**Universitatea "Babeş-Bolyai", Cluj-Napoca**

Facultatea de Fizică
&
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# Study of structure and properties of new solid forms of 5-fluorouracil with piperazine

Ph.D. Thesis Summary

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**CONTENTS**

**Keywords**: 5-Fluorouracil; piperazine; solid forms; XRD; FTIR, X-ray diffraction, polymorphs, co-crystal; salt; XPS

**SUMMARY**
The main objective of this doctoral dissertation was to identify and investigate new solid forms - polymorphs, salts or co-crystals - of a pharmaceutically active compound used in various cancer treatments, namely 5-fluorouracil. The dissertation contains four chapters describing the purpose, methods of preparation and analysis of the samples and the original results obtained in the s performed studies.

## EXPERIMENTAL RESULTS

### XRD checking of crystalline forms resulted by slow evaporation of 5-fluorouracil solutions obtained with different solvents

Polymorphism has important consequences in the development of drugs. The existence of multiple crystal forms with differences in the solid-state properties can translate into significant effects on the bioavailability of the active drug substance [1].

The 5-fluorouracil is an efficient agent largely used in the clinical practice for treatment of solid tumors. It is an antimetabolite drug of pyrimidine class with antiviraland anticancer activities [2] and is one of the most effective chemotherapeutic agents administered in colorectal cancer treatment [3-5]. This chemotherapeutic agent has N–H donors and C=O acceptors (Fig. 1) and exhibits the diversity of hydrogen bonding motifs from a crystal engineering viewpoint [6].

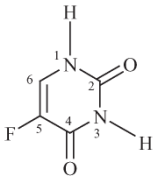


**Fig. 1 Chemical structure of 5-fluorouracil**

Nevertheless, 5-fluorouracil is sparingly soluble in water and slightly soluble in alcohol, and therefore the testing of its solubility in different solvents, as well as the identification of 5-fluorouracil polymorphs could be of biomedical interest. Such results are important also in the approach to obtain co-crystals or salts with proper coformers for achieving new pharmaceutical solid forms which may be may influenced by a particular solvent system[7].

In this study we carried out apreliminar investigation on solubility and polymorphism of 5-fluorouracil compound by initial solvent screening for 14 solvents.

The test on the solubility and the identification of 5-fluorouracil (noted 5-FU) polymorphs was accomplished by solving 5-FU in different solvents, followed by crystallization at room temperature by slow solvent evaporation, and X-ray diffraction analysis of the resulted crystalline phases. The X-ray diffraction (XRD) patterns were recorded with a Shimadzu XRD-6000 diffractometer with graphite monochromator. The measurements were performed at room temperature, in 2θ range between 3–35°, with Cu $K_\alpha$ radiation ($\lambda$ = 1.54178 Å, operating conditions 40 kV and 30 mA).

An amount of about 20 mg 5-FU,provided by Alfa Aesar,was added to 1000 µl of 14 solvents and the mixtures were heated at 40ºC. The 1000 µl of each solvent was progressively supplied in 5 steps of 200 µl each one. Under these conditions the 5-FU dissolution was obtained only in water. Consequently, to the other solvents were added amounts of 200-500 µl water (Table 1).

With respect to the solubility test, excepting the solvents based on n-heptane, toluen, 3 dimethyl-2butanone and dichloromethane which led to suspensions, in the case of all other solvents we could obtain 5-FU solutions after water addition to about 20 mg 5-FU and 1000 µl solvent (Table 1).

***Table 1*** Solubility test data

| Crt.nr. | Solvent | 5-FU (mg) | Added water (µl) | Observation |
|---|---|---|---|---|
| 1 | water | 20.8 | 200 | solution |
| 2 | ethanol | 19.9 | 400 | solution |
| 3 | dioxane | 19.8 | 400 | solution |
| 4 | acetonitrile | 20.4 | 200 | solution |
| 5 | 2,2,2 trifluoroethanol | 19.7 | 500 | solution |
| 6 | n-heptane | 19.6 | 400 | suspension |
| 7 | ethylacetate | 20.0 | 400 | solution |
| 8 | toluen | 19.5 | 400 | suspension |
| 9 | 3dimethyl-2butanone | 20.7 | 400 | suspension |
| 10 | dichloromethane | 20.2 | 500 | suspension |
| 11 | tetrahydrofuran | 20.6 | 500 | solution |
| 12 | etoxyethanol | 19.8 | 500 | solution |
| 13 | methanol | 20.3 | 500 | solution |
| 14 | 2-butanone | 20.1 | 500 | solution |

The X-ray pattern difractionrecorded from the powdered samples obtained after slow evaporation show that the only sample which clearly delivers a diffrent diffractogam from that of

5-FU is obtained for the sample resulted from the 5-FU solution with acetonitrile (Fig. 2), while all other samples deliver XRD patterns with the characteristic lines of 5-FU (Figs. 3 and 4).

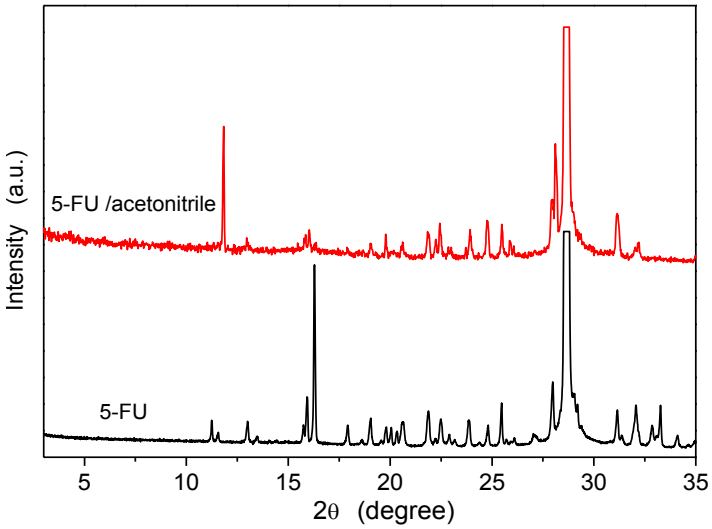


**Fig. 2** XRD patterns of 5-FU and crystalline form resulted after evaporation of 5-FU solved in water solution with acetonitrile.

