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STABILITY PREDICTION OF CEFAZOLIN SODIUM AND CEPHALORIDINE IN SOLID STATE

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ABSTRACT

The stability of commercially-available solid pharmaceutical preparations containing cephaloridine and cefazolin sodium was evaluated with an accelerated isothermal degradation method at three different temperatures (37°, 45° and 60°C). A specific and sensitive differential pulse polarographic method was used for cephalosporin determination. Data obtained from high-temperature studies were processed using Arrhenius relation to predict shelf-life. The greater thermal stability of cephaloridine than cefazolin sodium was found, in contrast to what can be deduced from official monographs. Differential scanning calorimetry and X-ray diffraction were used to characterize the solid state of cephalosporin antibiotics.

INTRODUCTION

Our lab was recently commissioned to control the possible degradation of some lots of cephaloridine (CPH) and cefazolin sodium (CFZ) for injection (packaged in single-dose vials for sterile dry powders). These had been accidentally left inside a container in a trading port for two months during summer. The B.P. [1] requires that CPH should be

stored at a temperature of 8° to 15°C. For CFZ, instead, no specific storage conditions are recommended by the U.S.P. [2]; this should mean that thermal stability of CFZ is greater than CPH. However, no evidence of the above prescriptions are reported in the literature. While several papers are available on CPH and CFZ stability in aqueous vehicles, little information has been reported on their degradation in the solid state [3-5].

Several analytical methods for the determination of cephalosporin antibiotics have been described, including HPLC [6, 7], derivative spectroscopy [8-10] and voltammetry [11-14]. A screening of the literature showed that in terms not only of sensitivity, precision and accuracy, but also of selectivity and simplicity, electroanalytical methods were in many cases superior to other methods including chromatography and spectrophotometry [13]. Differential pulse polarography (DPP) was thus selected as a stability-indicating technique for assaying CPH and CFZ in this accelerated stability study. Differential scanning calorimetry and X-ray powder diffraction were also employed for solid phase characterization.

MATERIALS AND METHODS

Materials - Pure CPH (in the polymorphic δ form) and CFZ were kindly provided by Glaxo (Verona) and Firma (Florence), respectively, who also guaranteed their pharmaceutical grade and supplied the necessary cephalosporin commercial samples for injection. All other chemicals were of analytical-reagent grade. pH 2.2 Britton-Robinson universal buffer was used as supporting electrolyte. The buffer solution was 0.04 M in boric, phosphoric and acetic acids. All solutions were made using purified water passed through a Millipore Milli-Q system.

Polarographic Apparatus and Procedure - CFZ and CPH were assayed by a differential pulse polarographic technique [11, 14] opportunely modified. A Metrohm 646 VA Processor was used; this was connected to a 647 VA stand, which incorporates a multi-mode mercury electrode assembly (DME or HMDE), as working electrode. The three-electrode

system was completed with an Ag/AgCl reference electrode and a platinum rod as auxiliary electrode. A built-in teflon-coated rod stirrer was used for mixing. Polarography was performed in the differential pulse mode with a 50 mV pulse being applied, using a scan rate of 10 mV s^{-1} and a forced drop time of 0.6 s. Scans were performed from -0.3 to -1.25 V. Highly purified nitrogen was passed through the solution to remove dissolved oxygen. Since the temperature coefficient was less than 1.0 % $degree^{-1}$ (between 18° and 38°C), no thermostating of the cell was necessary. Cephalosporin stock solutions were prepared by dissolving 30 mg of CPH or 50 mg of CFZ in water and making them up to 5 mL with water. At the time of the analysis a calibration graph was prepared by micropipetting a series of sufficient volumes of the stock solution for achieving final concentrations of 10-40 μg mL^{-1} of CPH and 16-66 μg mL^{-1} of CFZ into the polarographic cell, containing 15 mL of the Britton-Robinson buffer. The polarographic solution was briefly stirred and deoxygenated after each addition. The resulting current was calculated on the basis of peak height (mm) at -1.012 V for CPH and at -0.69 V for CFZ, measured with reference to the supporting electrolyte polarogram. For each analyte concentration the DPP assay was repeated twice averaging the results. Samples stored in the ovens were assayed under the same conditions by using an in-cell concentration of 24 μg mL^{-1} for CPH and 40 μg mL^{-1} for CFZ. A Metrohm 701 KF Titrino was used for Karl Fisher determination of cephalosporin water content.

