ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2011, *56*(10-11), 959-966

THERMAL STABILITY AND BIOLOGICAL INTERACTIONS OF SOME CEPHALOSPORINS

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Received June, 2, 2010

The thermal behaviour of cephalosporins, cephalexine, cefadroxil and cefoperazone, was studied helped by a hyphenate technique (TG/DTG/ /FT–IR UATR). By comparison of the FT–IR spectra of initial *vs.* char samples, were identified the compounds which resulted from thermal decomposition and which may present a high toxicity. The biological effect was tested on the evolution of *Drosophila Melanogaster*, using the three cephalosporines and the corresponding residue till 220°C. As biological answer the fertility index, the male/female ratio and some significant anatomical changes were observed. The cephalexine seems to be the compound with the highest positive influence on fertility and the insignificant changes of the anatomical characteristics were observed. On the other extreme, the cefoperazone presented important changes in the biological answer.

INTRODUCTION

Cephalosporins are the most frequently prescribed class of antibiotics. They are structurally and pharmacologically related to the penicillins. These substances have a *beta*-lactam ring structure that interferes with the synthesis of the bacterial cell wall and so are bactericidal. All bacterial cells have a cell wall that protects them. Cephalosporins disrupt the synthesis of the peptidoglycan layer of bacterial cell walls, which causes the walls to break down and eventually the bacteria dies.[1-3]

The cephalosporins are derived from the cephalosporin C which is an acid-stable molecule with antibacterial activity and is produced from Cephalosporium acremonium.

The fruit fly, *Drosophila melanogaster*, is the cheapest and suitable material for demonstrating the particular laws of genetic heredity. The analytical methods were developed to produce a large number of fruit flies, to reduce mortality in small quantities, but also to control the environmental conditions for its growing.

Diploid number of chromosomes of these species is 8, forming four pairs. With only four pairs of chromosomes is of great help for geneticists in determining gene of *Drosophila melanogaster*, in linkage groups and in establishment of chromosome features, many genes are sharply segregated in accordance with a simple theoretical report. The genes behaviour associated with sex chromosome also get to interest in genetic studies of sex linkate characters.[4] *Drosophila melanogaster* is a prolific body with relatively short development cycle and with an inexpensive laboratory maintenance. Thus, a pair of genitors can produce nearly 200 offspring, allowing statistical interpretation of results. A new generation can be obtained at 20°C in about 14 days and, thus, in a year one could study a large number of successive generations (over 24).[5,6]

The purpose of this study was to assess the thermal behaviour for three cephalosporines, respectively the biological effect was tested on the evolution of *Drosophila Melanogaster*, using both three cephalosporines and the corresponding residue till 220°C.

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This study is in connection with our previous papers on the thermal behaviour of some cephalosporins,[7,8] where an elevated kinetic analysis allowed to obtained believable data for a life time prediction.

Cephalosporins' formulas are presented in Fig. 1.

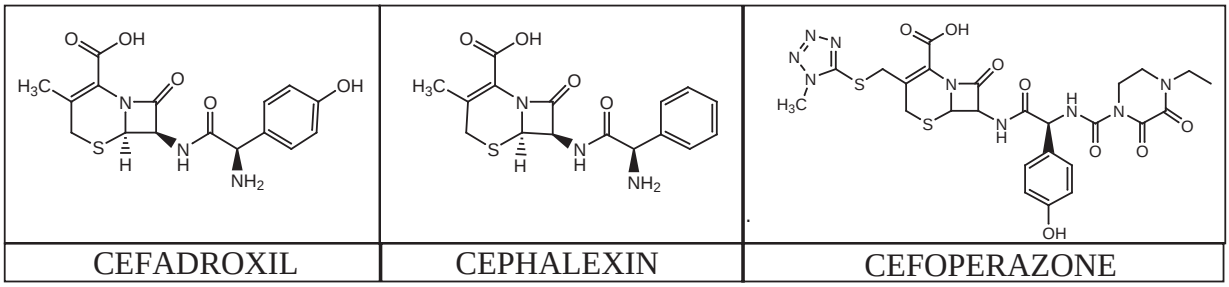


Fig. 1 – The chemical structure of the used cephalosporins.

