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Note

The preparation and examination of polymorphous vincristine sulphate

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Abstract

The examination of the polymorphism of tumour inhibitory vincristine sulphate with a bis-indole skeleton is described. The changes in molality with temperature were followed thermo-osmometrically. It could be shown by X-ray diffraction that a sample separated by cooling the solution had an amorphous structure and the proportion of the X-ray-amorphous part of the substance along with the crystalline phase increased when the temperature of the solution was lowered from 50°C to 40°C to 30°C. At lower temperatures, a mixture of molecular associations of different masses coexists in dynamic equilibrium. Polymorphic modifications of various compositions, characterized by different ratios of the crystalline and amorphous phases in the solid substance, can be obtained during the course of crystallization. © 1998 Elsevier Science B.V.

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1. Introduction

In the 1960s the therapeutic significance of a group of bis-indole alkaloids isolated from *Vinca rosea* L. (Apocynaceae) was recognized after the anti-tumour activity of vinblastine and vincristine (Eckhardt, 1969) had been discovered in 1960 and 1964, respectively.

Due to the advantageous therapeutic experience, vincristine sulphate was included in both the American (USP XIX) and the British Pharmacopoeias (BP73 BPC 73). An overview of papers dealing with the pharmacoanalytical properties of the substance was published (Burns, 1972). It is curious that data about the crystal structure of vincristine sulphate, which certainly does influence its biopharmaceutical properties, are missing,

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whereas the X-ray powder diagram of the free base, liberated by NH_4OH from its aqueous solution, extracted with an organic solvent and recrystallized, was published (Burns, 1972).

In our previous paper (Dávid et al., 1996) a comprehensive examination of the polymorphism of vincristine sulphate was carried out.

Since the crystal structure of any solid substance is determined by its physicochemical characteristics (Haleblian and McCrone, 1969; Csonka-Horvay et al., 1971; Haleblian, 1975; Graf et al., 1982; Burger et al., 1985; Rácz, 1989a; Ghan and Lalla, 1992; Labhasetwar et al., 1993; Romero et al., 1993; Rocco, 1994), and until now in the case of vincristine sulphate has not been completely investigated, the purpose of the present study was to prepare and analyse the various polymorphic forms of vincristine sulphate.

2. Materials and methods

2.1. Materials

In the experiments a sample of vincristine sulphate of USP 23 grade (VINC-SO_4) received from G. Richter Ltd. (Budapest), as well as a USP standard for comparative X-ray measurements, were used. All other reagents and solvents used were of analytical purity.

2.2. Methods

2.2.1. Examination in solution

The temperature dependence of the UV spectrum of VINC-SO_4 in chloroform solution at a concentration of 4×10^{-2} mg/cm^3 was examined, using a Unicam SP 8-200 UV/VIS spectrophotometer, in a temperature-stable cuvette (thickness 1 cm). There are too few data available in the literature concerning the temperature dependence of the UV spectrum. In the case of VINC-SO_4 the changes in the UV spectrum reflect the chemical structural characteristics of the substance, the ionic or associated forms of which are excitable with higher or lower energy.

Changes in the molality of the VINC-SO_4 solution with temperature were examined thermo-osmometrically (Takács and Dávid, 1966) using suitable equipment (Dávid et al., 1963). It is known that, in an atmosphere isothermally saturated with vapours of the solvent, the hanging drop of a solution compared with the hanging drop of the pure solvent shows a temperature difference that is proportional to the molality of the solution. Using a Siemens K-l7 glass thermistor couple, the molalities of VINC-SO_4 solutions were measured thermo-osmometrically by the no-compensation method on a Wheatstone bridge (nA sensitivity) with reference to a calibration straight line obtained with a series of 10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1} molal solutions of benzoic acid (analytically pure, weighed to within 0.1 mg accu-