Thin Layer Chromatography - Stored cephalosporin samples were qualitatively evaluated with TLC. Precoated silica gel plates (F_{254} HPTLC, Merck) were used. The developing solvent system was prepared by shaking a mixture of butanol-acetic acid-water (4:1:5, v/v/v) and taking the organic phase after settling. Visualization was accomplished with fluorescence quenching under short-wave UV light at 254 nm.

X-Ray Diffraction - X-ray powder diffraction patterns were taken with a Philips PW 1130 apparatus over the 5-40° 2θ and 2-40° 2θ range, for

CPH and for CFZ, respectively, at a scan rate of 2° min^{-1}, using filtered Co/Kα radiation.

Differential Scanning Calorimetry - DSC was performed with a Mettler TA 4000 apparatus equipped with a DSC 20 cell. Samples were weighed (Mettler M3 microbalance) in pierced Al pans (5-10 mg) and scanned at 10 K min^{-1} between 30° and 250°C. Scans were also carried out at 30 K min^{-1} (in the same temperature range) after a pretreatment of each sample kept at 120 °C for 10 min.

Accelerated Stability Study - Commercially-available representative samples of the same lot of CPH and CFZ for injection were divided into four groups and stored in a refrigerator at 4 °C (reference samples) or in thermostated ovens at 37°, 45° and 60°C in their own glass seal ampoules. The samples were not stored at controlled humidity, because a new sealed sample was opened at the time of each analysis. Besides in preliminary studies Karl Fischer assays, carried out on cephalosporin samples at zero time and after 3 months' storage at 4 °C and 60 °C, gave a constant water content of 2.2 and 1.47 % for CPH and CFZ, respectively, in good agreement with official monograph values. The accelerated stability study was carried out for 6 months. Sealed samples were removed at specific time intervals (for the first and the second month every 15 days and then every 30 days), cooled, opened and carefully mixed to guarantee homogeneous sampling. Two stock solutions of each sample were prepared (6 mg mL^{-1} for CPH and 10 mg mL^{-1} for CFZ) and assayed (averaging the results) with the above polarographic procedure.

RESULTS AND DISCUSSION

CFZ and CPH exhibited single well-resolved polarographic peaks at -0.69 V and -1.01 V, respectively (Fig. 1); these were suitable for precise and accurate determination. The calibration graphs were linear in the concentration range of 16-66 μg mL^{-1} for CFZ and 10-40 μg mL^{-1} for for CPH. At each analysis time a new calibration graph was obtained

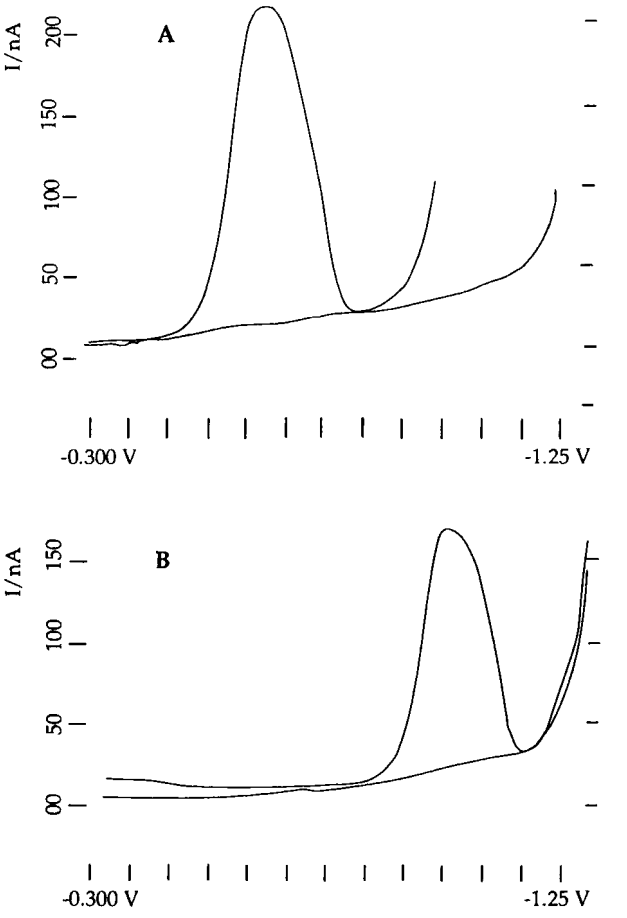

FIGURE 1
Differential pulse polarograms of cefazolin sodium (A, 33.5 μg mL^{-1}) and cephaloridine (B, 20.6 μg mL^{-1}).

for both antibiotics and the resulting equations for 95% confidence limits for the intercept and slope are reported in Table 1. The precision of DPP determinations was good: for five measurements at the concentration level of 20 μg mL^{-1} the relative standard deviation was 1.0 % for CFZ and 1.3 % for CPH.