RESULTS AND DISCUSSION

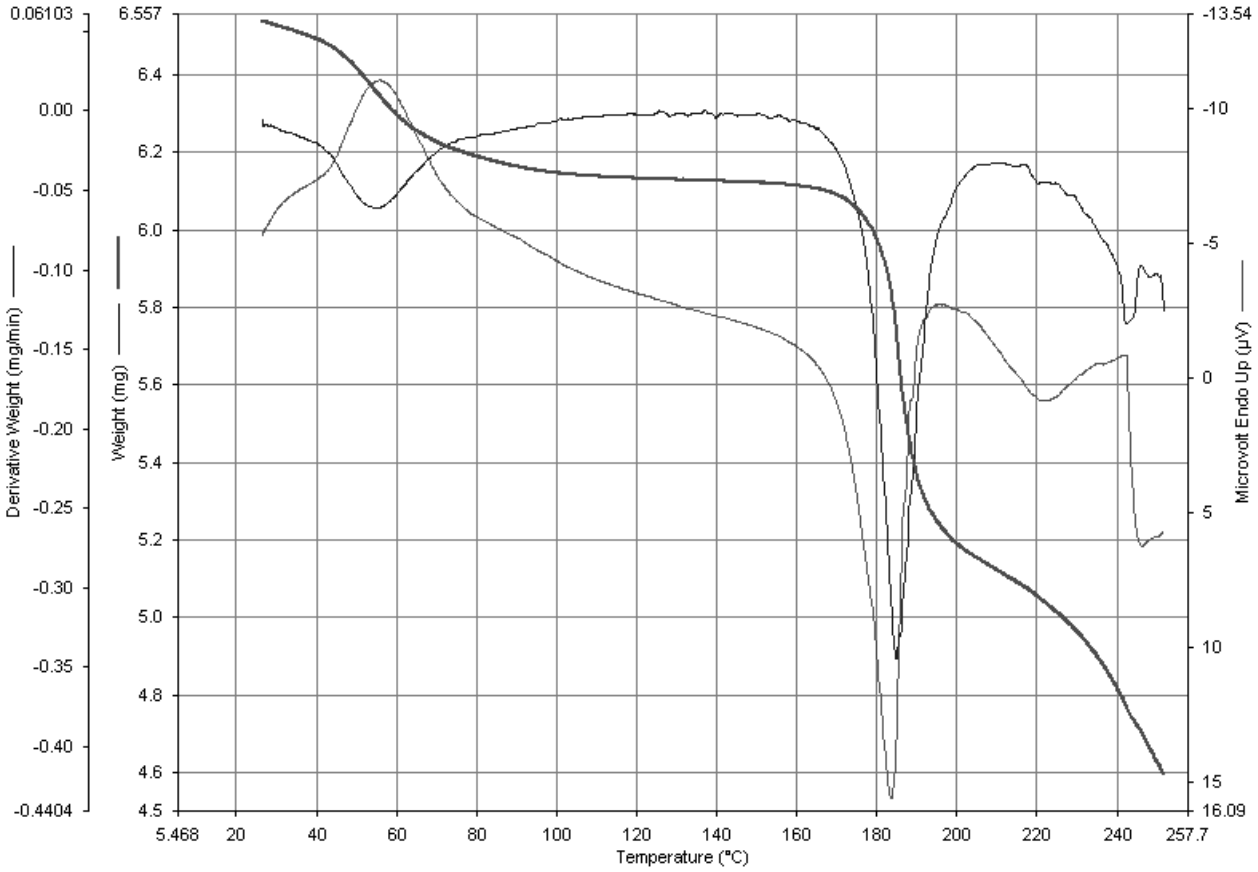
a) Thermal Analysis

The results of TG/DTG/DTA curves obtained for the three cephalosporins under monohydrate state during heating at β=7°C·min^{-1} in air atmosphere are presented in Fig. 2.

