Journal of Thermal Analysis and Calorimetry, Vol. 57 (1999) 61-73

THERMAL ANALYSIS, MICROCALORIMETRY AND COMBINED TECHNIQUES FOR THE STUDY OF THE POLYMORPHIC BEHAVIOUR OF A PURINE DERIVATIVE

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Abstract

The polymorphic behaviour of the purine derivative MKS 492 was studied with investigations of suspensions of selected samples in different solvents and of samples obtained by crystallizations. The samples were analyzed by DSC, TG and X-ray diffraction. Six different crystalline modifications called A, B, B', C, D and E and an amorphous form were identified. Four pure crystalline modifications, A, B, C and D have been manufactured and characterized by DSC, X-ray, IR, solubilities, densities, hygroscopicity and dissolution measurements. The four forms A, C, D and E are monotrop to the form B. The form B is enantiotrop to the form B', which revealed the highest melting point of all known polymorphs. This form B' is only stable at high temperature. Temperature resolved X-ray diffraction was very helpful for proper interpretation of the thermal events. The melting peaks of the forms A and C and the endothermic peak corresponding to the enantiotropic transition B into B' occur in a narrow range of temperature. The form B which is the most stable one at room temperature has been chosen for further development. Quantitative methods to determine the content of the forms A, C and D in samples of form B or to determine the content of form A, B and D in form C have been developed by using X-ray diffraction. Limits of detection are 1 or 2%. For the quantitative determination of the amorphous fraction, X-ray diffraction and microcalorimetry are compared. For high amounts of the amorphous fraction, the X-ray diffraction method is preferred because it is faster. Microcalorimetry is very attractive for levels below 10% amorphous content. The lowest limit of detection is obtained by microcalorimetry, about 1%.

Keywords: amorphous, combined techniques for polymorphism, DSC, MKS 492, polymorphism, purine, quantitative determination of amorphous and polymorphs, solvent mediated transitions, temperature resolved X-ray diffraction, TG, thermodynamic relation between polymorphs, xanthine

Introduction

The study of the polymorphic behaviour of new chemical entities is a domain of the preformulation work [1]. Thermal analysis, microcalorimetry and combined techniques such as thermal microscopy, DSC-TG, DSC-IR, DSC-Raman and temperature resolved X-ray diffraction are suitable for the identification of the thermodynamic stable form at room temperature [2] and for the monitoring of a reproducible process in order to obtain a pure crystalline modification. Additionally solvent mediated transformations are used in order to add complementary information for the establishment of the relationships between different forms. MKS 492 is a xan-

1418–2874/99/ $ 5.00

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

thine derivative. Substances containing this purine structure are used in the treatment of asthma and cardiac insufficiency [3]. This work summarizes the complex polymorphic behaviour and the analytical study of this substance.

Experimental

MKS 492 is the physiologically active *R*(+)–enantiomer (Sandoz; Basle, Switzerland). Calibrated Perkin Elmer DSC-7 with robot system, calibrated TGA 7 Perkin Elmer, calibrated instrument Scintag XDS 2000 with autosampler or with heating cell for the X-ray diffraction experiments and a micro DSC II of Setaram for the microcalorimetric studies were used. Equilibration studies were performed in suspensions at controlled temperature by using small bottles and a vibromixer E-1 of the company B. Braun Biotech International for the shaking of the suspensions [4] in order to induce solvent mediated transitions. The suspensions were analyzed after 1 day equilibration. Solubilities were determined by HPLC or by gravimetry. Dissolution rates were performed by the flow through method [5] and intrinsic dissolution by using the Vankel VK 7000 instrument. Densities were measured by gas pycnometry by using helium as gas.

The study revealed the identification of six different crystalline modifications and an amorphous form.

Form A was obtained as research sample using ethanol/diethyl ether as crystallization solvents. Form B was manufactured in production scale from ethyl acetate with addition of ter-butyl methylether at 60°C. It is also obtained by crystallization or suspension from water or polar organic solvents at 25 or 50°C. Form C was obtained from ethyl acetate with addition of terbutylmethylether at 25°C in production scale. Form D was obtained after equilibration of forms A or C in acetonitrile, methanol or acetone. Form E was crystallized from a hot saturated solution in methylene chloride. The amorphous form was obtained by lyophilization or fast evaporation of a solution in methanol. Form B' is obtained by heating form B and is observed only at high temperature.