The products stored at 4°, 37°, 45° and 60°C were assayed over a period of 180 days. As regards CFZ, only the samples stored at 60°C showed a decomposition of over 10% at the end of this time interval,

TABLE 1

Calibration Data of DPP Analysis of Cephaloridine and Cefazolin Sodium at 95% Confidence Limits for the Intercept and Slope.

Days	Concentration range, $\mu g\ mL^{-1}$	Sensitivity, $mm\ mL\ \mu g^{-1}$	Intercept, mm	Correlation coefficient
CEPHALORIDINE				
15	10.25 - 40.79	3.30 ± 0.0680	-2.79 ± 1.89	0.9996
30	10.32 - 41.06	3.42 ± 0.0323	-3.02 ± 0.906	0.9999
45	10.32 - 41.06	3.44 ± 0.0540	-4.31 ± 1.52	0.9998
60	10.30 - 41.10	3.28 ± 0.166	-5.45 ± 4.68	0.9997
90	11.10 - 44.40	3.30 ± 0.0510	-4.91 ± 1.56	0.9998
120	10.32 - 41.10	3.03 ± 0.0377	-2.27 ± 1.06	0.9998
150	9.45 - 37.60	3.17 ± 0.0750	-2.56 ± 1.93	0.9994
180	10.18 - 40.53	3.13 ± 0.0350	-2.00 ± 0.982	0.9999
CEFAZOLIN SODIUM				
15	16.87 - 67.15	2.32 ± 0.0160	-4.10 ± 0.759	0.9999
30	17.30 - 68.87	2.45 ± 0.00487	-2.55 ± 0.233	0.9999
45	17.57 - 69.93	2.51 ± 0.00410	-3.86 ± 1.96	0.9997
60	16.80 - 66.90	1.60 ± 0.0190	-1.41 ± 0.889	0.9998
90	17.57 - 69.93	2.32 ± 0.0213	-2.83 ± 1.02	0.9999
120	17.20 - 68.50	2.20 ± 0.0280	-2.08 ± 1.32	0.9999
150	17.17 - 68.34	2.33 ± 0.0320	-3.59 ± 1.49	0.9998
180	17.80 - 70.90	2.36 ± 0.0270	-3.74 ± 1.32	0.9999

while for those stored at 45° and 37°C, the percentage of decomposed drug was about 10% and 5%, respectively. Although insufficient decomposition had occurred to establish the reaction order unambiguously, all data were consistent with first-order kinetics, even if good correlation coefficients were also found for zero-order kinetics (Table 2).

TABLE 2

Linear Correlation for Zero-Order and First-order Treatment of Data Obtained from the Stability Study of Cefazolin Sodium.

T °C	Reaction order	Linear equation	Correlation Coefficient
37	0	y = 101 - 0.0235 x	-0.988
45	0	y = 101 - 0.0610 x	-0.984
60	0	y = 99.3 - 0.114 x	-0.967
37	1st	y = 4.61 - 0.000235x	-0.988
45	1st	y = 4.61 - 0.000643x	-0.985
60	1st	y = 4.60 - 0.00128 x	-0.973

Typical plots of the solid-state decomposition of CFZ at various temperatures are shown in Fig. 2A. First-order decomposition rate constants K (day^{-1}) were evaluated by least-squares analysis from the slopes of these curves. By plotting the log of degradation constants versus the reciprocal of the absolute temperature, a linear relation was obtained (Fig. 3). Degradation then displayed apparent Arrhenius behaviour with an apparent activation energy of 14 kcal/mol. The linearity of the plot (r = 0.960; P = 0.05) allowed extrapolation of the degradation rate constant from the high temperatures to 25°C. This was found to be 1.07×10^{-4} day^{-1} (RSD = 5.2%). The expected shelf-life calculated from these results was about 2.7 years.

CPH samples, stored for the same time interval and under the same conditions, showed less degradation than the corresponding CFZ samples. Appreciable decomposition was found only for the products stored at 60°C (Fig. 2B), while for the samples stored at lower temperatures the percentage of decomposed drug after six months did not reach 5 %. Besides, in this case the data obtained could not be processed by the Arrhenius relation. A problem of physical stability was however observed; on ageing, the powder developed an amber color, well before its loss of potency exceeded 10%, thus indicating that some decomposition had occurred.