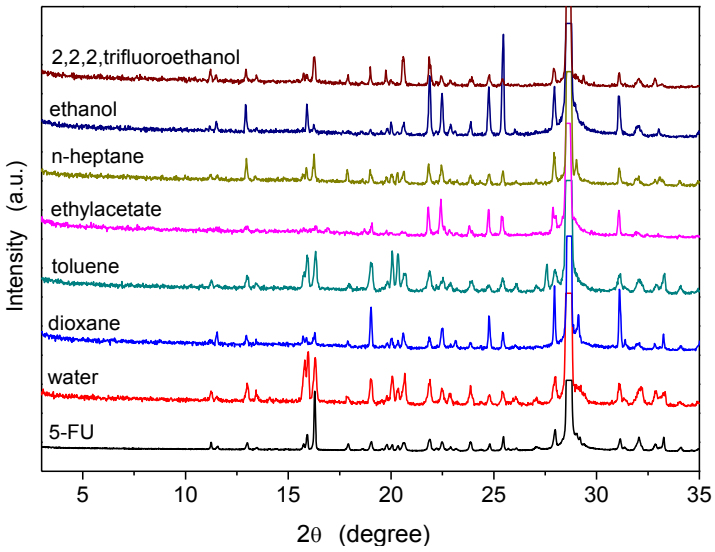


**Fig. 3** XRD patterns of crystalline forms resulted after evaporation of 5-FU solved in water and in water solutions with dioxane, toluene, ethylacetate, n-heptane, ethanol and 2,2,2-trifluoroethanol.

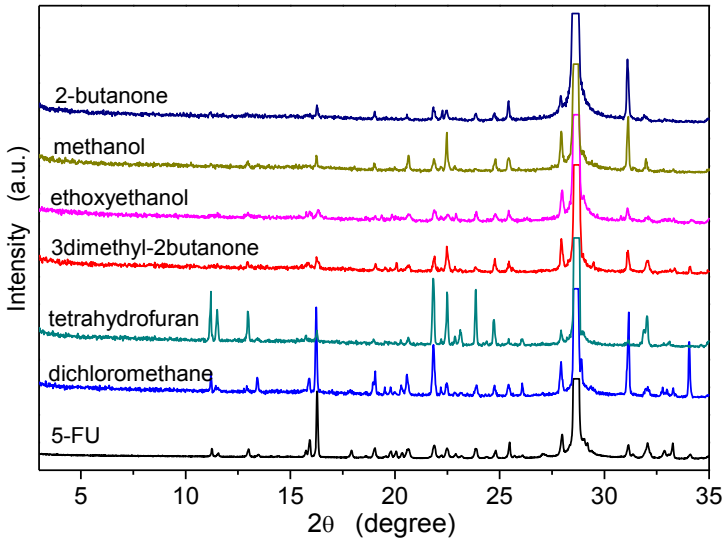


**Fig. 4** XRD patterns of crystalline forms resulted after evaporation of 5-FU solved in water solutions with dicloromethane, tetrahydrofuran, 3-dimethyl-2butanone, ethoxyethanol, methanol and 2-butanone.

The main characteristic signals in the diffraction line of 5-fluorouracil occur as a very intense peak at 2θ = 28.7°and a much weaker one at 2θ = 16.3°. Due to the very high intensity of the peak recorded at 2θ = 28.7°, this was truncated in all XRD patterns.By checking the XRD patterns of the samples resulted by slow evaporation of 5-FU aqueous solutions with different solvents, it is first remarked a very pronounced enhancement of the diffraction line at 2θ = 28.7° and the mentening of 5-FU signature (Figs.3 and 4), excepting the crystalline form resulted from the solution with acetonitrile (Fig. 2). At the same time, one notices differences related to relative intensities of the diffraction peaks and this result can be primary assigned to occurance of 5-FU hydrated forms, because in hydrated forms the lattice constants are modified [8]. For the crystalline form obtained from acetonitrile aqueous solution the XRD pattern indicated the formation of a 5-FU polymorph, with crystaline structure different from that of 5-FU before solvation.

The solubility of 5-fluorouracil (5-FU) chemotherapeutic agent was tested in 14 solvents: water, dioxane, toluene, ethylacetate, n-heptane, ethanol, 2,2,2-trifluoroethanol, dicloromethane, acetonitrile, tetrahydrofuran, 3-dimethyl-2butanone, ethoxyethanol, methanol and 2-butanone. By heating at 40$^{o}$C, an amount of about 20 mg FU can be solved in 1.2 ml water or in aqueous solutions obtained by addition of 0.2-0.5 ml water to 1 ml solvent, excepting n-heptane, toluen, 3 dimethyl-2butanone and dichloromethane, in which, under these solubility test conditions appeared suspensions.

The structure of the samples resulted by slow evaporation of 5-FU solutions and suspensions was examined by checking their XRD patterns. Only for the crystalline form obtained from acetonitrile aqueous solution the XRD pattern indicates the formation of a 5-FU polymorph, while in all other cases the crystalline forms may are structurally similar to 5-FU before solvation and could be assigned to hydrated forms of 5-FU.

The 5-FU polymorph obtained by slow evaporation of acetonitrile aqueous solution could pay attention for further investigations related to new solid forms of 5-FU active drug substance.

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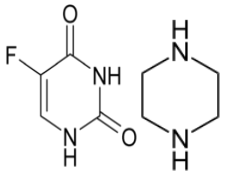
## New solid state forms of antineoplastic 5-fluorouracil with anthelmintic piperazine

Two new solid forms of antineoplastic agent 5-fluorouracil with anthelmintic piperazine were obtained by liquid assisted ball milling and slurry crystallization methods. The N-H hydrogen bonding donors and C = O hydrogen bonding acceptors of 5-fluorouracil allow to form co-crystals with other drugs delivering improved properties for medical applications, as proved for other compounds of pharmaceutical interest. Only one of the new 5-fluorouracil with piperazine solid forms was obtained as co-crystal, namely that prepared by liquid assisted grinding process, and the other one, prepared by slurry method, resulted as a salt. Both new solid forms were investigated using X-ray powder diffraction, differential thermal analysis and Fourier transform infrared spectroscopy.




***5-fluorouracil*** ***piperazine***

XRPD patterns were obtained by using a Shimatzu XRD-6000 diffractometer with graphite monochromator in the incident beam, 2D=6.708 Å. The experimental conditions were: the 2θ range between 3–40°, Cu K$\alpha$1 radiation ($\lambda$ = 1.5406 Å) (40 kV; 30 mA), measurements were performed at room temperature. The samples were mildly pre-ground in an agate mortar in order to control crystals crystallites size and to minimize the preferred orientation which creates a systematic error in the observed diffraction peak intensities.

DTA/TGA analysis, were performed with a Simultaneous Thermogravimetric and Differential Thermal Analyzer, Shimadzu DTG-60H. The sample was heated in the range of 24–500°C with a heating rate of 10°C/min in alumina sample cell (diameter 5.8 mm × 2.5 mm) under dry nitrogen purge (70 ml/min).

In order to detect vibration modes of the functional groups, the all samples it was investigated by Fourier transform infrared spectroscopy, using a JASCO 6200 FTIR spectrometer (number of scans, 256; resolution 4 $cm^{-1}$; range 4000–400 cm−1).The KBr pellets were prepared by mixing

In order to detect vibration modes of the functional groups, the all samples it was investigated by

1.2 mg of sample with 150 mg KBr, and pressing the mixture into a 13 mm disks at 12 tones pressure. The spectra were analyzed using Spectra Analysis software.