Results

Polymorphism program part 1

Behaviour in solid state, equilibrations and crystallizations in solvents

The DSC curves of forms A, B, C, D at the heating rate of 10 K min^{-1} are given in Fig. 1. The first two batches which have been produced were identical and the form was called crystalline modification A. The DSC curves at different heating rates showed only one peak. Batch 1 has a melting enthalpy of 93 J g^{-1}. Batch 2 is inhomogenous as demonstrated by a great discrepancy of the melting enthalpies obtained by several measurements (from 42 up to 90 J g^{-1}). This behaviour was due to the presence of amorphous material, determined by X-ray diffraction. A pre-treatment of 3 h of the samples of batch 2 at 105°C lead to the transformation of this amorphous part into the form A.

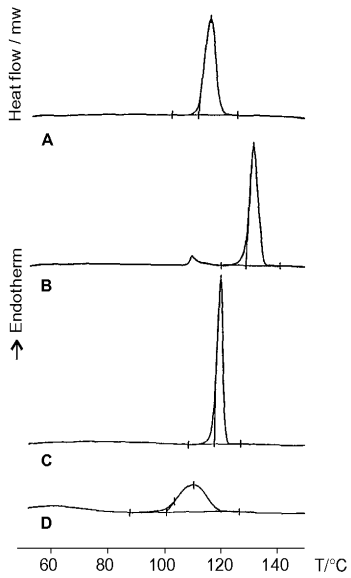

Fig. 1 DSC curves of forms A, B, C and D of MKS 492 at the heating rate of 10 K min^{-1} starting at 40°C. A – form A, onset T_f of A=111°C; B – form B, onset T_f of B'=128°C; C – form C, onset T_f of C=118°C; D – form D, onset T_f of D=101°C

Polymorphic behaviour of MKS 492 was first studied with batch 1 by using crystallizations experiments as well as suspensions at 25 and 50°C. DSC curves after suspension in water and crystallization in ethanol/water 1:1 are given in Figs 2a and 2b.

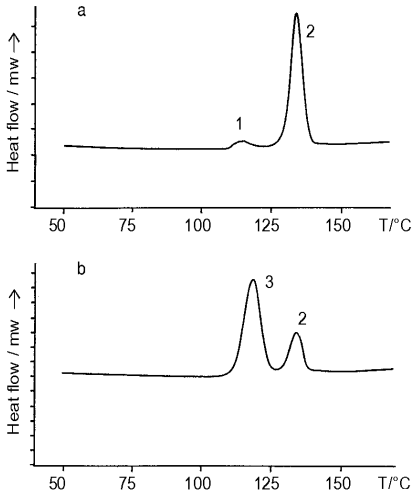

Fig. 2 DSC curves at the heating rate of 20 K min^{-1}. a – Sample obtained after suspension of form A at 50°C in water, TG <0.1%; b – Sample of form A after crystallization in ethanol:water 1:1, TG <0.1%

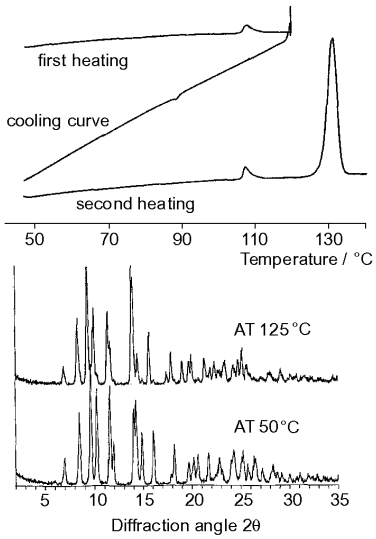

Fig. 3 Study of the endothermic peak 1 of Fig. 2a. Demonstration of reversible transformation B↔B'. Upper curves: DSC first heating curve up to 120°C and cooling curve, then second heating curve until 140°C; Bottom: temperature resolved X-ray diffraction experiment: X-ray diffraction pattern obtained at 50 and 125°C independently of heating or cooling experiment

Three peaks are identified. A new form, called form B, is responsible to the higher melting peak 2. Are peaks 1 and 3 the remaining form A?

The study of the DSC heating-cooling curves (Fig. 3a) and of the temperature resolved X-ray diffraction (Fig. 3b) of the sample treated in water demonstrate that this sample is a pure form B which undergoes a reversible enantiotropic transition in a second form called B'.

