J Therm Anal Calorim (2011) 105:1007–1013
DOI 10.1007/s10973-010-1012-5

Study of polymorphism of organosulfur and organoselenium compounds

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Received: 14 May 2010 / Accepted: 9 August 2010 / Published online: 31 August 2010

Abstract The thermal behavior of a series of organosulfur and organoselenium compounds has been studied by means of differential scanning calorimetry, X-ray diffraction, and thermomicroscopy in order to investigate their polymorphism. In this study, the polymorphism of some of the products has been established. The results of the experiments show that there are four types of thermal behavior for compounds studied. The physicochemical characterization of sulfur and selenium compounds showed differences in structural parameters and fusion temperatures among polymorphic forms.

Keywords Thermal analysis · Calorimetry · DSC · Polymorphism · Organoselenium · Cytotoxics

Introduction

Methods of thermal analysis are well-established techniques used in research laboratories within the pharmaceutical industry and have been extensively reviewed [1–5]. Thermal analysis techniques are especially useful for studying the behavior of the polyphasic system drug substances and excipients, and they find a unique place for delivery systems. Since changes in temperature and moisture occur due to processing and storage, changes in the solid state may have a considerable effect on the activity, toxicity, and stability of the compounds.

Owing to the different internal organization within the solid state, polymorphs may show different melting points, solubilities, chemical reactivity, or stability [6]. These can have an impact on pharmaceutical properties, such as dissolution rate and bioavailability. The effect of polymorphism on the bioavailability of a drug has been associated with different rates of dissolution of the polymorphic forms [7].

Unfortunately, polymorphism is common among pharmaceutical substances, and the evaluation of polymorphism for new drug entities is important in early preformulation studies. Statistically, about 85% of APIs (active pharmaceutical ingredients) exhibit polymorphism and 50% have multiple polymorphic forms [8].

The development of a polymorphic compound can be favored since the early stages of drug development [9]. Variations in the crystallization process, nature of the solvent, crystallization temperature, heat exchange rate, and stirring speed may potentially affect the formation of polymorphs [2]. The determination of the polymorphism of a substance is of great importance due to the strong influence of the crystalline form on the physicochemical properties, bioavailability and stability of drugs [10], and, in some compounds with biological activity, can even become metastable forms, being twice as active as the stable form [11].

An important point in studying the thermal behavior of polymorph forms is knowledge regarding the thermodynamically more stable forms at a certain temperature and under a given pressure; therefore, the less stable form can be converted into a more stable form during storage or under certain stress conditions.

The influence of certain technological pharmaceutical processes in the emergence of polymorphisms is important

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because of the involvement of said processes in the preparation of solid dosage forms. It has been observed that some pharmaceutical compounds undergo transitions during drying, spraying, grinding, and crushing processes as well as in the preparation of tablets [12].

The determination of polymorphism studies of drugs combines several techniques. The methods used include IR spectroscopy, X-ray diffraction, thermomicroscopy, and thermal methods, including differential scanning calorimetry [13, 14].

The potential drugs studied in this article correspond to organosulfur and organoselenium compounds with potent in vitro cytotoxic activity in prostate cancer cells [15]. Some of them exhibited a better antitumoral profile than etoposide, a drug that is used as first-line treatment in prostate cancer. In addition, in the past years selenium derivatives are emerging as interesting antitumoral and proapoptotic compounds [16].

Experimental

Materials

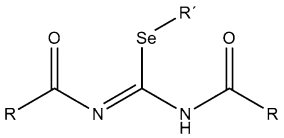
The compounds were synthesized according to a general method described by our research group [15]. The compounds had a symmetrical disubstitution with a great structural variety. In addition, compounds showed the presence of a sulfur or selenium atom with an alkyl substituent (Table 1). All of the compounds were synthesized with a high grade of purity because they had been evaluated as cytotoxic agents in biological assays. The purity of final compounds was assessed by elemental analyses and found to be >95% in all cases. Each product was identified by infrared spectroscopy, ^{1}H-NMR spectroscopy, and mass spectrometry. For some of them we have realized additional determinations such as solubility, alterations against the light, and thermomicroscopy studies.