The X-ray diffraction powder patterns for 5-fluorouracil, piperazine and the two new solid forms of 5-fluorouracil with piperazine (Fig.1) evidence structural differences between the starting materials and the obtained co-crystal and salt, and point out the presence of new crystalline phases. For 5-fluorouracil the characteristic diffraction line [17] occurs very intense at 2Θ = 28.7°and a very weak diffraction peak is recorded at 2Θ = 16.3°, while for piperazine the most intense diffraction line is at 2Θ = 20.6° and several relative intense peaks are recorded at 15.8°, 17.4°, 18.6°, 20.0°, 22.6° and 27.4°. The diffraction lines occurring in the diffractograms patterns recorded from 5-fluorouracil and piperazine are missing or shifted in the new solid form diffractograms patterns.

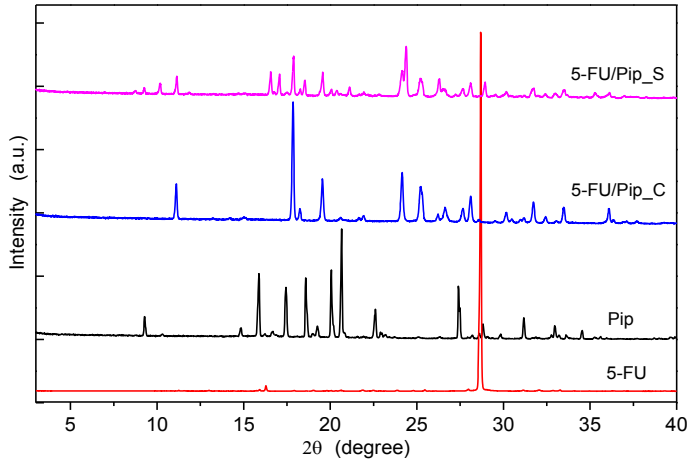


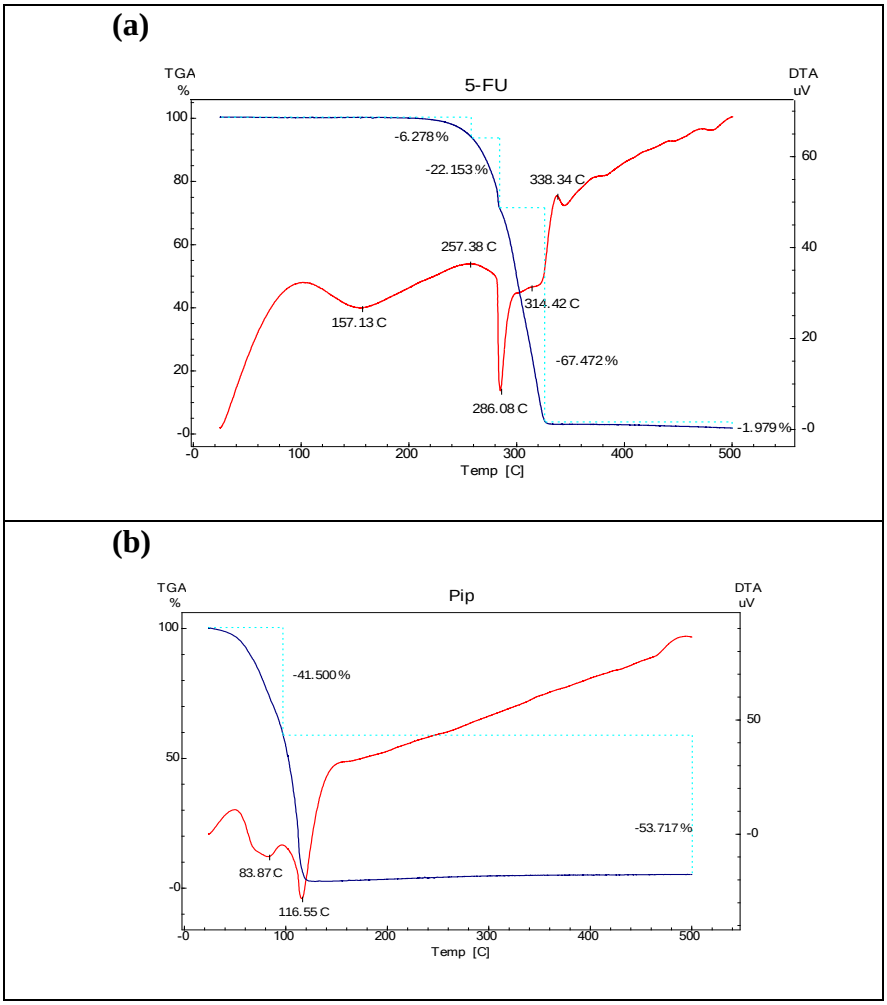

**Fig. 1 XRPD patterns of 5-fluorouracil and piperazine, and of their new solid forms**.

For the solid form obtained as co-crystal (5-FU/Pip_C) a new diffraction line occurs at 2Θ = 11°, while for the solid form obtained as salt (5-FU/Pip_S) new peaks are recorded between 2Θ = 8.6° and 2Θ = 11°. As well new diffraction lines observed neither in 5-Fu nor in Pip components occur between 24.4° and 27.4°. The diffraction patterns of the two components before and after their combination by ball milling or slurry method prove the formation of different structures with respect to the starting compounds, and the sestructures characterize two new solid forms of 5-fluorouracil with piperazine.

The DTA-TGA curves for 5fluorouracil (Fig. 2a) show good thermogravimetric stability up to its melting evidenced in DTA run by the endothermic event centered around la 286 °C. The weaker

endothermic event at 157°C is related to the begin of a structural decomposition. TGA thermogram indicates a weak mass loss of 6.3% in the narrow temperature a range between 220 and 260°C, and a substantial mass loss, about 90%, showing that the 5-fluorouracil undergoes a rapid decomposition [17, 18]. The DTA curve of piperazine(Fig. 2b) shows two endothermic events; the first one around 84°C is assigned to water removal, and the second one around 116°C to piperazine melting and decomposition with a very large mass loss pointed out in TGA curve. For the new solid forms of 5-fluorouracilwith piperazine, the first endothermic events recorded before the melting at about 285°C for co-crystal and 281°C for salt (Fig. 3) are assigned to decomposition processes [6, 19] accompanied by mass loss.

**Fig. 2 DTA/TGA traces of 5-fluorouracil and piperazine**



Hydrogen bonds between proton donors and proton acceptors play an essential role in determining the properties of molecular systems. The infrared spectra are very sensitive to the position of the proton, and this may provide information on co-crystal or salt formation [20].