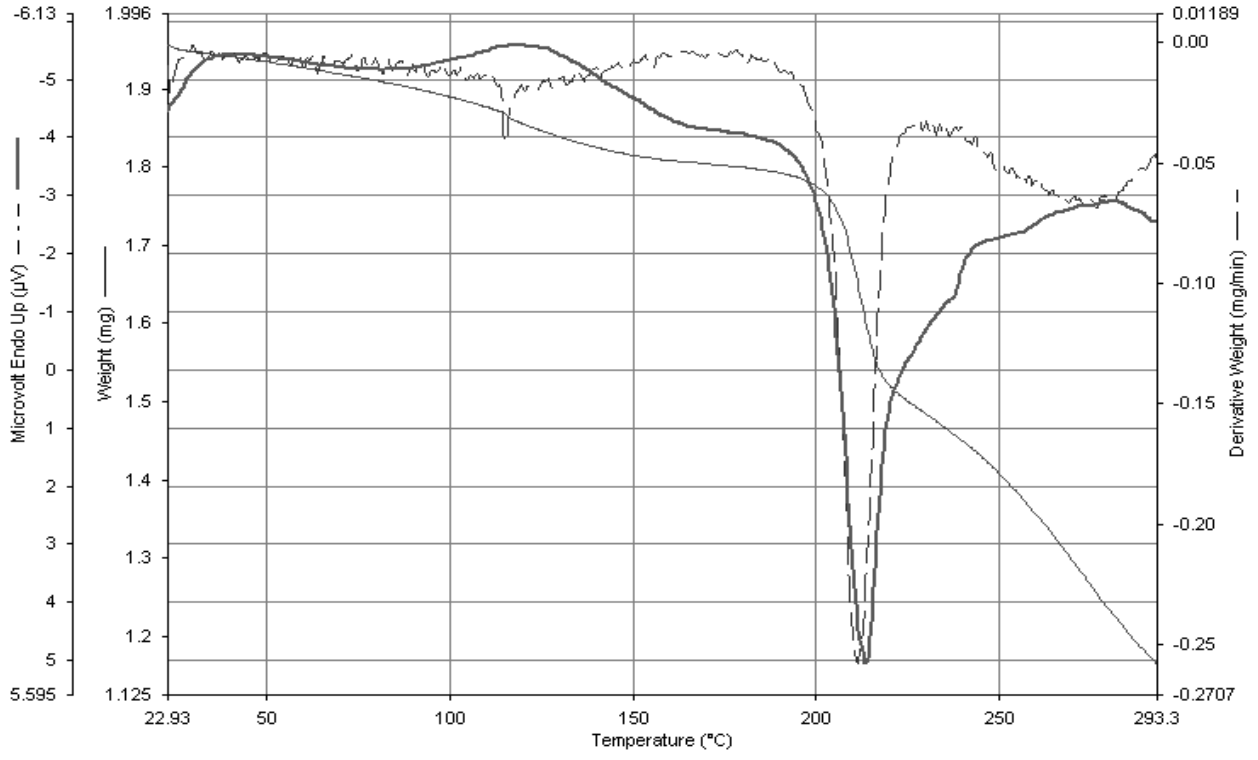
The three cephalosporins which were thermal analysed (cephalexin, cefadroxil, cefoperazone) differ in terms of structure in a lesser extent (only a hydroxyl group – cephalexin and cefadroxil), respectively in a large proportion, by means of presence of many radicals bounded by dihydrotiazinic cycle, in the cefoperazone's case. After examining and comparing the thermogravimetric curves of the three active substances, it appears that they comply in the same manner, with some observations: (i) cephalexin monohydrate decomposes into three stages characterized by weight loss, which can be identified by DTG curves; (ii) for cefadroxil monohydrate, the process which involved the destruction of cefadroxil molecule (cephem structure) starts at a temperature of 190°C compared to the cephalexin's case for which the starting process' temperature is 158°C; (iii) cefoperazone monohydrate is characterized by heating curves which contain several peaks on the same temperature range compared with the two previous cases. This fact is explained if it takes into account the diversity of radicals contained in the structure of this active substance, which are more bulky compared with the corresponding active substances' substituents from the same class.

After the comparison of the FTIR spectra of the three cephalosporins before (Fig. 3) and after heat treatment (Fig. 4), one can observe the disappearing of the 3450 cm^{-1} band, that corresponding to the hydration water, the disappearing of those from the 1050–1200 cm^{-1} range, characteristic to the *β*–lactamic cycles, and also the disappearing of those from the 1900–2170 cm^{-1} range corresponding to the heteroatom–hydrogen bonding and of those which underline the carbonyl and carboxyl groups (ν = 1760; 1690 cm^{-1}).[9, 10]

As shown in Fig. 4, it can be seen that the spectra of the three substances after the heat treatment are quite similar (even if there are minor differences between the peak values, they are not fundamentally different kinds of vibration), having the same basic features as: the presence of the alkyl groups, i.e. of a various substituted aromatic compounds which are of a high toxicity.

