The results relative to the values of the percentages of calcium obtained in successive thermogravimetric experiments, performed in oxygen and static air, in order to evaluate the repeatability of the method, are presented in Table 4, while in Table 5 the data of theoretical and experimental calcium percentage values are compared with reference to thermogravimetric analysis in oxygen or static air. Then the problem of the control of disodium fosfomycin was faced as the residue values obtained, although consisting only of pyrophosphate, were found to be markedly lower than the theoretical value (Table 2). On the basis of a previous study [5,6] it was hypothesized that this disagreement could be due to losses during the thermogravimetric analysis because of ejections of matter from the crucible, following extremely violent decomposition processes. In similar circumstances the problem was solved by adding to the analysed mixture an opportune substance that, on melting, could amalgamate the analysed com-

TABLE 4

Precision of calcium or sodium analysis (by TG): for calcium fosfomycin in static air or in oxygen stream; for disodium fosfomycin in static air with succinic or glutaric acids added

% Ca calc.	% Ca found (in static air)	Mean	% Relative SD	% Ca found (in oxygen)	Mean	% Relative SD
Calcium fosfomycin						
22.76	22.76			22.81		
	22.81			22.60		
	22.72	22.76	0.1	22.13	22.97	0.9
	22.77			23.30		
	22.76			22.99		
% Na calcd.	% Na found (in static air with SA added)	Mean	% Relative SD	% Na found (in static air with GA added)	Mean	% Relative SD
Disodium fosfomycin						
22.26	25.21			25.26		
	25.25			25.35		
	25.21	25.26	0.2	25.16	25.26	0.3
	25.35			25.27		
	25.26			25.27		

SA = succinic acid.
GA = glutaric acid.

TABLE 5

% Calcium or sodium content of examined calcium and disodium fosfomycin and of a commercial drug. Comparison of the results obtained by TG in nitrogen or oxygen stream and in static air with and without the addition of succinic acid (reported values are the mean of at least three determinations)

Antibiotic salt	% Ca or Na calcd.	% Ca or Na found [a]	% Ca or Na found [b]	% Ca or Na found [c]	% Ca or Na found [d]	% Difference between found and calculated values			
						TG [a]	TG [b]	TG [c]	TG [c,d] h8
Calcium fosfomycin	22.76	24.33	22.97	22.76	22.77	+6.9	+0.9	0.0	+0.04
Disodium fosfomycin	25.26	8.59	9.52	8.58	25.26	−66.0	+62.3	−66.0	0.0
Commercial drug containing disodium fosfomycin with 8.33% (w/w) SA associated	23.16	23.28	23.11	23.19	-	+0.5	−0.2	+0.1	

SA = succinic acid.

[a] In nitrogen.

[b] In oxygen.

[c] In static air.

[d] In static air, with SA added.

pounds, thus avoiding losses of it; obviously this added substance must leave no residue at all. In previous papers [5,6] with residues consisting of sodium or potassium sulphate, the added substance was ammonium sulphate or persulphate that, apart from being decomposed completely at temperatures lower than 500°C, also behaves as a furnisher of sulphur, so that this element is in excess of sodium or potassium during all the decomposition process. Unfortunately, analogous salts of phosphorus, easily available commercially and characterized by a high purity level, such as hydrogen diam-