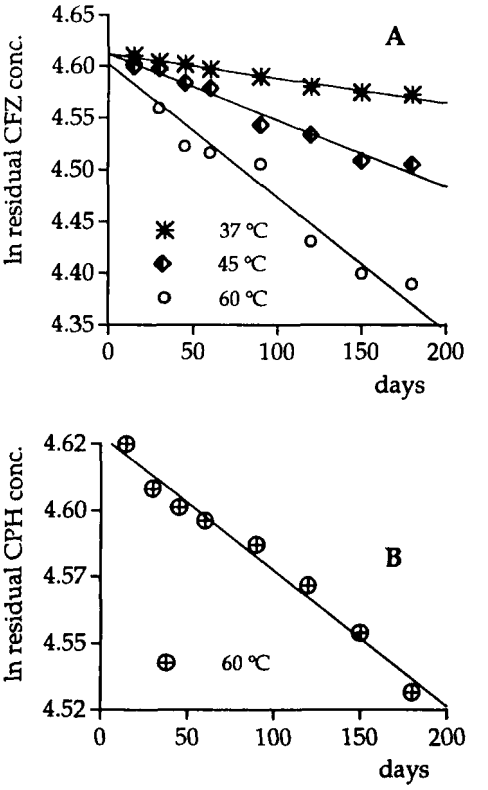

FIGURE 2
Apparent first-order plots for decomposition of cefazolin sodium (A) stored at 37°, 45°and 60° C, and cephaloridine (B) stored at 60 °C.

The results obtained from accelerated solid-state stability studies showed the greater thermal stability of CPH than CFZ, in contrast to what can be deduced from B.P. and U.S.P. prescriptions about their storage conditions [1-2]. Samples of CFZ stored for six months at 60°C were examined by TLC and revealed degradation to two decomposition products. Samples of CPH, stored in the same conditions, did not show any noticeable spots, confirming its poor degradation. Accordingly, analyses carried out on the above-mentioned lots of CPH and CFZ which had accidentally been left during summer in a metal container (inside which the temperature might have exceeded 60°C) gave recoveries in the range 97.1-87.1% for CPH and 90.0-80.2% for CFZ.

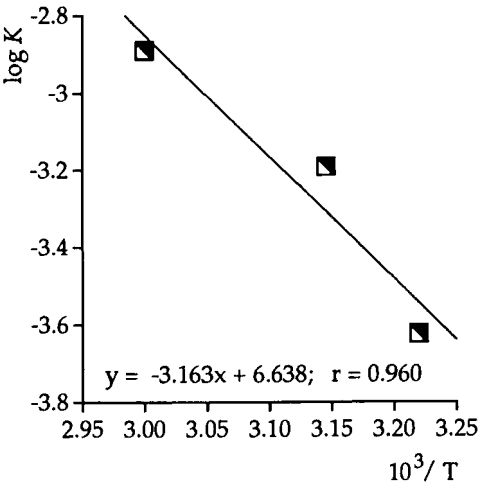

FIGURE 3
Arrhenius plot of first-order rate constants for the thermal decomposition of cefazolin sodium.

It is very difficult to interpret solid-state degradation results, because we may not be aware of all the variables that control such degradation. In fact, besides temperature and moisture, several other factors such as particle size, extent of crystallinity, traces of impurities, presence of polymorphic forms, may all be important variables. In order to obtain further information on CPH and CFZ solid-state characteristics, DSC and X-ray diffraction analyses were then performed on degraded and reference samples. The X-ray powder diffraction patterns of CFZ and CPH samples stored 6 months at 4 °C are shown in Fig. 4 together with the corresponding samples stored for the same time interval at 60°C. Both CPZ and CPH were seen to be crystalline, even if the crystallinity of CPH was clearly higher. This was in agreement with the finding that the chemical stability of CPH was greater than that of CFZ. In fact, previous reports have found that the amorphous cephalosporins are considerably less stable than the crystalline forms [15-16]. The cephalosporins maintained their crystallinity almost unchanged on ageing, independently of the storage temperature. No significant variation was observed in the diffraction