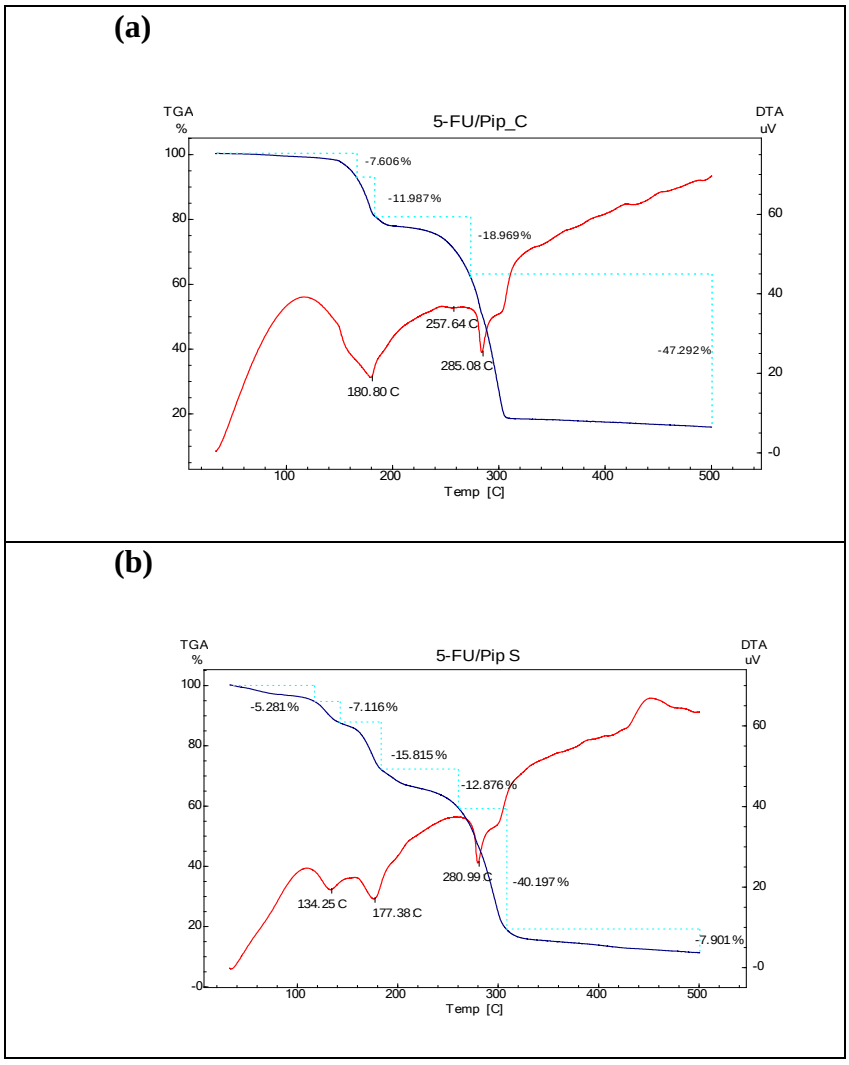



**Fig. 3 DTA/TGA traces of the new solid forms of 5-fluorouracil with piperazine.**

The FTIR spectra of 5-fluorouraciland piperazine, and of their two new solid forms 5-FU/Pip_C and 5-FU/Pip_S foremost indicate the changes of hydrogen bonding in the new solid forms. The formation of co-crystals of an active pharmaceutical ingredient and a coformer is based on several intermolecular interactions including hydrogen bonds, and unlike salt formation, no proton transfer occurs between the active pharmaceutical ingredient and coformer [ 21]. In the chemical structure

of 5-fluorouracil there are N-H hydrogen-bonding donors and C=O hydrogen-bonding receptors which could form hydrogen-bonding synthons in 5-fluorouracil co-crystals. Hydrogen bonds affect in particular the vibrational frequencies in compounds containing N-H and C=O functional groups. The changes in the vibrational modes of these groups could be accountable for the co-crystal or salts formation [6, 22]. The absorption bands assigned to vibrations of N-H, =C-H and C=O bonds in5-fluorouracil [23] appear for the investigated 5-FU simple at 3135 $cm^{-1}$, 3067 $cm^{-1}$ (Fig. 4) and 1666 $cm^{-1}$, (Fig. 5), respectively. For piperazine an intense absorption band assigned to N–H vibrations [19] occurs at 3210 $cm^{-1}$ (Fig. 4).The absorption bands in the region 3100–3000 cm−1 are assigned to = C–H stretching vibrations, and that at about 2940 cm−1 and 2840 cm−1 may be attributed to –CH2 vibrations. The O-H stretching band of water is recorded from both solid forms around 3440 $cm^{-1}$. The bands recorded at 1428 cm-1 and 1345 cm-1 arise from CH bending vibrations of the substituted pyrimidine compounds, and the absorption band at about 1246 cm−1 is due to the fluorine atom on the ring [17, 24, 25]

The bands assigned to N-H and =C-H vibrations in 5-FU, at 3148 cm-1 and 3074 cm-1, appear at higher wave numbers after co-crystal formation, at and this hypsochromic shift indicates that the intrinsic hydrogen bonding interactions in 5-FU are interrupted and some new hydrogen bonds are formed during co-crystallization [6, 16]. In fact, for 5-FU/Pip_C solid form is also noticed a similar blue shift to 1681 cm-1 (Fig. 5) in the position of the 1666 $cm^{-1}$ band assigned to C=O stretching vibrations, again on the account of hydrogen bonding interactions. Interrupting the hydrogen bonding and packing of a crystal could result in a higher-energy solid form that thereby could also enhance the effective aqueous solubility of a new co-crystal solid form [26] and this could be deemed also for co-crystals of 5-FU with Pip.

For 5-FU/Pip_S solid form the 3135 $cm^{-1}$ band of 5-FU does not more appear, and that at 3067 cm-1 and 1666 cm-1 are shifted to lower wave numbers at 3056 cm-1 and 1659 $cm^{-1,}$ respectively, due to salt formation by proton transfer, and on protonation the stretching vibrations are moving down [27]. The new infrared absorption bands at 3265 cm-1 and 3215 cm-1 could be related to 3210 cm-1 band assigned to N–H vibrations in piperazine after proton transfer for salt formation.