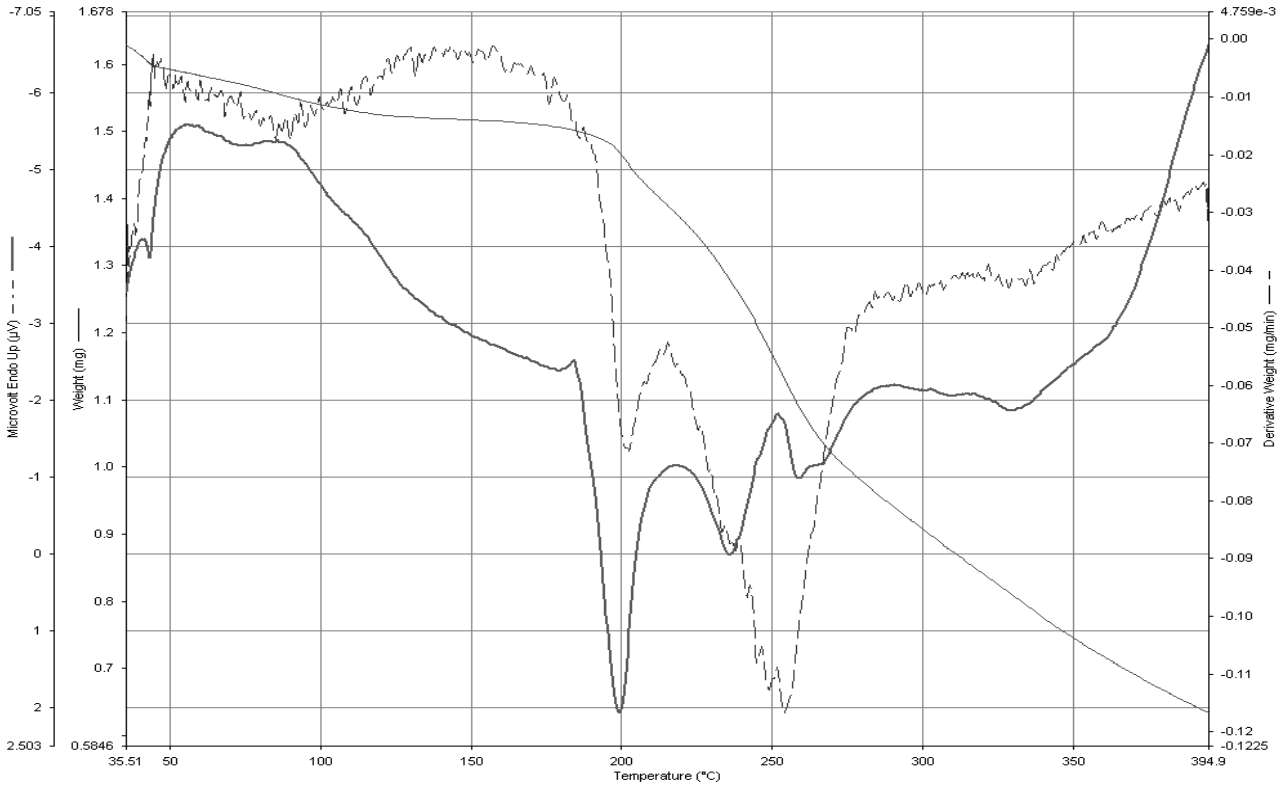


a)



b)

Fig. 2 – The thermoanalytical curves TG/DTG/DTA obtained in air at β=7°C·min^{-1} for a) cephalexin monohydrate; b) cefadroxil monohydrate; c) cefoperazone monohydrate.



c)

Fig. 2 (continued) – The thermoanalytical curves TG/DTG/DTA obtained in air at β=7°C·min^{-1} for a) cephalexin monohydrate; b) cefadroxil monohydrate; c) cefoperazone monohydrate.