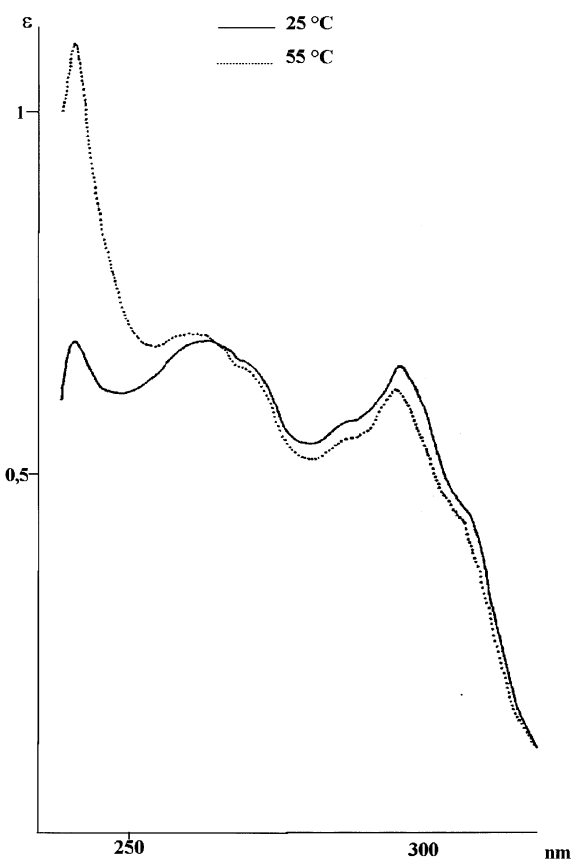

Fig. 1. Dependence of the UV spectrum of vincrisrine sulfate on temperature.

Table 1
Temperature dependence of thermo-osmometrically measured molalities of vincristine sulfate solutions and molecular masses of associates calculated therefrom

Measurement temperature (°C)	VINC-SO_4 (mol. mass 923.04) solution (mg/g)	Thermo-osmometric molality (mOsmol/l)	Calculated mol. mass (g)	Deviation[a] (%)
17.2	172.3	≈0.0015	114864	27
	91.2	0.001	91200	
26.4	172.3	0.020	8615	6
	91.2	0.010	9120	
31.2	172.3	0.049	3516	6
	91.2	0.025	3648	
35.0	172.3	0.090	1914	2
	91.2	0.047	1940	

[a] Refers to extreme values.

racy) in chloroform. From our previous studies it was established that benzoic acid in chloroform solution in the concentration range used does not undergo any dissociation/association that would disturb the molality value (decay which leads to decarboxylation). Therefore, the apparent molecular mass of the examined substance was determined in the usual way.

2.2.2. Preparation of modifications of the solid phase

The sample was precipitated by cooling the solution as follows. One part of methanol is saturated with VINC-SO_4 at 40°C and the latter is then precipitated by the addition of five parts of ethanol. The suspension is kept at about 0°C for 3 h when the precipitate is filtered and dried at 20°C under a reduced pressure of 60 Pa.

The samples are then crystallized by heating the solution as follows. One part of methanol is saturated with VINC-SO_4 at 50°C. After adding five parts of ethanol to the saturated VINC-SO_4 at the same temperature, the precipitated part is separated by filtration and dried as described above. Similar isothermic samples were also prepared at 40°C and 30°C.

2.2.3. Examination of the modification

X-ray diffraction was measured with a powder diffractometer (Philips PW 1060) using the Bragg–Brentano recording technique with Cu/K_{alpha} radiation generated by 30 kV, 30 mA.

The UV spectrum of the solid phases was recorded spectrophotometrically by the method of Csonka-Horvay et al. (1971) using the microcrystal layers left behind after the evaporation of the solvent from the chloroform solution of VINC-SO_4 dropped onto a hot (50°C) quartz slide. Because of the excessive light absorption by the optically disperse samples, it was necessary to reduce the transduction of the comparative light ray by the use of an optical grid (a bronze sieve fabric). In order to make the survey of spectrum characteristics easier, the pictures of the 2.17×10^{-4} mol/dm^3 chloroform solutions were made in a cuvette with 2 cm thick layers and Range 2 setting of the instrument, while those of the solid phases were made with the Range 1 setting.