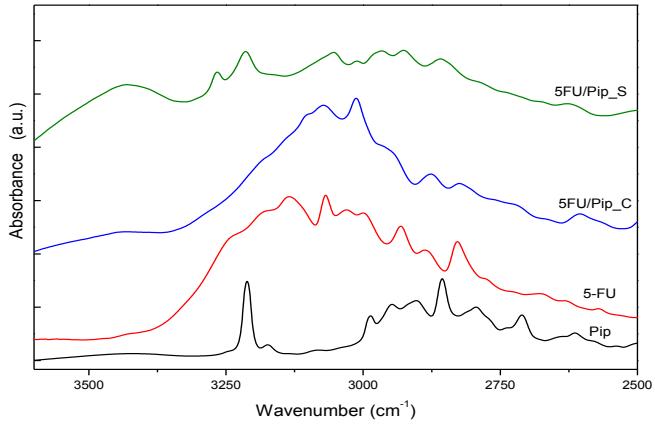



**Fig. 4 FTIR spectra of 5-fluorouracil and piperazine, and of their new solid forms, recorded in the spectral range from 4000 cm-1 to 2500 cm-1.**

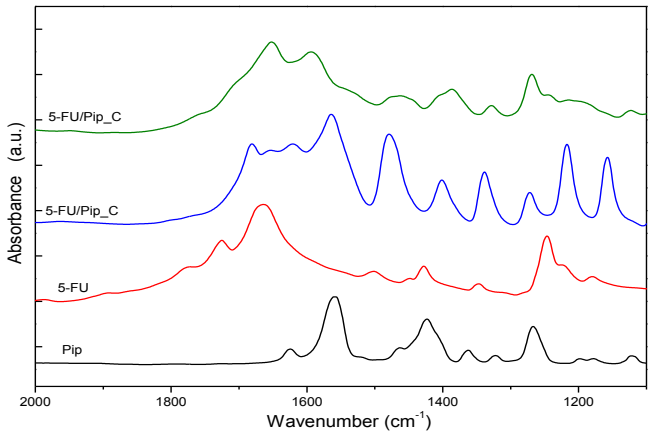



**Fig. 5 FTIR spectra of 5-fluorouracil and piperazine, and of their new solid forms, recorded in the spectral range from 2000 $cm^{-1}$ to 1100 $cm^{-1}$.**

For the first time are reported results on solid forms of antineoplastic 5-fluorouracil with anthelmintic piperazine. Two new forms were obtained by liquid assisted ball milling and slurry methods as co-crystal and salt, respectively. The XRD and FTIR results, preliminary to an extended study, point out the structural differences between the involved components and their new solid forms. The infrared absorption bands assigned to N-H, =C-H and C=O vibrations support the co-crystallization achieved by liquid assisted ball milling of the components, while the solid form prepared by slurry method results in a salt by proton transfer.

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## XPS investigation of new solid forms of 5-fluorouracil with piperazine

The present study reports the results obtained by X-ray photoelectron spectroscopy (XPS) for new solid forms of antineoplastic agent 5-fluorouracil with anthelmintic piperazine obtained by liquid assisted ball milling and slurry crystallization methods as co-crystal and salt, respectively.

The interest for these new solid forms of 5-fluorouracil with on other drug consists in obtaining new compounds with potential pharmaceutical and biomedical applications. The XPS results bring additional information on the atomic environments in the newly obtained solid forms of 5-fluorouracil with piperazine beside the earlier reported X-ray diffraction and infrared spectroscopy data. The deconvolution of N 1s core level spectra allows distinguishing unequivocally the protonated (salt) from hydrogen-bonded (co-crystal) nitrogen species based on the N 1s binding energy associated with the protonation of nitrogen atoms. The N 1s component around 402 eV observed only for the solid form obtained following the slurry route proves in this case the formation of a salt on basis of positively charged nitrogen resulted by protonation of secondary amine groups. Moreover, for the solid form obtained following the ball milling route, one notices a large negative shift of N 1s binding energy that supports the development of a co-crystal.The detailed procedures used for the synthesis of 5-fluorouracil&piperazine solid forms were previously presented [11].

The XPS spectra of the resulted samples were recorded with a SPECS PHOIBOS 150 MCD system equipped with monochromatic Al-$K_\alpha$ source (1486.6 eV), hemispherical analyser, multichannel detector and charge neutralization device. Samples were fixed on a double-sided carbon tape and care was taken to ensure that the sample particles covered the tape. The experiments were performed by operating the X-ray source with a power of 200 W, while the pressure in the analyse chamber was in the range of $10^{-9}$- $10^{-10}$ mbar. Charge neutralization was used for all samples. The binding energy scale was charge referenced to the C 1s at 284.6 eV. High resolution spectra were obtained using analyzer pass energy of 20 eV. Elemental compositions were determined from survey spectra acquired at pass energy of 100 eV. Analysis of the data was carried out with Casa XPS software. A Shirley background was used for all curve-fitting along with the Gaussian/Lorentzian product form (70% Gaussian and 30% Lorentzian).

As already mentioned, previous XRD and FTIR results [11] on new solid forms of 5-fluorouracil with piperazine, prepared by two different routes, signalised that the liquid assisted grinding route lead to co-crystal formation, while the slurry compounding route to salt formation. The structural

changes occurring during the two synthesis processes were further analysed using XPS technique, which is commonly used in the analysis of materials providing information on the elemental composition, atomic environments and bondings on the outermost layer of a sample [15-17].

The elemental composition on the surface of the samples (Table 1) provided by the XPS wide-scan spectra (Fig. 1) differ from the expected composition calculated for the pure 5-fluorouracil ($C_4H_3FN_2O_2$) and piperazine ($C_4H_{10}N_2$).Anyway, by XPS analysis is not possible to detect hydrogen because its 1s photoelectron has a very small cross-section for photoemission. At the same time, changes may occur due to samples exposure to atmospheric oxygen and carbon contamination

For piperazine the theoretical atomic ratios N/C and O/C are 0.5 and 0, respectively. The enhanced values experimentally obtained by XPS analysis for both carbon and oxygen on the surface of the piperazine sample, which before analysis was exposed to atmosphere, lead to N/C=0.05 and O/C=0.23 ratios. Oxygen contaminations up to 8 at % at were reported for samples which should not contain oxygen and are assumed to arise from the presence of oxygen containing adsorbates such as tightly bound water [18, 19].

Moreover, piperazine is strongly hygroscopic and prolonged exposure to the air leads to the formation of a hexahydrate [20, 21], but this would be not an obstruction to co-crystals or salts formation.

For 5-fluorouracil sample the theoretical atomic ratios are N/C=0.5 and O/C=0.5. On sample surface these values are lower, i.e. N/C=0.43 and O/C=0.38, due to carbon contamination.

For both 5-FU/Pip_C and5-FU/Pip_S solid forms these ratios are relatively close, N/C=0.35 and O/C=0.24, and N/C=0.32 and O/C=0.21, respectively.