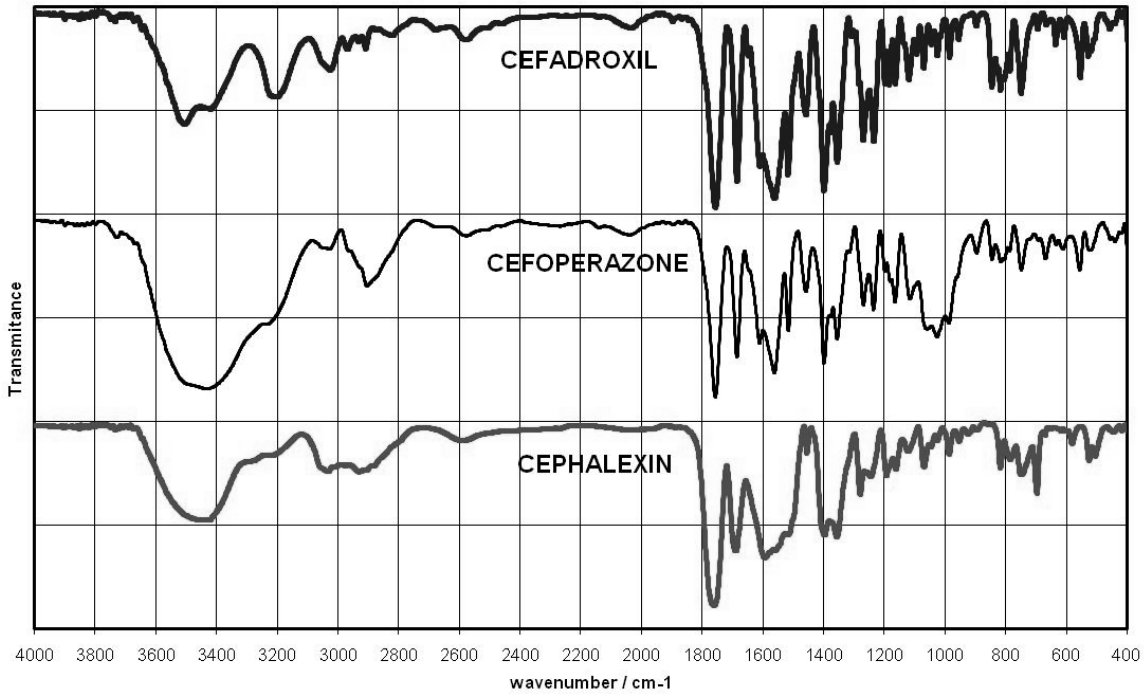
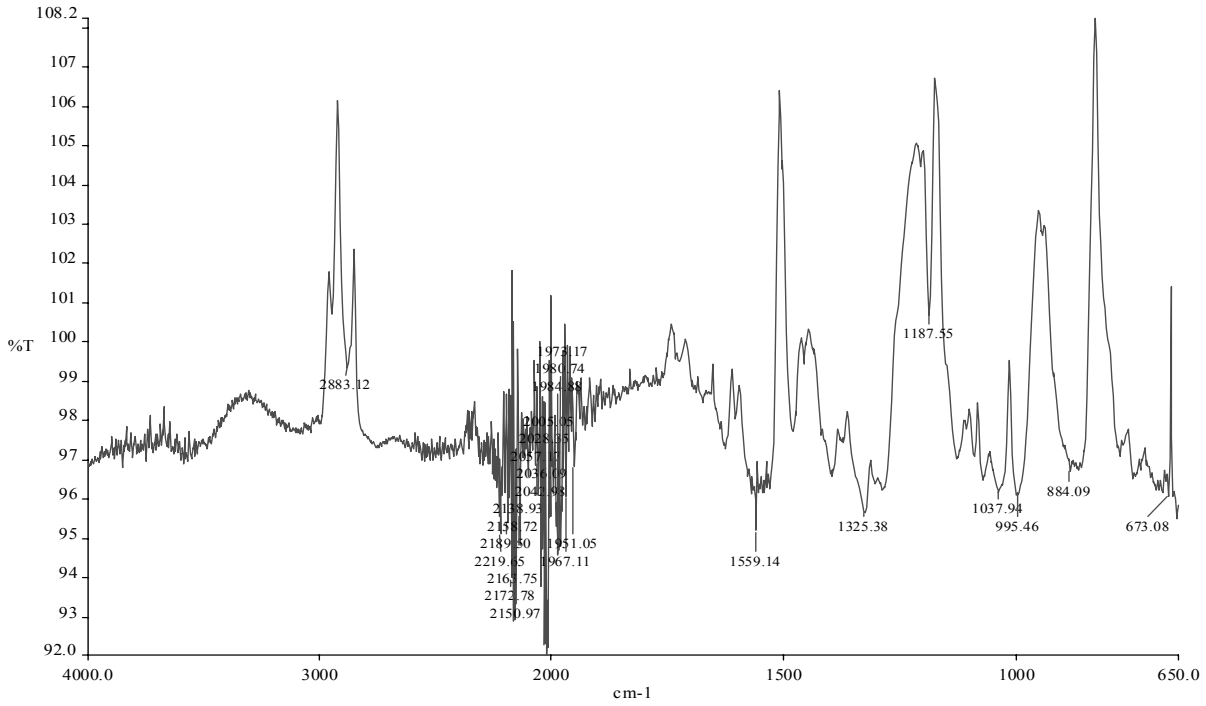