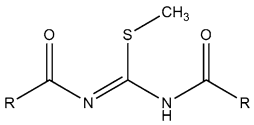
Methodology

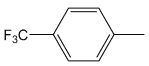
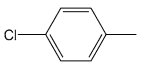
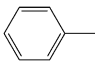
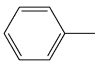
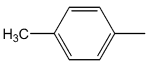
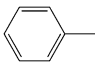
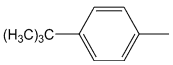
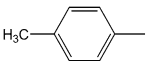
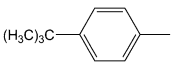
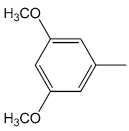
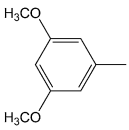
Calorimetric studies were carried out with a Perkin-Elmer DSC Diamond. The calorimeter is calibrated with indium and zinc (provided by Perkin-Elmer and manufactured according to guideline ISO35) at 10 K min^{-1} and with a nitrogen flow of 20 mL min^{-1}. The gases connected to the equipment are nitrogen and air with a purity of 99.999%.

Calorimetric analyses are carried out in aluminum capsules for volatiles of 10 μL, at a heating rate of 10 K min^{-1}, using a sample of approximately 3 mg, in order to establish the T_{onset}, T_{max}, and the enthalpy of fusion ΔH_f. All of the experiments were performed at least three times, and the values were expressed as the mean ± standard deviation.

Table 1 Selenium (Series A) and Sulfur (Series B) compounds studied





Series A			Series B	
R	*R*′	Ref.	*R*′	Ref.
	–CH_3	Se-3		S-1
	–CH_3	Se-6		S-2
	–CH_2–CH_3	Se-7		S-4
	–CH–$(CH_3)_2$	Se-8		S-5
	–CH_3	Se-10		
	–CH_3	Se-11		
	–CH_3	Se-12		
	–CH_3(–CH_3)$_2$	Se-13		

The *patterns of X-ray diffraction* are recorded on a powder Bruker D8 Advance model diffractometer with a Göbel mirror and parallel beam optics. The equipment for measuring consists of an X-ray generator with Cu anode of 2.2 kW, a slit of 1° in the primary beam and 2° Soller slit, a Göbel mirror for Cu tube with collimator 0.15°, and a NaI detector (thallium doped).

Results and discussion

Calorimetric study

Studying the thermal behavior of organoselenium and organosulfur derivatives [17] evidenced that some of these compounds have shown an interesting calorimetric behavior with two endothermic peaks in the DSC thermograms. The thermal behavior of these organoselenium and organosulfur compounds (series A and B, Table 1) has been studied before beginning the process of degradation in order to evidence the possible polymorphism of these compounds (Table 2).

For this study, samples of selected compounds are exposed to successive cycles of heating–cooling. The results below show that there are four types of thermal behavior for the series A and B:

- *Behavior I* Compounds that do not alter the thermal behavior after an initial fusion–recrystallization cycle (Fig. 1). Under these conditions there is no evidence of polymorphic behavior. The compounds are S-2, Se-6, Se-7, Se-12, and Se-13.
- *Behavior II* Compounds that solidify into an amorphous solid form after a first heating–cooling scan (Fig. 2). The compounds are S-5 and Se-11.
- *Behavior III* Compounds that show a new polymorphic form at a T_{onset} lower than the endothermic temperature at the first melting–recrystallization scan (Fig. 3). The compounds are S-4, Se-8, and Se-10. Se-8 is the only compound of the series that shows two polymorphic forms in the first heat scan. In every case, in the successive cycles of melting–recrystallization, the higher temperature polymorph disappears, obtaining lower temperature polymorph.
- *Behavior IV* These compounds have three polymorphic forms: an initial polymorphic form with a higher T_{onset}, a new polymorph with an intermediate T_{onset} in the second heat scan, and another new polymorphic form with the lowest T_{onset} in the third scan. The compounds are S-1 and Se-3 (Fig. 4).