The third batch of the drug substance was form C. Form C has only one melting peak, a fact already observed for the form A. Through investigations on polymorphism also forms D and E could be generated.

Form C was easy to be produced. This form was stable after storage at 50 or at 30°C at a relative humidity of 75%. This form remained unchanged after granulation as demonstrated by DSC and X-ray diffraction measurements. However it was very difficult to obtain form C without form B in up-scale manufacture. Which form is really the thermodynamic stable form at room temperature?

Polymorphism study part 2

Study of the relationships between the forms of MKS 492

Once the forms manufactured, they are characterized and their thermodynamic relationships studied. Form E transforms into form B in all experiments performed in suspensions and was not studied further.

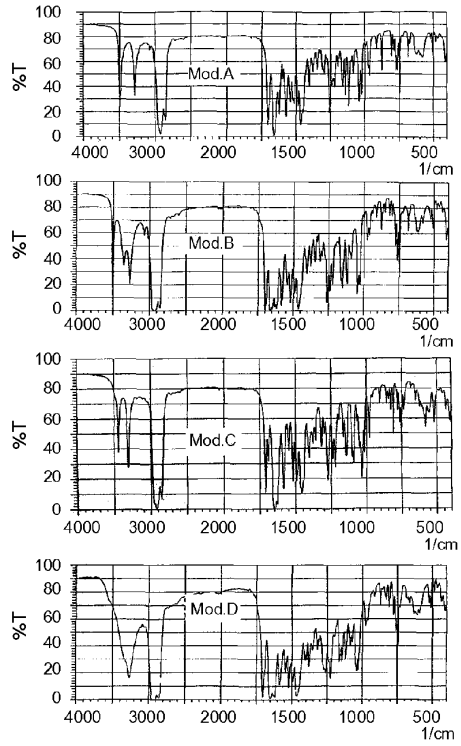

Fig. 4 IR spectra of forms A, B, C and D in Nujol

The DSC curves of forms A, B, C and D are given in Fig. 1, the IR spectra are given in Fig. 4 and the X–ray diffraction patterns in Fig. 5. The X-ray diffraction of form B' is obtained only at high temperature and is given in Fig. 3b.

It was interesting to apply the Burger's rules [6] knowing the melting temperature and the melting enthalpy of each form. The Burger's rules are based on the approximation of the Gibbs free energy functions, namely regarding the enthalpy of fusion as constant with temperature [7]. The Gibbs free energy functions had been introduced for both cases, namely for temperature independent and temperature dependent enthalpies of fusion, as estimations for the stability regions of polymorphic forms in case of organic substances by Marti [7]. It has been shown by the study of a great number of polymorphic systems, that in normal cases only the higher approximation gives reliable stability regions [8]. In case of monotropy, the form with the highest melting point has the highest melting enthalpy. In case of enantiotropy between two forms, the higher melting form has the lower melting enthalpy. Form D was clearly unstable with a very low melting enthalpy.

DSC measurements were performed with several samples of form A, B and C. It appears that milling gives some amorphization and that the temperature of the endothermic peak B→B' at about 110°C and its enthalpy values vary depending on the batch studied and depending on the heating rate. This findings are from a point of

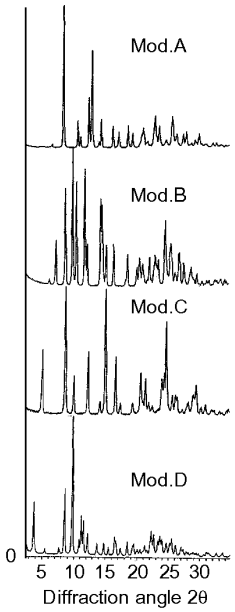

Fig. 5 X-ray diffraction patterns of forms A, B, C and D

view of polymorphic transition not very surprising. Any such transition is depending on the composition of each sample in respect to kinetic relevant chemical and physical impurities especially also with the fact that milling creates an intimate mechanical contact between impurities and amorphous part of the drug substance. The values obtained with the most crystalline sample of form B which was not milled before the DSC experiment were 6 J g^{-1} for the endothermic peak of transition B→B' and 92 J g^{-1} for the melting enthalpy of the form B'. One might estimate a value of 98 J g^{-1} for the enthalpy of fusion of the form B. (With milled samples the temperature of transition increases and the enthalpy value decreases.)