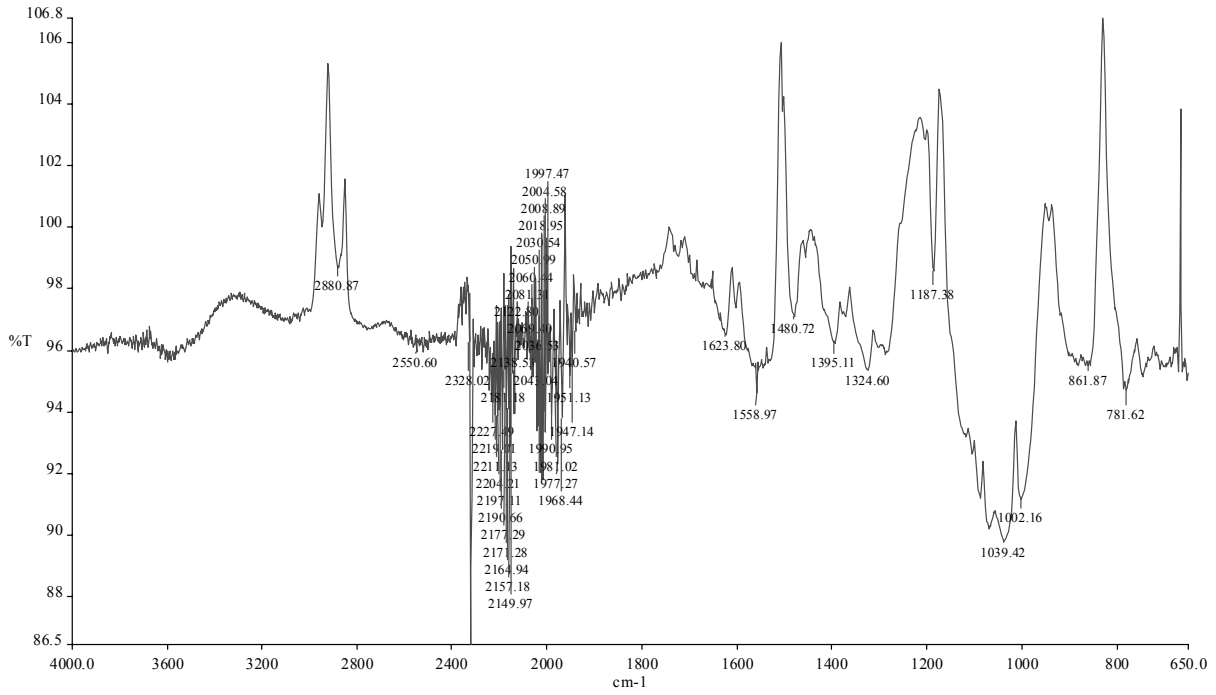