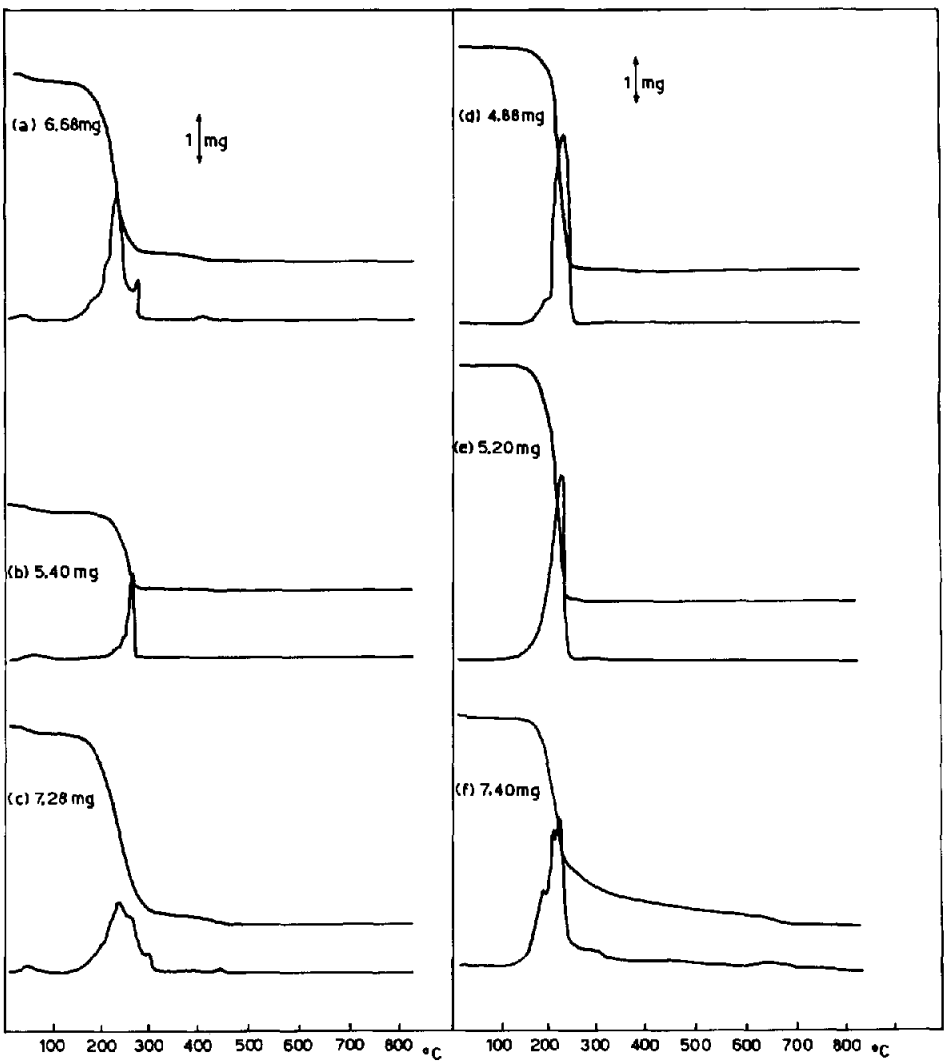


Fig. 5. TG and DTG curves of (a) disodium fosfomycin with 44.3% (w/w) SA, (b) a commercial drug containing disodium fosfomycin with 8.33% (w/w) SA associated, (c) disodium fosfomycin with 42.9% (w/w) GA, (d) succinic acid, (e) glutaric acid, (f) calcium fosfomycin with 46.2% (w/w) SA. Static air; heating rate 10°C min^{-1}.

monium or dihydrogen monoammonium phosphate, were found to be useless as they decomposed slowly and were accompanied by residue formation even at temperatures higher than 800°C. After many tests with different substances, that have not obtained the desired effect, finally the addition of succinic acid to disodium fosfomycin was found to be very useful in order to avoid any deflagration during thermoanalysis and so any loss of matter above 400°C; on the other hand, succinic acid is often associated with disodium fosfomycin in many commercially available pharmaceutical preparations. In Fig. 5 the thermogravimetric curves in static air of disodium fosfomycin added with succinic acid and of a commercial drug, containing disodium fosfomycin with 8.33% (w/w) succinic acid associated, are reported. The relative TG data are summarized in Table 2. It can be observed that the decomposition occurs in this case without any particular rapid or violent process; this is confirmed by the behaviour of the DSC curve of the drug (Fig. 4). It is possible to distinguish two processes very similar to those described in the case of calcium fosfomycin. Nevertheless in this case the amount of carbon at the end of the first process is the least, and it is lost at a temperature much lower than that in the case of calcium fosfomycin. It can also be noted that on the addition of succinic acid to calcium fosfomycin, again a residue of only calcium pyrophosphate is obtained at a temperature lower by about 270°C than that without any addition, as shown by the thermogravimetric curve of Fig. 5 and by the TG data reported in Table 2. In this table it is also important to observe that, with the addition of succinic acid, the sodium pyrophosphate experimental and theoretical percentages, in the final residue of disodium fosfomycin, also agree well. In order to analytically characterize the method completely, in this case some experiments for the evaluation of the repeatability and accuracy of the procedure were also performed. The analytical results of the repeatability and of the sodium percentages found and calculated are reported in Tables 4 and 5, respectively. Once the positive antideflagrant effect of succinic acid had been ascertained, it was easy to foresee that other compounds that are also able to