The sample of form C has a melting enthalpy of 89 J g^{-1}. Form A has a melting enthalpy of approx. 93 J g^{-1}. One may assume that forms A and C are monotrops to B.

The density of form B is slightly higher than the densities of form A and C, what confirm this hypothesis.

For proper interpretation, the results of solubility, as well as equilibration studies of mixtures of the forms A, B and C were also taken into consideration.

After 24 h vibration in water at 20, 25, 30, 35, 40 and 50°C, all forms A, B, C, D give the form B. A and C transform into B also at pH 3, 5 and 7. After equilibration 24 h in most polar organic solvents, form A and form C transform into form B. The transformations are faster if seeds crystals of B are added. Crystallization from polar organic solvents gives form B. Form C was obtained by crystallization from toluene or methylethylketone. In ethanol/terbutylether mixtures B+C were obtained.

The results of the solubilities measured by HPLC at 20, 30 and 40°C in water after different time of equilibration of suspensions are given in Table 1, the results ob-

tained in ethyl acetate in Table 2. Form B is less soluble than forms A and C at all temperatures. The lowest solubility of form B was found also in ethanol, isopropanol, acetone (Table 3). The same experiment carried out with mixtures of A and B or C and B or D and B in water shows a fast transformation of A, C or D into B, resulting in practically the same solubility values (Table 1).

Table 1 Solubilities results in water

T/ °C	Time/ min	Form A/	Form B/	Form C/	Form D/	A+B/	C+B/	D+B/
					mg ml^{-1}			
	10.	2.71	1.71	2.16	1.77	1.57	1.67	2.15
20	20.	2.76	1.62	2.02	1.76	1.55	1.60	2.00
	40.	2.77	1.68	2.07	1.87	1.60	1.67	1.65
	10.	2.96	1.76	3.03	2.21	1.86	1.77	2.22
30	20.	2.98	1.58	2.76	2.34	1.70	1.59	2.04
	40.	3.00	1.59	2.82	2.26	1.68	1.66	1.96
	10.	3.56	1.61	4.51	2.36	1.69	1.58	1.60
40	20.	3.61	–	4.31	–	–	–	–
	40.	3.58	–	4.63	–	–	–	–

Table 2 Solubilities results in ethyl acetate

T/ °C	Time/ min	Form A/	Form B/	Form C/
			mg ml^{-1}	
	10	16.9	9.3	16.2
20	20	17.1	9.3	16.6
	40	17.0	9.4	17.2
	10	21.2	9.4	21.4
30	20	22.7	12.2	22.6
	40	22.7	14.1	22.6
	10	33.1	12.5	34.8
40	20	35.1	14.7	35.3
	40	35.2	17.9	35.3
	10	98.8	21.6	88.4
60	20	99.9	52.6	95.6
	40	100.5	73.8	99.2

In conclusion B is the stable form for temperatures under about 110°C. A, C, D are monotrop to B. B' is enantiotrop to B.

The results of solubilities and equilibrations studies agree with the stability estimations found from the melting points and the melting enthalpies of A, C, D and B. But without the equilibrations, DSC alone would not have given a clear conclusion between A and B since the differences of melting enthalpies are less than 10% and also because the melting point of B is unknown.

Kinetic aspects

The results of the stability studies of form C as well as the experiments performed in suspensions for A, B, C and mixtures thereof discussed above revealed the influence of the kinetic aspect of the transformation of C or A into B. The DSC curves of forms A, C and D did not show any transformation into B after the melting point even at very slow heating rates. Form A shows a small peak of form C at 0.6 K min^{-1} heating rate. In order to check the kinetic aspect, DSC curves of mixtures were performed. As demonstrated in Fig. 6, recrystallization from melt into form B' is observed if seeds of B are present in the sample. The mixtures of the polymorphs A and B (Fig. 6a) or polymorphs C and B (Fig. 6b) in the ratio 1:1 are revealing a melting-recrystallization transformation indicating a partially parallel and consecutive transformation into B'. The comparison of the theoretical values of the melting endotherm of form B' if no transformation occurs and the measured values demonstrate clearly that a recrystallization occurs from the melt. For the mixture A+B (1:1), the value of the melting energy without recrystallization of B' is 49 and the value measured is 84 J g^{-1} indicating a strong recrystallization. For the mixture B+C (1:1), the theoretical value is also 49 and the measured value is 83 J g^{-1}. These findings comply with the exotherms observed after the melting of A and C in the mixtures containing B. For the mixture A+C (Fig. 6c) no exotherm is observed after the melting. But a higher value of the melting endotherm of C is observed (theory 46