Fig. 3 – The infrared spectra (FTIR) of the three cephalosporins before the heat treatment (KBr pellets).

a)


%T
cm-1
2883.12
1559.14
1187.55
1325.38
1037.94
995.46
884.09
673.08

b)


%T
cm-1
2880.87
2550.60
1623.80
1558.97
1480.72
1395.11
1324.60
1187.38
1039.42
1002.16
861.87
781.62

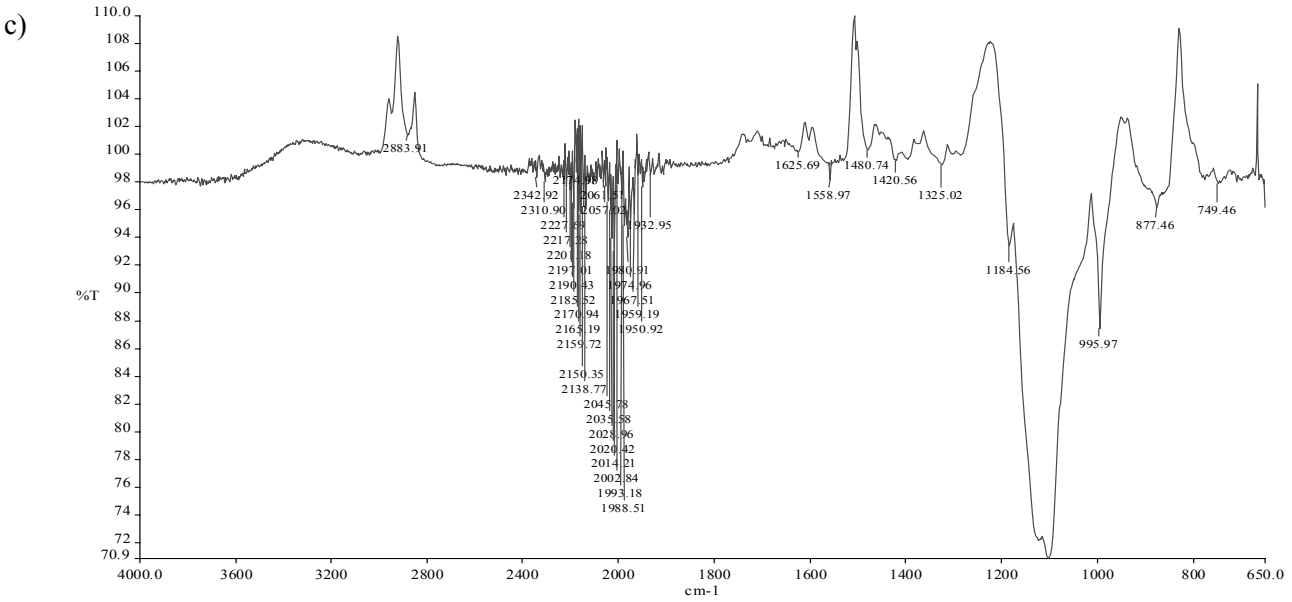


Fig. 4 – Infrared spectrum (FTIR) of the three cephalosporins – after heat treatment at 220°C a) cephalexin monohydrate; b) cefadroxil monohydrate; c) cefoperazone monohydrate.

b) Biological Answer

Reference results are presented in Fig. 5. The control egg/adult cycle was 11 days, during which following observations were made: a big number of eggs since the second day; the larva appears after three days; the first adult appears after 11 days.

The samples with cephalosporins present a similar egg-adult cycle, with an insignificant variation of 1–2 days (see for example Fig. 6).

More important is the variation observed in the fertility coefficient (see Table 1) defined as ratio of the number of fruit fly adults resulted in egg-adult cycle at the end of experiment and number of fruit fly adults introduced at the beginning of the experiment.

The cephalexine has an obvious positive antibiotic activity, the corresponding fertility coefficient being twice in comparison with the reference and the other two cephalosporins. A smaller fertility coefficient by cefoperazone, together with the appearance of new adults with discoloured eyes is a sign of a possible secondary toxic effect of that compound.

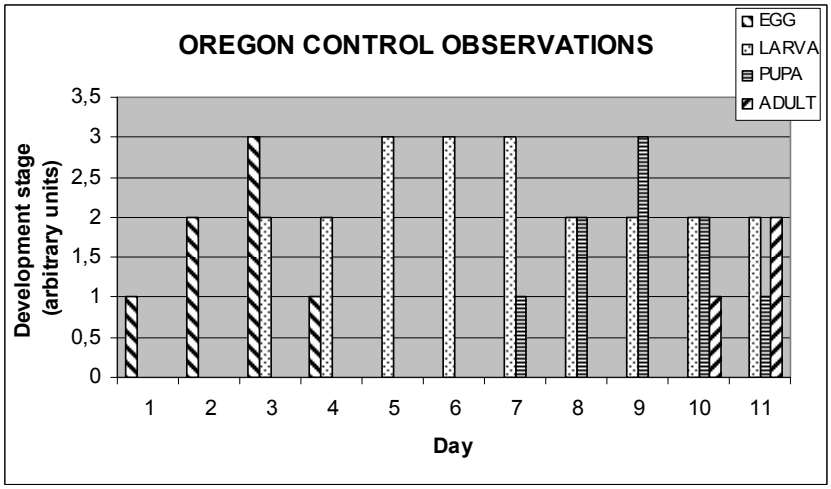
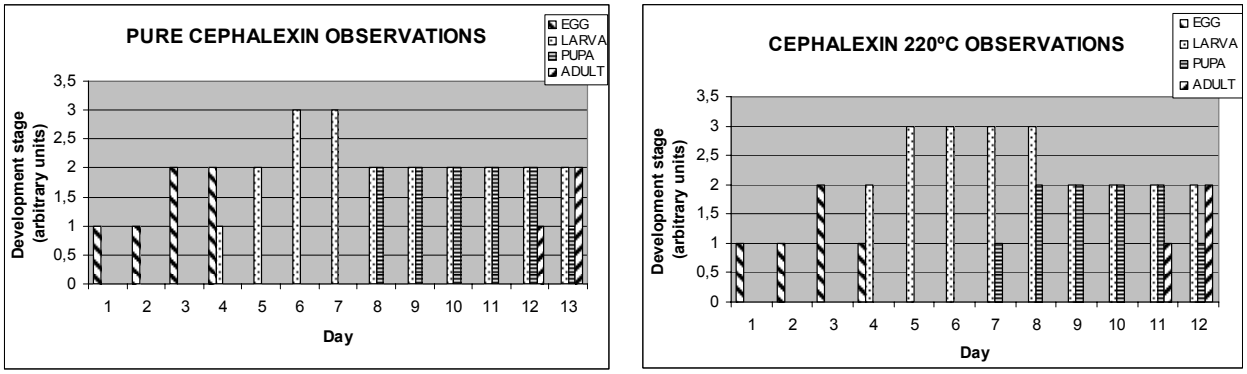


Fig. 5 – The egg-adult cycle *Drosophila melanogaster* using the reference sample.