TABLE 6

% Sodium content of examined disodium fosfomycin. Comparison of the results obtained by TG in static air, with different dicarboxylic acids added (reported values are the mean of at least three determinations)

Carboxylic acid added	% Na calcd.	% Na found	% Difference between found and calcd. values
Oxalic acid	25.26	25.40	+0.6
Malonic acid	25.26	25.33	+0.3
Succinic acid	25.26	25.26	0.0
Glutaric acid	25.26	25.26	0.0

behave similarly to succinic acid can exert the same function, if added. In particular oxalic, malonic and glutaric acids were found to be useful in the thermoanalysis of disodium fosfomycin in static air. Some accuracy data are given in Table 6. The best results are obtained with succinic and glutaric acids, the thermograms of which are reported in Fig. 5. These two acids also seem to be preferable as they, after the fusion, boil on decomposition, while oxalic and malonic acids tend to sublimate [11].

CONCLUSIONS

In the case of calcium fosfomycin it must be preferable to operate in oxygen or static air, rather than in a nitrogen stream, if a stoichiometrically defined residue, which is quantitative and without carbon, is to be obtained at a temperature lower than 1000°C; while in the case of disodium fosfomycin, independent of the chosen atmosphere, the final residue is stoichiometrically defined but not quantitative, because of the violent, strongly exothermal decomposition, expecially in oxygen. From the analytical point of view it can be observed that by thermogravimetric analysis the nature and the percentage of water in the compound can be rapidly and accurately determined; and, at least for calcium fosfomycin, the method of purity control, based on a comparison between calculated and experimental calcium percentage values, can be profitably applied, which could also be foreseen, on the other hand, from the high thermal stability of pyrophosphate, even at temperatures higher than 1000°C and the fact that the high molecular weight of pyrophosphate produces a final residue of high weight percent, in comparison with the amount of the initially analysed compound. The experimental results obtained (Tables 4 and 5) confirm these assumptions also from the points of precision and inaccuracy (within 1%), by operating in oxygen or static air. The data of Table 5 also show that the method, if applied without any addition to the sample, fails in the case of disodium fosfomycin (inaccuracy is quite high), while if succinic acid in an appropriate concentration is added, the method can also be applied in this case, with good accuracy and precision, of the same order as that observed for calcium fosfomycin. The addition of succinic acid to calcium fosfomycin is not convenient, as it is positive only in being able to produce a residue of pyrophosphate alone at lower temperature, but with no improvement of analytical data precision and accuracy (which anyway is quite good), while a more complex experimental procedure is required. On the basis of the data of Tables 4 and 6 it can be concluded that of the other dicarboxylic acids that can be added in place of succinic acid, glutaric yields the best results, of the same quality as succinic acid itself. Lastly it can be osberved that the results obtained on analysing a commercial drug containing disodium fosfomycin and succinic acid show, as in this case, that a very simple,

indirect and accurate experimental control of the percentage of disodium fosfomycin in the drug is possible, without the employment of any separation procedure, by the determination of the percent sodium content found in the thermogravimetric residue.

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