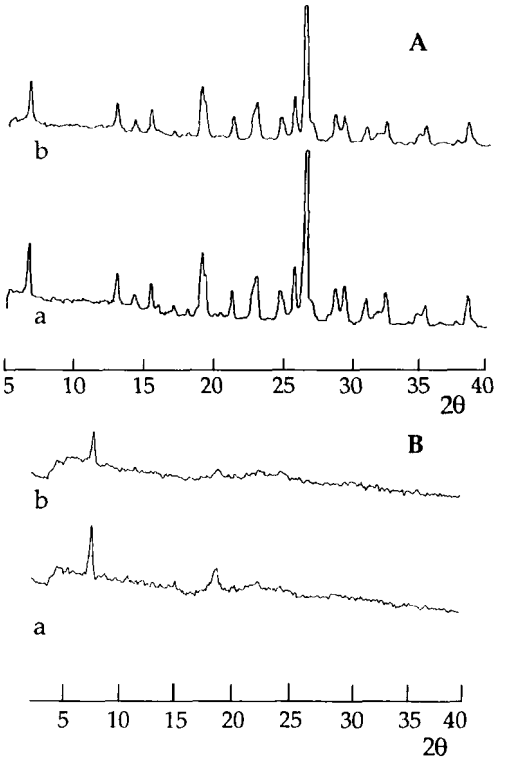

FIGURE 4
X-ray diffraction powder patterns of cephaloridine (A) and cefazolin sodium (B) stored for six months at 4° (a) and 60°C (b).

patterns of the samples stored at the various temperatures, and no effect of drug degradation was visible, even between the samples stored at the two considered limit temperatures (4° and 60°C).

The results of thermal analysis recorded at 10°C min^{-1} in the 30-250°C range on CFZ after 6 months' storage at 4°, 37°, 45° and 60°C, respectively, are given in Fig. 5A. No differences were observed between the sample stored for 6 months at 4°C and a sealed reference sample, opened just before running (not shown). All the samples showed a large endothermal effect in the 90-120°C range due to water loss, then an endothermal peak immediately followed by a sharp exothermal effect which is due to the drug degradation process. The

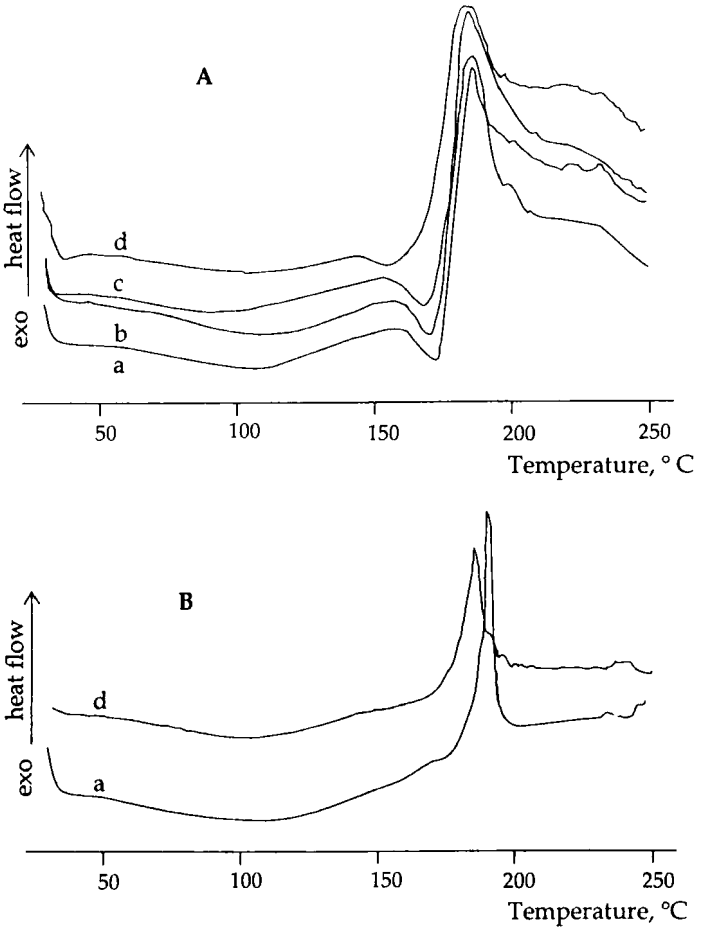

FIGURE 5
DSC curves of cefazolin sodium (A) and cephaloridine (B) six months stored at different temperatures: 4°C (a), 37° (b), 45° (c), 60° (d); heating rate 10 K min^{-1}.