(a) (b)

Fig. 6 – The egg-adult cycle *Drosophila melanogaster* using the samples which contained the cephalexin before (a) and after heat treatment at 220°C (b).

Table 1

The fertility coefficient for *Drosophila melanogaster* using the pure active substances

Sample	Reference	Cephalexine	Cefoperazone	Cefadroxil
Fertility coefficient	3.025	6.066	2.716	3.483

The effect of thermal degradation, i.e. the toxicity of the degradation products, is demonstrated in Table 2. For cephalexine residue, the fertility coefficient was reduced by half the value of pure compound; for cefoperazone, the small increasing of the fertility coefficient after the heat treatment is probably due to the loss of the azine side chain, well-know for the intrinsic toxicity. For cefadroxil, it is also observed an increasing of toxicity of residue in comparison with the pure compound, but not so dramatic like for cephalexine.

Regarding the male/female ratio (see Table 3) no significant changes were observed, therefore an obvious effect on the sex chromosomes is difficult to be sentenced.

Table 2

The fertility coefficient for *Drosophila melanogaster* using the active substances char at 220°C

Sample	Reference	Cephalexine char	Cefoperazone char	Cefadroxil char
Fertility coefficient	3.025	3.166	3.033	2.666

Table 3

The male/female ratio for *Drosophila melanogaster*

	Reference	Cephalexin pure	Cephalexin 220ºC	Cefadroxil pure	Cefadroxil 220ºC	Cefoperazone pure	Cefoperazone 220ºC
Male / female ratio	0.919	0.845	0.863	0.960	0.982	1.322	1.133

EXPERIMENTAL

The active substances which were utilized in this article are cefadroxil, cephalexin and cefoperazone, three cephalosporins obtained from Antibiotice Iaşi, Roumania.

Thermogravimetrical analysis (TG and DTG) was performed on Perkin–Elmer DIAMOND equipment in temperature range 25–250°C, using an air atmosphere and under dynamic conditions in order to study the thermal stability of the active substance. Samples with the mass in the range of 2 to 5 mg were put into aluminium crucibles, at a heating rate, β=7°C·min^{-1}.

The IR spectra of the active substances before the heat treatment were obtained at room temperature in the range 4000–400 cm–1 in KBr pellets using Jasco FTIR–670 Plus spectrophotometer, respectively the spectra corresponding to the samples after the thermal decomposition were recorded on a Perkin Elmer Spectrum 100 device using the U-ATR technique.

Drosophila grows in nature on fruits which began to ferment. In laboratory conditions, a good culture's medium must contain sugar or a source of sugar, the yeast and a substance such as gelatine or agar to give some texture to the environment. It was used the culture's medium with cream of wheat with added gelatin and sugar. The medium was boiled for 15 minutes, then it was added 0.5 ml propionic acid and it was anointed with a mixture containing yeast in order to prevent the development of fungi on the culture medium. Over this culture's medium, were added the three cephalosporin's

substances which were analyzed (cephalexin, cefoperazone, cefadroxil), and the residues from thermal treatment of active substances at temperature of 220ºC, the amount added in all cases being 35mg.

For each sample were used two blank and five tubes containing the analyte. In each tube were introduced wild type *Drosophila melanogaster* adults, four females and eight males each. During three weeks it was observed the life cycle of fly samples in the normal culture and in the analyte culture.

CONCLUSIONS

The thermal analysis coupled with the determination of IR spectra by the UATR technique is a rapid and versatile way for estimation of the potential toxicity due to a thermal decomposition of pharmaceuticals.

The biological answer obtained by the evolution of *Drosophila melanogaster*, even if qualitative, is enough for the estimation of the toxicity induced by the thermal decomposition of a compound, in our cases some pharmaceuticals.

For the studied cephalosporins, the relative simplest structure of cephalexin is the most thermostable one and also with the best influence on the life cycle of *Drosophila melanogaster*. The azine side group of cefoperazone determines a certain toxicity of the pure compound, which is reduced after the disappearance of this side group, due to the thermal degradation.

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