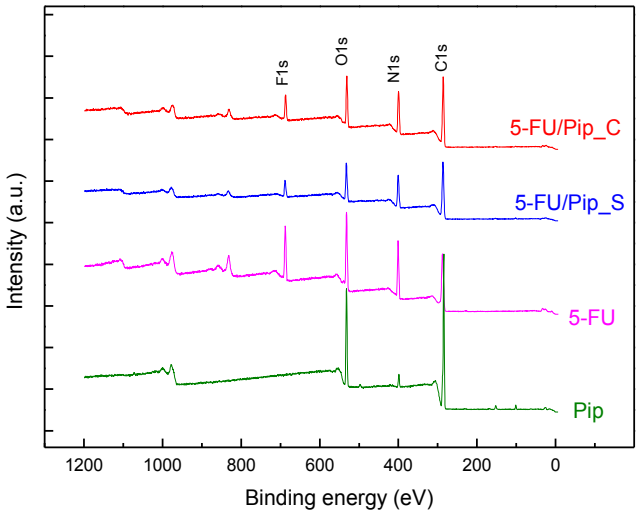



**Fig. 1 XPS survey spectra**

**Table 1 Elemental composition determined by XPS analysis on the outermost layer of samples**

| Sample | Elemental concentration (at %) | | | |
|---|---|---|---|---|
| | **C** | **O** | **N** | **F** |
| 5-FU/Pip_C | 59.7 | 14.3 | 20.9 | 5.1 |
| 5-FU/Pip_S | 62.7 | 13.4 | 19.7 | 4.2 |
| 5-FU | 50 | 18.9 | 21.5 | 9.6 |
| Pip | 77.7 | 18.5 | 3.8 | - |

For evidencing structural changes related to achievement of new solid forms, more important appears the analysis of the core level spectra of C 1s and N 1s photoelectrons (Fig. 2).

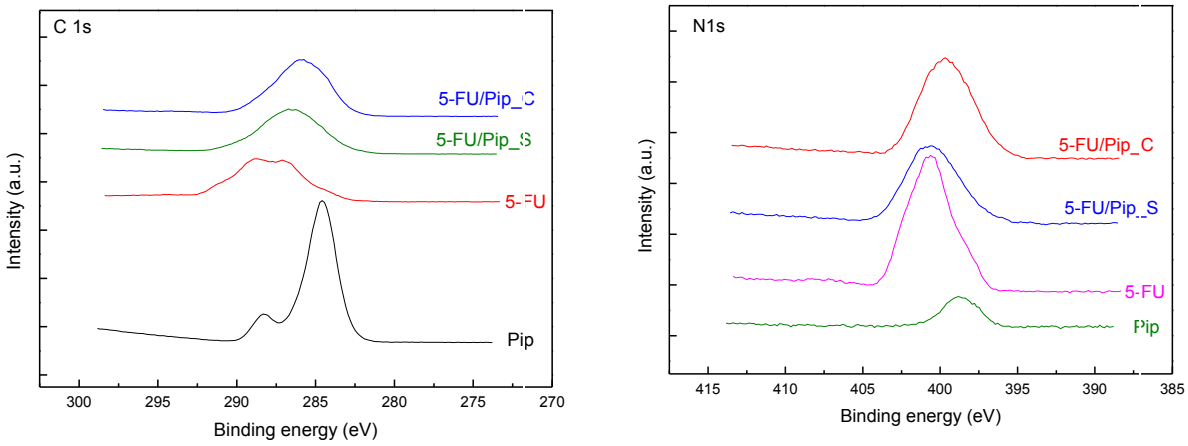
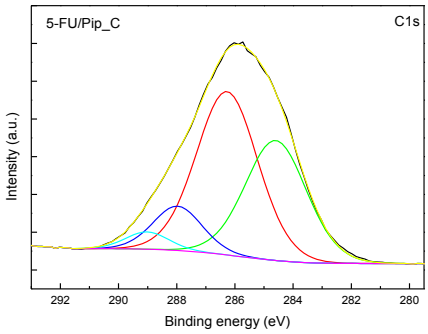
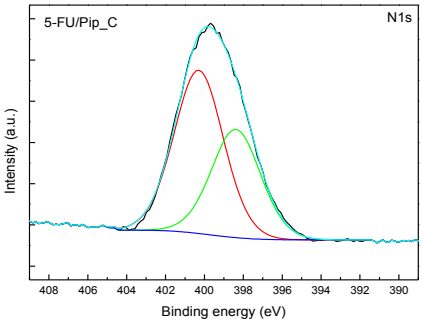
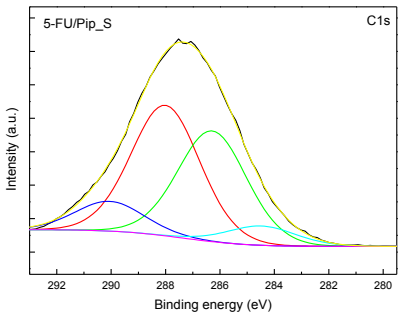


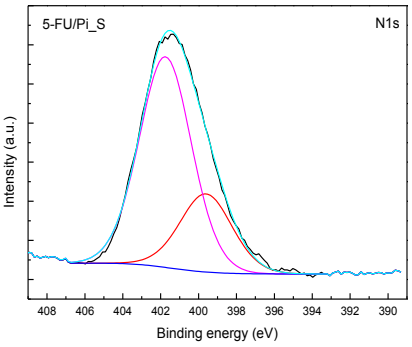

**Fig. 2 C 1s and N 1s high resolution spectra**

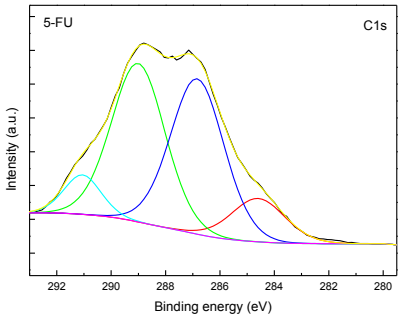
The C 1s core level spectra of 5FU/Pip solid forms are shifted to lower binding energies relative to 5-fluorouracil on account of piperazine. For deconvolution of C 1s core level spectra (Fig. 3) the components used were positioned closed to 284.6, 286.2, 288.3 eV and 291 eV. The 284.6 eV component is assigned to C–C, C–H bonds, including the carbon contamination, the 286.2 eV component to C–N, C-O bonds, including oxygen contamination, that at 288.3 eV to C=O bonds, and 291 eV component is related to C-F bonds [22-26]. The deconvolution of piperazine C 1s core level spectrum (Fig. 3) reveals three components with binding energies at 284.6, 286.2 and 288.3 eV, while C 1s core level spectra of 5-fluorouracil and 5FU/Pip solid forms reveals a fourth component at 291 eV assigned to carbon atoms involved in C-F bonds.