endothermal peak is attributable to the incipient melting of the drug, which was however not well evaluable because decomposition begins at once. The observed onset temperature of the endothermal melting peak for CFZ stored at 4°C was 171.9°C, and the apparent fusion heat was 9.5 J/g. The thermal curves of the other aged samples showed a gradual broadening in the melting peak and a shift to lower values with the increase in storage temperature (Fig. 6). Parallelly, a reduction

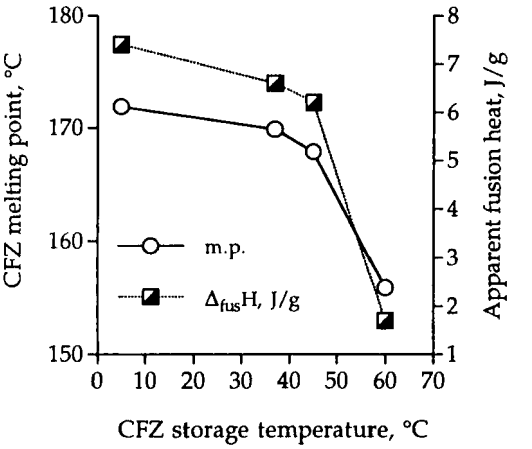

FIGURE 6
The effect of storage temperature on the melting point and the apparent heat of fusion of cefazolin sodium.

in the apparent fusion heat was observed (Fig. 6), and both the phenomena appeared to be related to the percentage of decomposed drug. A good correlation (r = 0.999) was in fact observed between the lowering of the fusion peak after a given time of storage at a given temperature and the corresponding percentage of decomposed drug measured polarographically (Fig. 7). In an attempt to reduce interference due to the evaporation of adsorbed water, and to "stabilize" the samples, thus aiming at a better separation of the melting from the degradation process, samples of CFZ were preconditioned by heating at 10°C min^{-1} up to 120°C, and by keeping them at this temperature for about 10 min. Then the samples were slowly cooled down to room temperature and finally run at a scan rate of 30°C min^{-1} . The thermograms recorded under these conditions showed a very similar general trend to the previously-described series. The only important difference observed, apart from the flat profile in the dehydration range, was a significant shift (about 10°C) to higher values of the onset temperature of both melting and decomposition peaks, which however remained very close. This phenomenon, which was common to all samples, was only due to the higher scan rate.

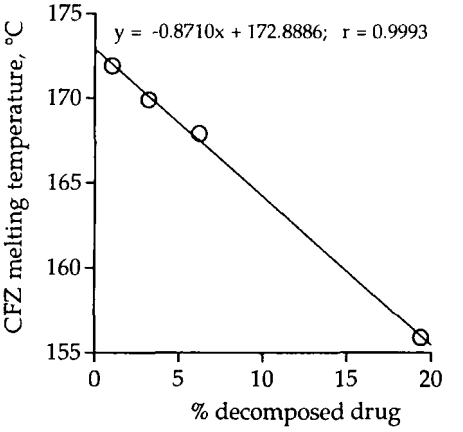

FIGURE 7
The relation between the melting point and the decomposed percentage of cefazolin sodium.

Unfortunately, because of the absence of a well-defined melting process, it was not possible to evaluate the purity of CFZ calorimetrically.

Fig. 5B shows the DSC plots of CPH samples after 6 months of storage at 4°C and at 60°C, respectively. Here, too, no differences were observed between the sample stored in freeezing and a reference sealed sample, opened just before scanning (not shown). All the samples showed a large endothermal effect in the 90-120°C range, due to the drug dehydration process, followed by an exothermal peak attributable to the drug decomposition. No important differences were observed in the thermal behaviour of the samples stored at the various temperatures, apart from a slow decrease in the decomposition temperature (from 191° to 185°C for the samples stored at 4° and 60°C, respectively). The total absence of a melting process did not allow any further evaluation.

CONCLUSIONS

CPH and CFZ samples did not show any decline in potency, as measured by chemical assay, when stored at 4°C, and both the drugs

appeared suitable for use as sterile powders to be reconstituted prior to parenteral administration.

Stability studies carried out at high temperatures however showed the greater thermal stability of CPH as compared to CFZ, contradictory to what can be deduced from the storage prescriptions of official monographs [1, 2]. The clear temperature dependence of the decomposition rate constant demonstrated for CFZ solid samples suggests the need for an official recommendation for a defined storage temperature range, like that already existing for CPH.

ACKNOWLEDGEMENTS

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