Table 2 T_{onset} values for degradation process for selenium (Series A) and sulfur (Series B) compounds studied

Series A		Series B	
Ref.	T_{onset}/K	Ref.	T_{onset}/K
Se-3	487.1	S-1	502.9
Se-6	489.0	S-2	497.8
Se-7	475.8	S-4	503.5
Se-8	474.0	S-5	511.4
Se-10	488.5		
Se-11	492.2		
Se-12	492.2		
Se-13	487.4		

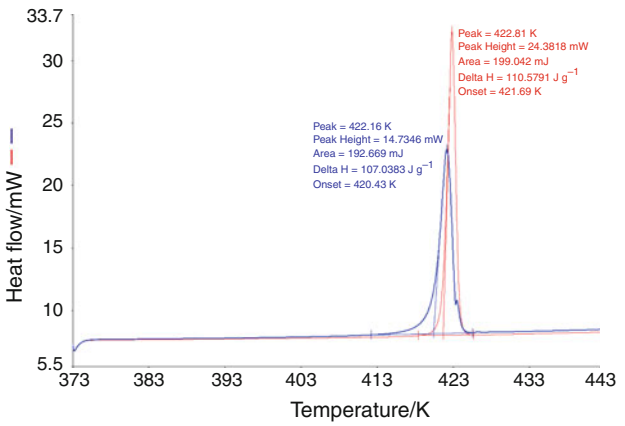


Fig. 1 DSC of compound S-2. *Red line* represents the first scan and *blue line*, the second scan. (Color figure online)

Study of the polymorphic forms of compounds S-4 and Se-10 (behavior III) and S-1 (behavior IV)

Differential scanning calorimetry

Two of the compounds that more clearly showed polymorphism in the DSC study were the compounds S-4 and Se-10, which are sulfur and selenium analog compounds. The polymorphic behavior of these compounds and S-1 has been studied by differential scanning calorimetry, thermomicroscopy, and X-ray diffraction powder.

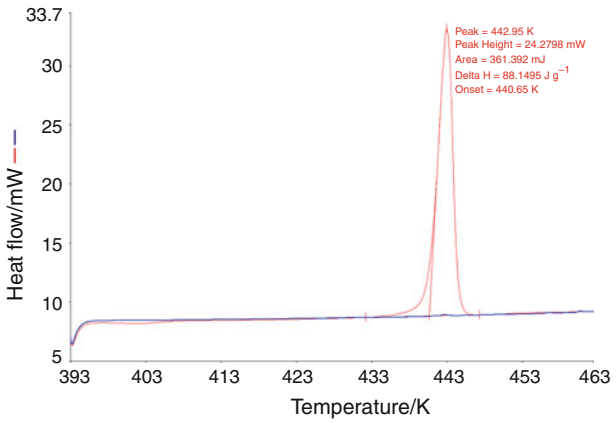


Fig. 2 DSC of compound S-5. *Red line* first scan; *blue line* the second scan. (Color figure online)

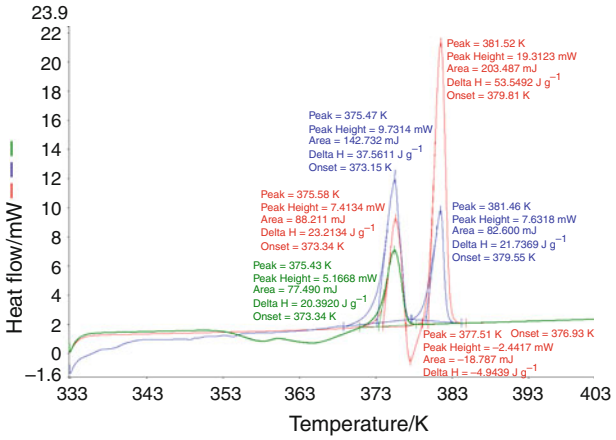


Fig. 3 DSC of compound Se-8. *Red line* first scan, *Blue line* second scan, *Green line* third scan. (Color figure online)

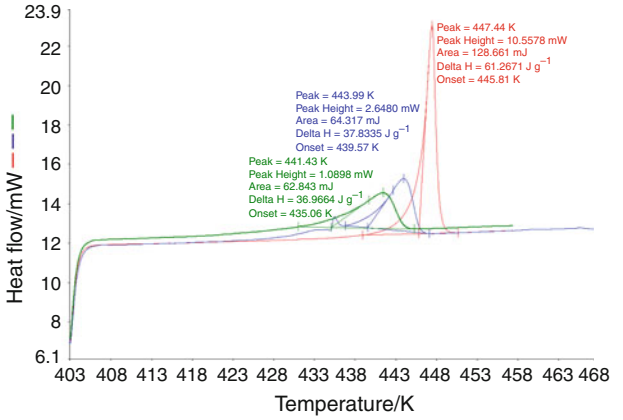


Fig. 4 DSC of compound Se-3. *Red line* first scan, *Blue line* second scan, *Green line* third scan. (Color figure online)

All samples were heated until temperatures 20 K below T_{onset} for degradation process, in order to asses that compounds were not degraded (Table 2). After melting the samples, they were cooled at room temperature and were left at room temperature enough time to be able to hypothesize that the compounds recrystallized before successive thermal processes.