Thermal analyses of 5.20 mg of the crystalline samples and 5.75 mg of the amorphous samples were made in a Du Pont 990 micro-TG apparatus (Thermal Analyzer 951) applying a heating rate of 10°C/min and a nitrogen gas flow rate of 10 dm^3/h, as well as of 50-mg quantities in a MOM Q-Derivatograph at a heating rate of 5°C/min in a platinum crucible.

3. Results

3.1. Examination in solution

The change of UV spectrum in the solutions was reversible because the spectrum determined at

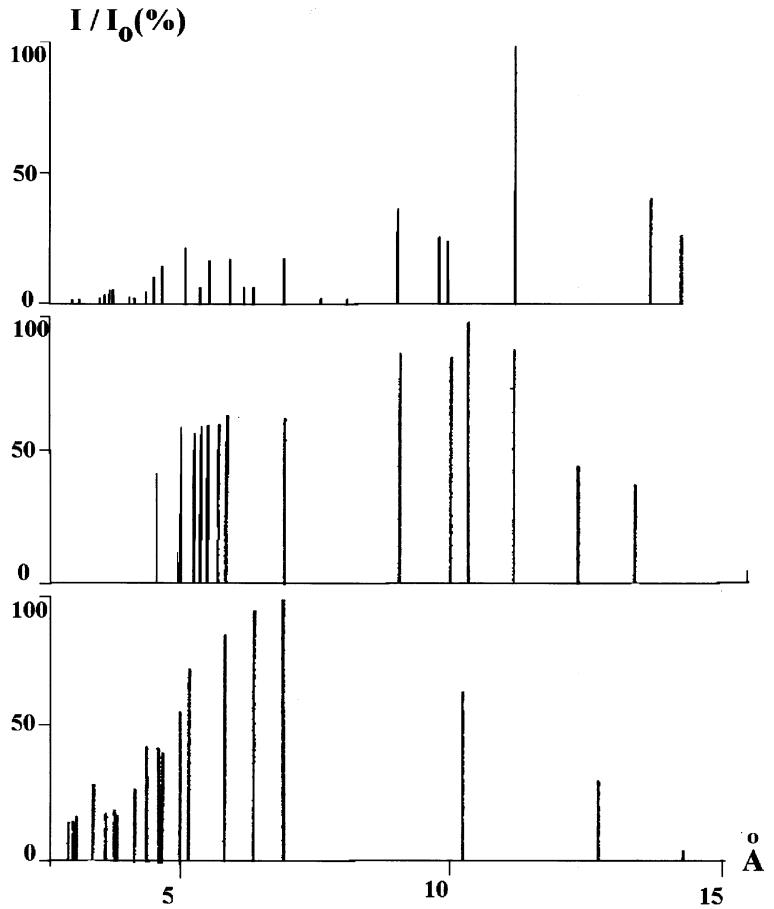

Fig. 2. X-ray diffraction powder diagrams of crystalline modifications of vincristine sulfate.

higher temperature returned to the original one in the recooled sample. Dependence of the spectrum on temperature is shown in Fig. 1.

The molalities of VINC-SO_4 solutions measured thermo-osmometrically and the calculated apparent molecular masses of the examined substances are given in Table 1. The accuracy of the data depends on the actual value of the measured molality. In general, the molecular mass can be determined with a difference of a few percent when the molality is 10^{-1}–10^{-2}, but the difference might be as great as 50% when molality is of the order of 10^{-4}. Because our expected conclusions were primarily qualitative in character, we found the accuracy of the method was sufficient.

3.2. Examination of the modification of the solid phase

The results of the X-ray diffraction show that the sample precipitated by cooling the solution was completely amorphous. The solid structures of the samples crystallized by heating are presented in Fig. 2. Reducing the 2θ angle values of the powder diagrams to Angstrom (Å) values, and considering the peak with the highest intensity of impulses/s (I_0) as 100%, we plotted the intensity values of the individual peaks (I) in percentage terms. In the powder diffractograms of the substances prepared at 50°C, 40°C and 30°C, lines of maximum intensity appeared at 11.05, 10.20 and 6.84 Å, respectively; these preparations may thus

be regarded as the modifications α, β, and γ. The increasing impulses/s value of X-ray diffraction as a function of the 2θ angle indicates that the proportion of amorphous matter along with the crystalline phase apparently increased with decreasing temperature in the samples prepared at 40°C and 30°C.