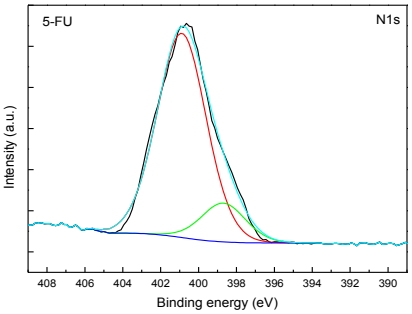

5-FU/Pip_C
C1s
Intensity (a.u.)
292
290
288
286
284
282
280
Binding energy (eV)


5-FU/Pip_C
N1s
Intensity (a.u.)
408
406
404
402
400
398
396
394
392
390
Binding energy (eV)


5-FU/Pip_S
C1s
Intensity (a.u.)
292
290
288
286
284
282
280
Binding energy (eV)


5-FU/Pi_S
N1s
Intensity (a.u.)
408
406
404
402
400
398
396
394
392
390
Binding energy (eV)


5-FU
C1s
Intensity (a.u.)
292
290
288
286
284
282
280
Binding energy (eV)


5-FU
N1s
Intensity (a.u.)
408
406
404
402
400
398
396
394
392
390
Binding energy (eV)

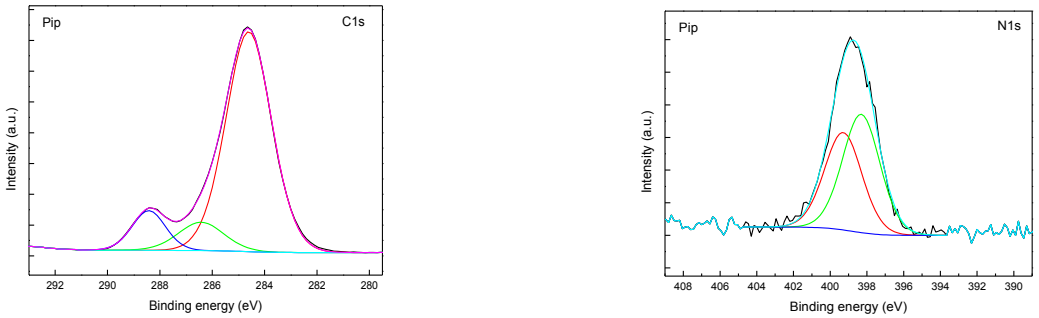



**Fig. 3 The deconvolution of C1s and N1s high resolution spectra for all the samples**

A first inspection of N 1s core level cpectra (Fig. 2) makes clearly visible a large negative shift of binding energy for 5-FU/Pip_C solid form obtained via liquid assisted ball milling route.

Based on the N 1s binding energy associated with the protonation of nitrogen atoms, the deconvolution of N 1s core level spectra (Fig. 3) allows to distinguish unequivocally the protonated (salt) from hydrogen-bonded (co-crystal) nitrogen species [28, 29].

Very recently it was reported that 5-fluorouracil may change under certain medium conditions the protonation degree of the amine groups [30]. The $–NH_2^+$ group resulting from the protonated secondary amine –NH is discernable in the signal delivered by nitrogen photoelectrons. The proton transfer to nitrogen is evidenced in XPS N 1s core level spectra by a positive shift of the binding energy [28, 29, 31].

The N1s envelope of 5-FU and Pip as well as of 5-FU /Pip -C solid form are well fitted with two components around 398.5, and 400 eV (Table 3). For 5-FU /Pip_S solid form, the two deconvolution components of N 1s photoelectron peak which appears shifted to lower binding energies are at 399.6 and 401.7 eV. The components around 398.5eV could be assigned to C-NH-C structural groups [25, 32, 33], that around 400 eV to structural bondings N-C=O [25] or to N-C=O, N-C-OH surface groups formed due to the previous atmospheric exposure of all the samples. The 401.7eV component evidenced only in N1s spectrum of 5-FU/Pip-S solid form is specific to protonated amine species $C-NH2^+$ [14, 24] and proves the salt formation. The negative shift evidenced for N 1s components in case of 5-FU/Pip_C solid form, compared to of 5-FU/Pip_S form, excludes the protonation of nitrogen atoms, and this shift is related to co-crystals development [25].

The analysis O1s spectrum is of interest because of the presence in such consistent amount of oxygen even for piperazine that does not contain oxygen in the structural formula. Information provided by deconvolution of O1s core level spectra (Fig. 4) can complete and support the information provided by analysis of C1s and N 1s spectra.

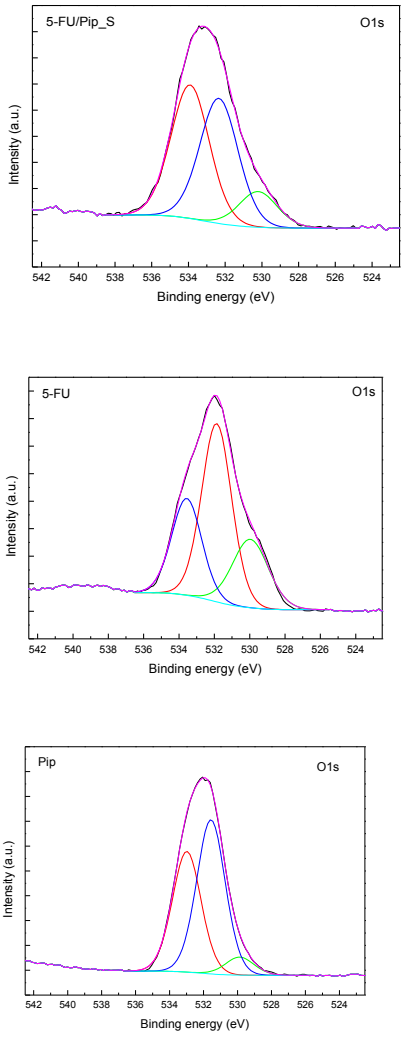



**Fig. 4 The deconvolution of O1s high resolution spectra for all the samples**

The O1s peak for all the studied samples can be deconvoluted with three components as follows: the component around 530eV assignable to contributions from oxygen double bonded with carbon in structural groups or/and surface species C=O, O=C-N or O=C–OH [24], around 532eV to oxygen single bonded with carbon in O-C=O (C-O ) groups [24], and the last one at 533 eV related to C–OH groups and/or chemisorbed water. [25, 26].

Solid forms of antineoplastic 5-fluorouracil with anthelmintic piperazine obtained by liquid assisted ball milling and slurry methods were characterised by X-ray photoelectron spectroscopy (XPS). The XPS results decidedly support the co-crystal formation by liquid assisted ball milling, and the salt by slurry method under the used synthesis conditions. Based on the N 1s binding energy associated with the protonation of nitrogen atoms, the deconvolution of N 1s core level spectra allowed to distinguish the protonated nitrogen species implied in salt from the hydrogen-bonded nitrogen species implied in co-crystal. The N 1s component around 402 eV observed only for the solid form obtained following the slurry route proves in this case the formation of a salt on basis of positively charged nitrogen resulted by protonation of secondary amine groups. For the solid form obtained following the ball milling route, one notices a large negative shift of N 1s binding energy that supports the development of a co-crystal. The XPS analysis technique proved to be a powerful tool in the investigation of the new solid forms obtained with 5-fluorouracil and piperazine pharmaceutical drug substances potentially considered to be of biomedical and pharmaceutical interest.