Figure 5 shows the DSC obtained for compounds S-4 (A) and Se-10 (B) (behavior III). In both the cases, during the first thermal scan, only an endothermic process (represented by the red curve in Fig. 5) occurs, typical of a melting process. However, after cooling the sample (the melt can recrystallize again), it can be observed that an endothermic process occurs at a lower temperature (blue curve in Fig. 5). This new process coincides with the emergence of a new polymorph in the process of recrystallization of the compounds (Tables 3, 4).

Figure 6 shows the DSC obtained for compound S-1 (behavior IV). We observed that for the heating–cooling treatments we obtained three different endothermic processes for each treatment with T_{onset} lower in second and third fusion–recrystallization process. These results seem to support the presence of three different polymorphic forms for behavior IV compounds (Table 5).

Thermomicroscopy

A thermomicroscopic study of products S-4 and Se-10 (behavior III) and S-1 (behavior IV) has been carried out in order to determine whether the polymorphism was visible [18, 19]. Simultaneous thermomicroscopy/DSC is useful for the study of phase diagrams [20]. Photographs were taken of the microscope image of the crystals of S-4 (a in Fig. 7), Se-10 (b in Fig. 7), and S-1 (a in Fig. 8) in their original form. The samples were then subjected to thermal heating until fusion. When the entire sample had melted and recrystallized, it was left at room temperature and the

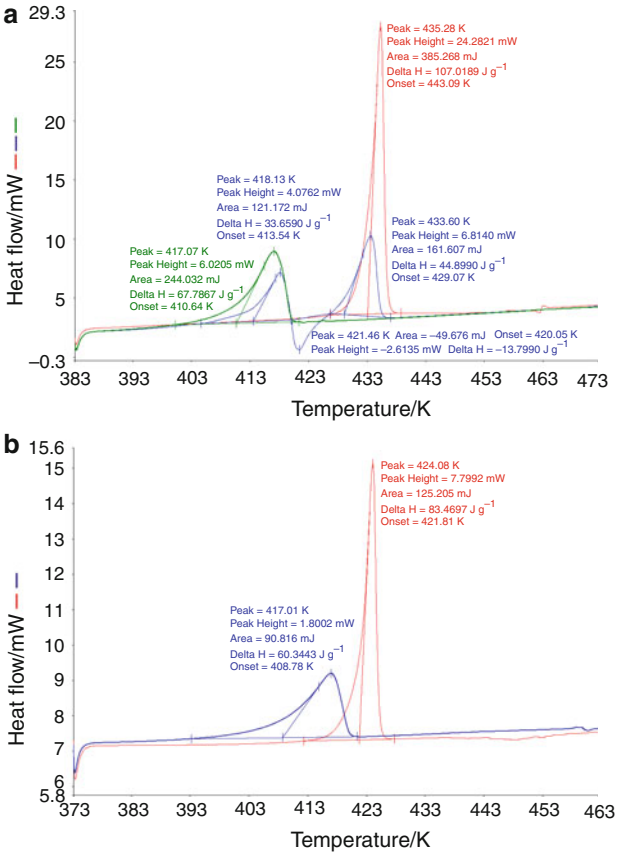


Fig. 5 **a** DSCs of compound S-4. *Red line* first thermal process; *blue line* second thermal process (after recrystallization of sample) and *green line* third thermal process (after another recrystallization again). Scanning rate of thermal processes: 10 K min^{-1}. **b** DSCs of compound Se-10. *Red line* first thermal process; *blue line* second thermal process (after recrystallization of sample). Scanning rate of thermal processes: 10 K min^{-1}. (Color figure online)

images of microscopic crystals of S-4 (c in Fig. 7), Se-10 (d in Fig. 7), and S-1 (b in Fig. 8) were photographed. The sample of S-1 was then subjected to a new thermal heating until fusion. When the entire sample had melted and recrystallized, it was left at room temperature and the microscopic image of the crystals of S-1 (c in Fig. 8) was photographed.