Fig. 3 illustrates the differences between the two settings for the 2.17×10^{-4} mol/dm^3 chloroform solution (Range 2) and solid phase (Range 1). The deviation, observable in the UV spectrum of the solid and the dissolved VINC-SO_4, confirms that the chemical structure of the substance is different for association/dissociation reasons.

The derivative thermogravimetric (DTG) and thermogravimetric (TG) curves resulting from the thermal analyses are shown in Fig. 4. The decrease of the mass below 100°C on the thermoanalytical TG graph indicates the loss of the solvent from the samples, which proceeds in two steps in a crystallized sample. The identical decay of the two samples over 120°C indicates, on the basis of the DTG graphs, a change of enthalpy.

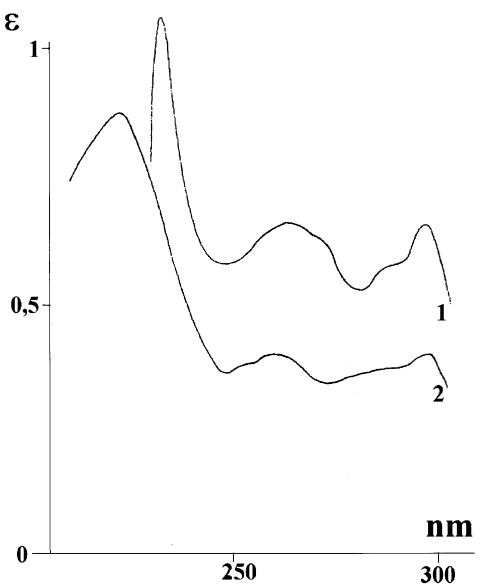

Fig. 3. UV spectrum of vincristine sulfate (1) in the solid phase, (2) in a 2.17×10^{-4} mol/dm^3 solution in chloroform.

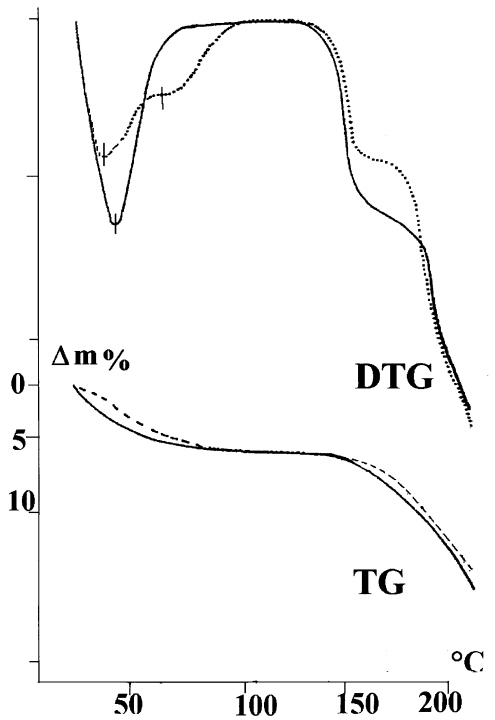

Fig. 4. Thermal analysis of vincristine sulfate. TG, thermogravimetric curve; DTG, derivative thermogravimetric curve.

4. Discussion

The examination of polymorphism helped elucidate the particular physicochemical properties of VINC-SO_4. Since it is customary to retrieve solid substances from their solutions by cooling, and under such circumstances VINC-SO_4 presents itself as an X-ray-amorphous solid, it may be assumed that the structural data for VINC-SO_4 are missing from the literature simply because the amorphous structure is an accepted characteristic property of solids.

Specific physicochemical properties that cannot be deduced from the structural formula but which are important for pharmaceutical formulation can be recognized by the effects of temperature changes on solutions (Rácz, 1989b).