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## Study about gamma irradiation for new co-crystal of 5-Fluorouracil with anthelmintic drug

In order to verify that the co-crystal could be influenced by the radiation emitted by Iodine, the 5-FU / Pip_C co-crystal sample was selected, which had been fused to a Iodine I-131 source at a source- like activity of 117.4 mCi (4.34 GBq), and at a distance of 1 m from the source for one hour, three hours, five hours, and 24 hours, respectively. Knowing the exposure time for each product, activity, and the stability of the isotope I-131 (0.22 mR / hr per mCi at 1 m or 7.647 E-5 mSv / hr per MBq at 1 m distance), the dose was absorbed by each individual sample.

The absorbed dose values are shown in the following table**(Table III.9)** After analysis, the X-ray and Fourier Transform Spectroscopy (FT-IR) spectroscopy were analyzed for the final identification of structural modifications in samples after dilution of the smear beam.

**Table III.9** The absorbed dose values

| Sample | Exposure time [h] | Dose rate [ mSv / h] | Absorbed Dose [mGy] |
|---|---|---|---|
| I_0h | 0 | 0.332 | 0 |
| I_1h | 1 | | 0.332 |
| I_3h | 3 | | 0.996 |
| I_5h | 5 | | 1.66 |
| I_24h | 24 | | 7968 |

Results of sample analyzes after XRD measurements are shown in **Figure III.28.** X-ray diffractograms obtained for the 5-FU and piperazine co-crystal were analyzed for up to 24 hours. It can be seen that the angular values 2θ at which the diffraction lines appear practically are not altered. This result confirms the stability of irradiation samples following gamma irradiation, the samples did not undergo structural changes in the crystalline structure, regardless of whether the exposure time to the radiation source was different.

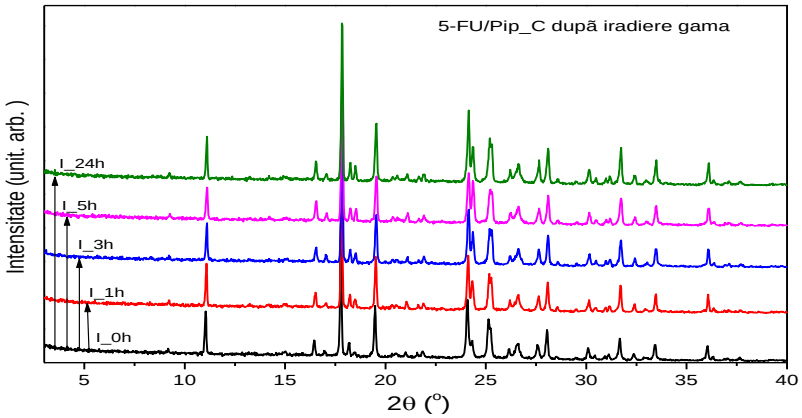


**Figure III.28** XRD Diffractograms for 5-FU / Pip_C, before and after exposure to gamma radiation

FT-IR spectroscopy confirmed the results of X-ray diffraction, namely that irradiation of the γ-radiation samples did not induce changes in the sample structure, as similar spectra were obtained even though the irradiation times were different. From **Figure III.30, it** is observed that the vibrations identified in the samples before gamma irradiation occur at near values in the spectra obtained for irradiated samples. R results show that the position of the absorption bands in the spectra obtained for the irradiated samples identified values close to those obtained for the non-irradiated samples.

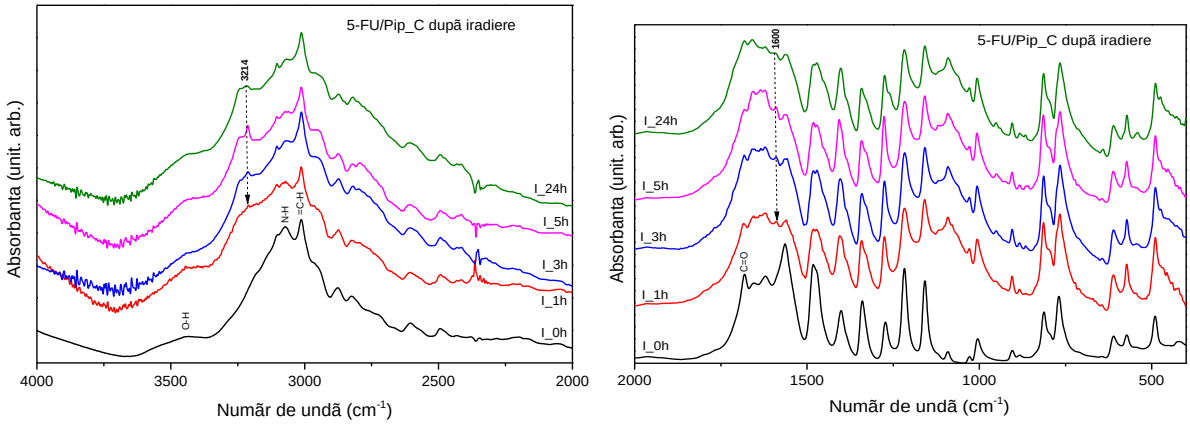


**Figure III.30** FT-IR spectra for the 5-FU / P-co-crystal co-crystal and after exposure to gamma radiation

These minor changes in sprays of irradiated samples may occur due to deformation of the crystalline grid under the action of gamma radiation when the crystallite size decreases, which cannot influence the properties of the substances.
The results obtained by X-ray diffraction and FT-IR spectroscopy revealed that no structural changes occurred in the obtained co-crystal, supporting the stability of this new form obtained by exposure to gamma radiation at various absorbed dose.

## CONCLUSIONS and perspectives

With the objective of obtaining, identifying and investigating new solid forms - polymorphs, salts or co-crystals - of 5-Fluorouracil, which is an active pharmaceutical compound used in various cancer treatments, the following conclusions are drawn from the research:

- New solid forms were synthesized between 5-fluorouracil (5-FU) and piperazine (Pip) which, before the research performed in this thesis, have not been studied in association.

- The solubility of 5-FU was tested in 14 aqueous solutions; in 10 cases solutions were obtained and in 4 suspensions resulted. The solid samples obtained in powder form after slow evaporation of the solvent recrystallization in the same structure, except in the case of aqueous acetonitrile solution.

- 5-FU solubilized in aqueous acetonitrile solution recrystallizes into a polymorph.

- The new solid forms of 5-FU with Pip were obtained by the method of mechanical mixing / grinding with solvent (solvent drop grinding - SDG) and by the method of suspension with the addition of antisolvent (slurring - SL).

- X-ray diffraction analyzes, correlated with the spectroscopic analyzes by FTIR and XPS, show that a co-crystal results from the SDG method and a salt from the SL method.

- Water dissolution tests indicate a higher solubility in the case of the new salt form.

- For the new solid forms of 5-FU irradiated Pip range at high medical doses (up to 8 mGy) no structural changes are highlighted.