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XRD CHECKING OF CRYSTALLINE FORMS RESULTED BY SLOW EVAPORATION OF 5-FLUOROURACIL SOLUTIONS OBTAINED WITH DIFFERENT SOLVENTS

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ABSTRACT. The present study aimed to obtain new crystalline forms of 5-fluorouracil (5-FU) chemotherapy drug after 5-FU solvation in different solvents, considering the crystallization by slow evaporation. The expectation was to obtain 5-FU polymorphs. 5-FU was tested in aqueous solutions of 14 solvents. After slow evaporation of solutions (11) or suspensions (3), the obtained crystalline forms were examined by checking their X-ray diffraction patterns. Only for acetonitrile solution resulted a polymorph of 5-FU.

Key words: *5-fluorouracil; solubility; X-ray diffraction; polymorphs.*

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INTRODUCTION

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 antiviral and 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].

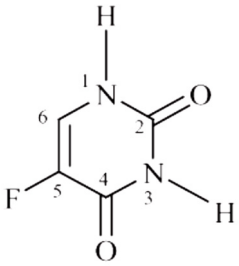

Fig. 1. Chemical structure of 5-fluorouracil

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 a preliminar investigation on solubility and polymorphism of 5-fluorouracil compound by initial solvent screening for 14 solvents.

EXPERIMENTAL

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 $K_{\alpha 1}$ radiation (λ = 1.5406 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 40°C. 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).

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

RESULTS AND DISCUSSION

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).

The X-ray diffractograms recorded from the powdered samples obtained after slow evaporation show that the only sample which clearly delivers a different 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).

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.

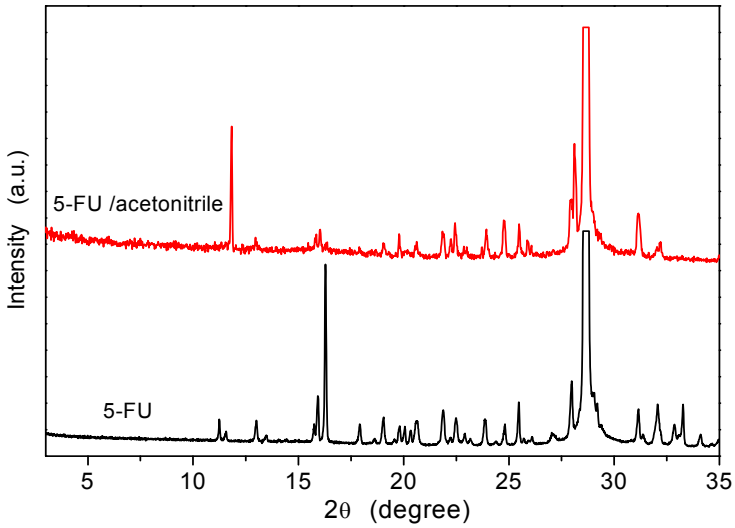

Fig. 2. XRD patterns of 5-FU and crystalline form resulted after evaporation of 5-FU solved in water solution with acetonitrile.

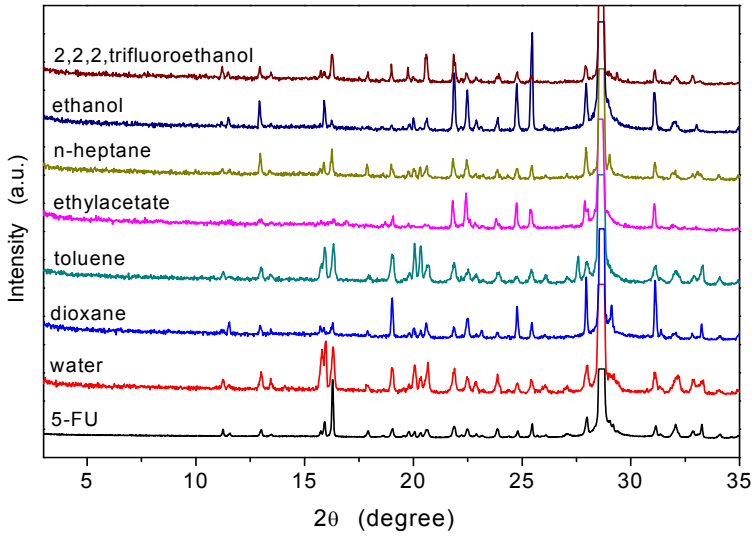

Fig. 3. XRD patterns of crystalline forms resulted after evaporation of 5-FU solved in water and in water solutions with dioxane, toluene, ethylacetate, n-heptane, ethanol and 2,2,2-trifluoroethanol.

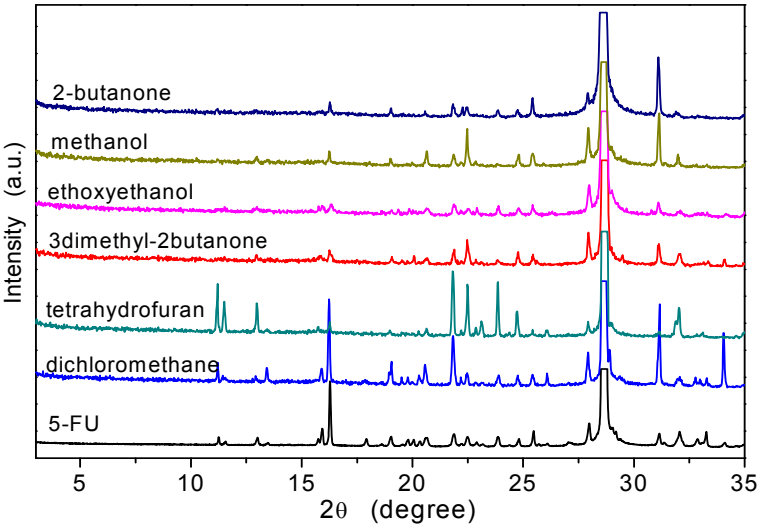

Fig. 4. XRD patterns of crystalline forms resulted after evaporation of 5-FU solved in water solutions with diclorometane, tetrahydrofuran, 3-dimethyl-2butanone, ethoxyethanol, methanol and 2-butanone.

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 $2\theta = 28.7^\circ$ 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.

CONCLUSIONS

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, diclorometh ane, 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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