From the spectrum of chloroform solutions of VINC-SO_4 taken at 25°C and 55°C (Fig. 1) it can be seen that on raising the temperature the 241 nm peak undergoes a marked hyperchromic shift

and a small bathochromic change while the 299 nm peak undergoes a small hypochromic shift and a minimal hypsochromic change. As has been mentioned before, the phenomenon is reversible; when the cuvette is cooled, the original spectrum returns. This means that by a change of temperature the chromophore groups of the molecule can be intramolecularly stimulated both simultaneously and in equilibrium by greater or smaller energies. In other words, VINC-SO_4 reacts to temperature changes of its solutions by changing the equilibrium between the chemical association and dissociation of its molecules.

The temperature dependence of the molalities of the solutions, which can be determined thermoosmometrically (Takács and Dávid, 1966) by direct measurement of the activity of the solution, was especially persuasive. The function values of the reversible association molecular masses pertaining to four different isotherms are shown in Fig. 5. It can be deduced from the measured data,

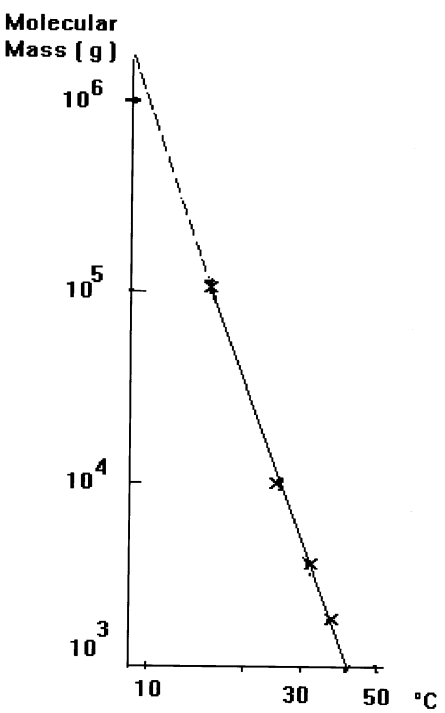

Fig. 5. Change of the molecular mass of vincristine sulfate associations dissolved in chloroform under the influence of temperature.

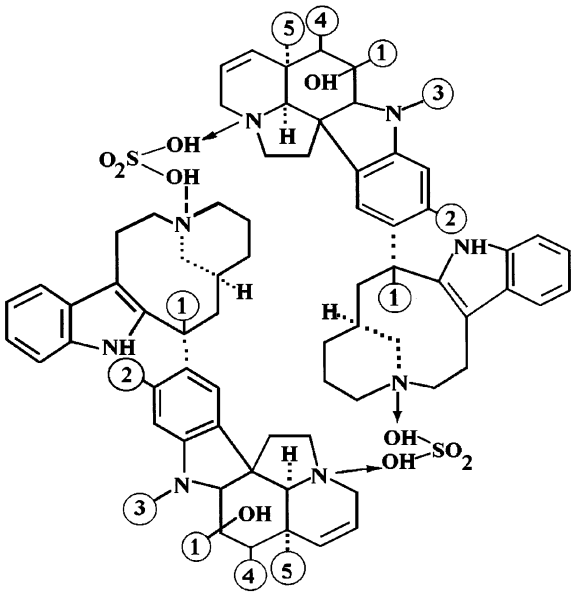

Fig. 6. Structural formulae of dimer associations of vincristine sulfate.

albeit qualitatively, that, due to a shift in the chemical equilibrium at 0°C, associations of molecules are formed in the solutions of VINC-SO_4 whose molecular mass may exceed several millions. This by itself is a sufficient explanation of the X-ray data on the solid body obtained by cooling. Macromolecular substances (e.g. organic polymers) are usually amorphous in the solid phase.

The TG curves of Fig. 4 show that all samples prepared in the described way suffered a similar decrease in mass, according to the DTG curves in Fig. 4. However, mass decrease of the crystalline and the amorphous substances occurred in substantially different thermal steps.

It is appropriate to choose the conditions for the crystallization or precipitation of a substance in such a way that the operation is conducted in the optimal solvent or solvent mixture, at the optimal concentration and at the suitable temperature.