As shown in Fig. 7, a clear difference can be observed in the crystalline appearance of the samples before and after the melting process. Originally, the crystals form of S-4 and Se-10 are rectangular sheet (a and b in Fig. 7). However, after the fusion process, both of them recrystallize into another crystalline form or another new polymorph form (c and d in Fig. 7). Thermomicroscopy study appears to confirm the possibility of polymorphic forms in these compounds.

As shown in Fig. 8, the crystalline appearance of the samples before (a in Fig. 8) and after first (b in Fig. 8) and second fusion–crystallization process (c in Fig. 8) for S-1 seems to be different. These thermomicroscopic images

Table 3 Fusion–recrystallization: sulfur compound S-4

	Form I			Recrystallization			Form II		
	T_{onset}/K	T_{peak}/K	ΔH/J g^{-1}	T_{onset}/K	T_{peak}/K	ΔH/J g^{-1}	T_{onset}/K	T_{peak}/K	ΔH/J g^{-1}
1st scan	–	–	–	–	–	–	433.09	435.28	107.02
2nd scan	429.07	433.60	44.90	420.05	421.46	−13.80	413.54	418.13	33.60
3rd scan	410.64	417.07	67.79	–	–	–	–	–	–

Table 4 Fusion–recrystallization: selenium compound Se-10

	Form I			Recrystallization			Form II		
	T_{onset}/K	T_{peak}/K	ΔH/J g^{-1}	T_{onset}/K	T_{peak}/K	ΔH/J g^{-1}	T_{onset}/K	T_{peak}/K	ΔH/J g^{-1}
1st scan	–	–	–	–	–	–	421.81	424.08	83.47
2nd scan	408.78	417.01	60.34	–	–	–	–	–	–

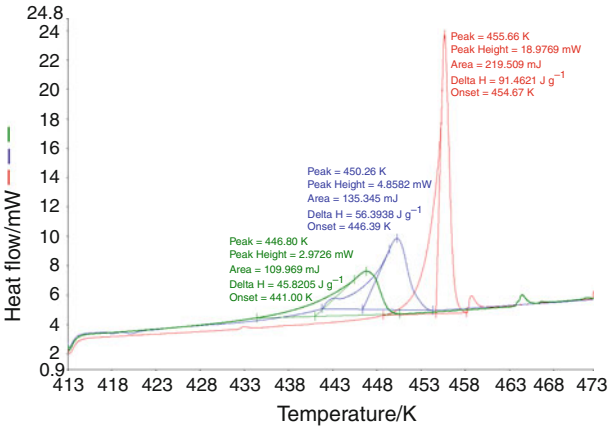


Fig. 6 DSCs of compound S-1. *Red line* first thermal process; *blue line* second thermal process (after recrystallization of sample) and *green line* third thermal process (after another recrystallization again). Scanning rate of thermal processes: 10 K min^{-1}. (Color figure online)

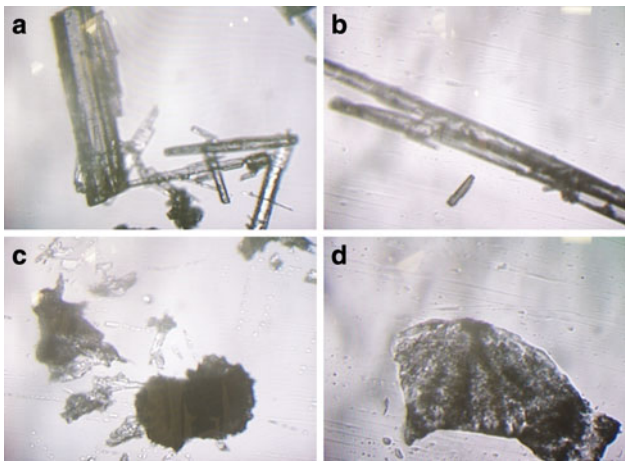


Fig. 7 Microscopic image of the compound S-4 (**a** before heat treatment, i.e., the original polymorph; **c** after heat treatment, i.e., the new polymorph) and Se-10 (**b** before heat treatment, i.e., the original polymorph; **d** after heat treatment, i.e., the new polymorph)

appear to confirm the possibility of different polymorphic forms for compound S-1 after heat treatment.

X-ray powder diffractometry

Due to the small quantity of product available, the conventional box method was not used, but the sample was attached to a glass surface. A dispersion of sample was prepared in an insoluble solvent and was fixed on a glass surface by evaporation (diethyl ether). The aim was to achieve a uniform and random distribution in order to avoid preferential orientations as much as possible.