Our observations are in good agreement with the chemical structure of VINC-SO_4. Even in the case of the most advantageous conformation, both its protonable tertiary nitrogen atoms are

separated from each other by an intramolecular distance greater than 900 pm, and the protons of the sulphuric acid molecule, even in the case of maximal polarization, cannot reach a distance greater than 500 pm. Salt formation between a single vincristine molecule and a single sulphuric acid molecule is thus hindered sterically. Even if the stoichiometric ratio [VINC]:[SO_4] is 1:1, only a dimer can be formed (schematically presented in Fig. 6). This fact is also made probable by the molality value measured above 35°C.

At lower temperatures, the dissolved VINC-SO_4 can be characterized by a dynamic equilibrium of molecular associations with different molecular masses according to the following scheme: –[VINC]-SO_4–[VINC]-SO_4–[VINC]-SO_4–[VINC]-SO_4–. Accordingly, various polymorphic forms can arise after crystallization or precipitation whose structure can be characterized by different ratios of the amorphous and crystalline phases. On the basis of the experimental results the substance in the solid phase can only be described by the general formula: $[VINC]_x$-$[SO_4]_x$, where $x = 2,3,\ldots n$.

The chemical properties of vincristine sulphate described above may have both pharmacotechnological and biological significance.

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References

Burger, A., Brox, W., Ratz, A.W., 1985. Different hygroscopicity of three different crystal forms of oxytetracyclin hydrochloride and effect on stability. Acta Pharm. Technol. 31, 230–235.

Burns, J.H., 1972. Vincristine sulphate. In: Florey, K. (Ed.), Analytical Profiles of Drug Substances, vol. 1. Academic Press, New York, pp. 463–480.

Csonka-Horvay, J., Dávid, A., Horváth, G., Náray-Szabó, G., 1971. Rotational isomerism in bis-[*p*-(3-methyl-butyloxy)-phenyl]-thiourea. Z. Naturforsch. 26B, 21–23.

Dávid, A., Horváth, G., Nemes, L., Hertelendy, L., 1963. Thermostatic process and equipment mainly for electroosmometric examinations. Hungarian Patent 152511G.

Dávid, A., Balogh, E., Csóka, G., Rácz, I., 1996. Methodological aspects of examination of the polymorphism of vincristine sulphate. Sci. Pharm. 64, 311–318.

Eckhardt, S., 1969. Treatment of lymph-node tumours with vinblastin. Ther. Hung. 17, 57–59.

Ghan, G.A., Lalla, J.K., 1992. Effect of compressional forces on piroxicam polymorphs. J. Pharm. Pharmacol. 44, 678–681.

Graf, E., Beyer, C., Abdallah, O., 1982. The physical instability of sulfamethoxazole aqueous suspension. Pharm. Ind. 44, 1071–1074.

Haleblian, J.K., 1975. Characterization of habits and crystalline modification of solids and their pharmaceutical applications. J. Pharm. Sci. 64, 1269–1288.

Haleblian, J.K., McCrone, W., 1969. Pharmaceutical applications of polymorphism. J. Pharm. Sci. 58, 911–929.

Labhasetwar, V., Deshmukh, S.V., Dorle, A.K., 1993. Studies on some crystalline forms of ibuprofen. Drug Dev. Ind. Pharm. 19, 631–641.

Rácz, I., 1989a. Drug Formulation. Wiley, New York, p. 155.

Rácz, I., 1989b. Drug Formulation. Wiley, New York, p. 32.

Rocco, W.I., 1994. Solid state characterization of stanozolol. Drug Dev. Ind. Pharm. 20, 1831–1849.

Romero, A.J., Savastano, L., Rhodes, C.T., 1993. Monitoring crystal modifications in systems containing ibuprofen. Int. J. Pharm. 99, 125–134.

Takács, G., Dávid, A., 1966. Über die Anwendung der Thermo-Osmometrie in der Arzneimitteluntersuchung. Informationsdienst APV 12, 154–156.