The scans were performed in a 2θ range 5–60°, and working conditions were 40 kV operating voltage, current 30 mA, and a scan rate of 0.02° 2θ s^{-1}.

Table 5 Fusion–recrystallization: sulfur compound S-1

	Form I			Form II			Form III		
	T_{onset}/K	T_{peak}/K	ΔH/J g^{-1}	T_{onset}/K	T_{peak}/K	ΔH/J g^{-1}	T_{onset}/K	T_{peak}/K	ΔH/J g^{-1}
1st scan	–	–	–	–	–	–	454.67	455.66	91.46
2nd scan	–	–	–	446.39	450.26	56.39	–	–	–
3rd scan	441.00	446.80	45.82	–	–	–	–	–	–

Figures 9 and 10 show the diffractograms obtained for compounds S-4 and Se-10 (behavior III), respectively. The XRD patterns of samples obtained from their synthesis and purification are shown in black. The diffractograms obtained for samples after melting and subsequent recrystallization are shown in red. To ensure that there is no mixture of polymorphic forms, the samples underwent the number of melting-recrystallization cycles needed for the obtainment of a single peak in DSC (as can be seen in Fig. 5a, b).

As it can be observed in Figs. 9 and 10, differences were found in the diffractograms obtained for the original sample, and after the melting-recrystallization process, differences were found in their peaks. Therefore, there are peaks that disappear (shown with blue arrows) and the relative intensities of several of these peaks change, a behavior typical of polymorphic forms.

X-ray powder diffractograms show distinct differences in the positions and relative intensities of reflection, clearly indicating different polymorphs I and II for the two compounds studied.

Figure 11 shows diffractograms for compound S-1 (behavior IV). The XRD patterns of original form obtained

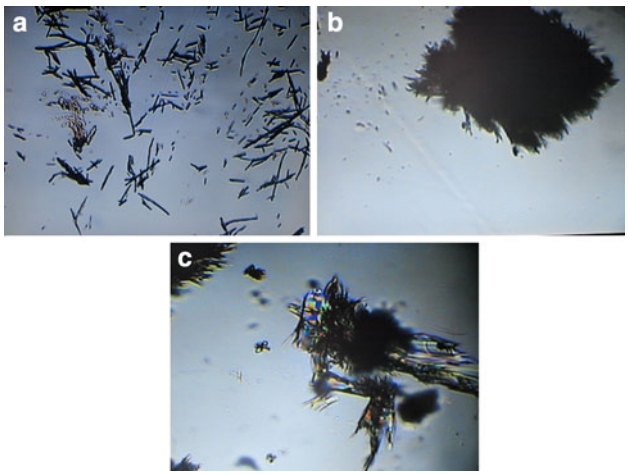


Fig. 8 Microscopic image of the compound S-1 (**a** before heat treatment, i.e., the original polymorph; **b** after first heat treatment, i.e., one new polymorph; **c** after second heat treatment, i.e., other new polymorph)

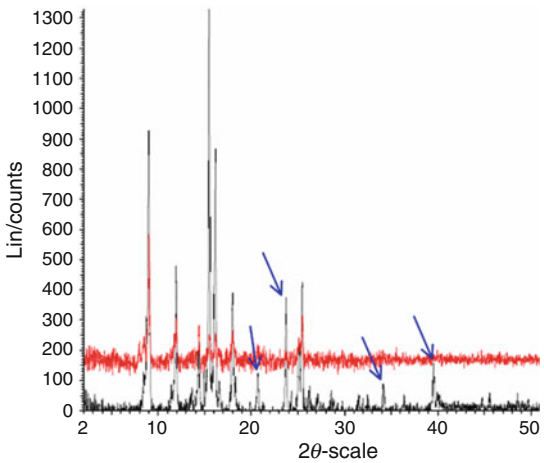


Fig. 9 X-ray diffractogram for compound S-4. *Black line* shows the diffractogram of the original polymorph and the red line shows X-ray diffractogram of the new polymorph obtained after several cycles of melting-recrystallization. *Blue arrows* indicate the peaks that disappear from one polymorph to another. (Color figure online)

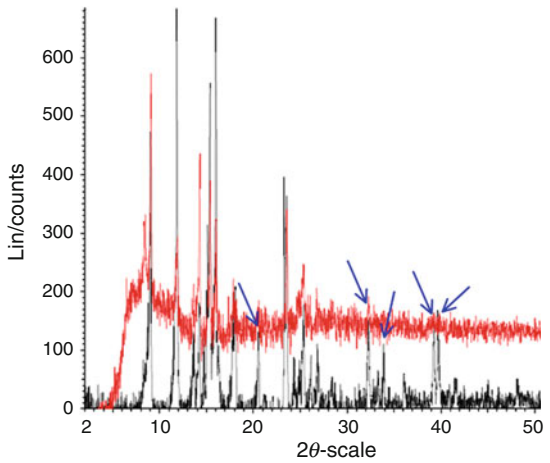


Fig. 10 X-ray diffractogram for compound Se-10. *Black line* shows the diffractogram of the original polymorph and the *red line* shows X-ray diffractogram of the new polymorph obtained after several cycles of melting-recrystallization. *Blue arrows* indicate the peaks that disappear from one polymorph to another. (Color figure online)

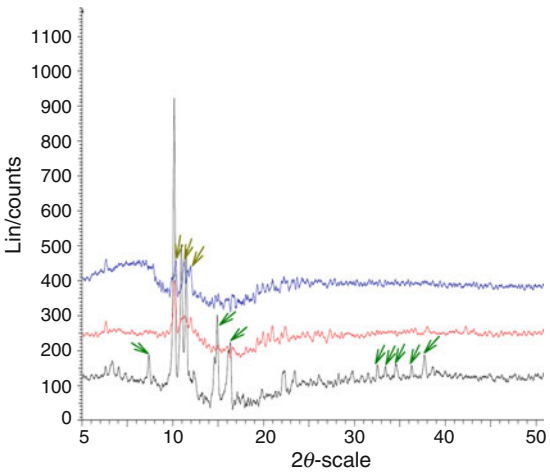


Fig. 11 X-ray diffractogram for compound S-1. *Black line* shows the diffractogram of the original polymorph. The *red line* shows X-ray diffractogram of new polymorph obtained after first heat treatment and blue line shows X-ray diffractogram of new polymorph obtained after second heat treatment. *Green arrows* indicate the peaks that disappear from original polymorph to the other ones. *Yellow arrows* indicate the peaks that change their relative intensity between first and second heat treatment. (Color figure online)

from its synthesis and purification is shown in black. The diffractograms after first and second melting-recrystallization processes are shown in red and blue, respectively. We can observe that there are some peaks in the original form of diffractogram that disappear (shown with green arrows) after first and second melting-recrystallization process. These findings indicate different polymorphs for the original form and after thermal treatments. However, XRD patterns for crystalline forms after first (red line) and second (blue line) melting-recrystallization process present minimum differences in the relative intensity of some peaks (shown with yellow arrows), suggesting some kind of difference in the crystalline package. Overall, these results seem to support the polymorphic behavior observed in DSC for this compound (Fig. 6), although we cannot assert a clear difference between the polymorphic forms after first and second heat treatment by X-ray diffraction assays.

Conclusions

The study of the physicochemical properties of polymorphics forms of a series of organosulfur and organoselenium derivatives has been carried out with a combination of differential scanning calorimetry, thermomicroscopy, and X-ray diffractometry.

These results of X-ray diffraction, together with those obtained by DSC and thermomicroscopy, lead to the conclusion that polymorphs are formed when compounds S-4, Se-10, and S-1 are heated above their melting point. The same or similar behavior was observed in more compounds of both series A and B.

The results show that there are four types of thermal behavior for series A and B. Some compounds (S-2, Se-6, Se-7, Se-12, and Se-13) do not evidence any polymorphic behavior (behavior I). Compounds S-5 and Se-11 solidify into an amorphous solid form (behavior II). Several compounds (S-4, Se-8, and Se-10) show a new polymorphic form at a T_{onset} lower than original one (behavior III). Finally, S-1 and Se-3 derivatives have three polymorphic forms with three different T_{onset} (behavior IV).

Calorimetric studies demonstrate that sulfur and selenium analogs have the same thermal behavior (S-2 and Se-6; S-5 and Se-11; S-4 and Se-10). These different thermal behaviors are cause by the substituent groups in the aromatic ring, although there is no relationship between electron-withdrawing or electron-donating groups and the thermal behaviors.

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