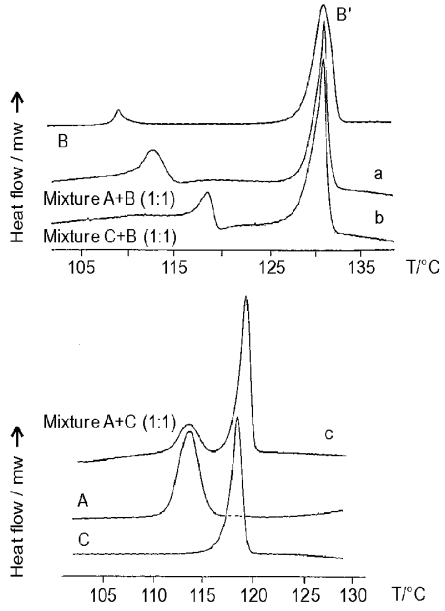

Fig. 6 Kinetic aspect: DSC curves at 2 K min^{-1} of polymorphic forms with seeds crystals added. Starting temperature: 100°C, mass 1.6 to 2.1 mg. a – mixture A+B 1:1. Calculated melting endotherm of B' per g of mixture: 49 J, found: 84 J; b – mixture C+B 1:1. Calculated melting endotherm of B' per g of mixture: 49 J, found: 83 J; c – mixture A+C 1:1. Calculated melting endotherm of C: 46 J, found: 68 J

Table 3 Characterization of the modifications of MKS 492

Characteristic	Form A	Form B	Form B'	Form C	Form D	Form E
DSC onset	111°C	–	128°C	118°C	109°C	92°C
Melting enthalpy J g^{-1}	93	98[1)]	92	89	65	44
Transition heat J g^{-1}	–	6		–	–	–
Temperature		108 to 112°C				
Weight loss by TG[2)]	<0.5%	<0.5%	–	<0.5%	2%	–
Morphology	needles	neddles		needles	plates + needles	
Hygroscopicity 1 day at 92% r.h.[3)], results of TG[2)]	<0.5%	<0.5%	–	<0.5%	hygros-copic	
Density g cm^{-3}	1.40	1.422		1.411		
Solubility water 20°C in % (w/w)	0.27	0.17		0.2	0.18	
Solubility ethanol in % (w/w) 25°C	2.4	1.5		2.5		
Solubility isopropanol 25°C in % (w/w)	1.3	0.9		1.5		
Solubility ethyl acetate in % (w/w)						
25°C	1.8	1.2		1.9		
50°C	5.7	3.3		5.6		
Solubility acetone 25°C in % (w/w)	7.1	4.3		7.2		
Dissolution rates in water at 37°C						
time in s for 50%	88	109		99	340	
time in s for 80%	219	315		330	972	
Intrinsic DR, 37°C in mg $min^{-1}cm^{-2}$						
in water		0.12		0.13		
in HCl 0.1 N		0.18		0.19		
Bands IR in cm^{-1}	3438	3502		3444	3274	3463
		3551		3309		3298
X-ray angle 2θ in degrees	12.7	7.1		4.9	3.8	9.3

[1)] Value not directly measured, however, estimated by the enthalpy of transformation (B→B') and the enthalpy of fusion of B

[2)] TG values are obtained between 25 and 150°C

[3)] 92% r.h. is obtained by using the atmosphere of the saturated sodium chloride salt

and value measured 68 J g^{-1}) indicating that recrystallization of A and melting of C occur in parallel ways.

The dissolution rates and the intrinsic dissolution rates of B and C are very similar (Table 3). Form C remains stable after granulation in the drug product. The results of the stability screening of form C showed that form C remain unchanged after 4 years in all climates, even tropical climate (30°C/75% r.h.). After tempering the batch of form C spiked with 2% water 1 month at 60 or 80°C, the transformation into B occurs.

In the up-scale the manufacturing of pure modification C was very difficult. The stable form B appeared. It was decided to proceed in the development with the stable form B. But if crystals of C appear in the conditions of the manufacturing process, the solvent mediated transformation C into B does not occur for kinetic reasons and both forms may be present. Therefore the crystallization process was adapted: temperature of the crystallization and addition of seeds of form B. Additionally a suspension in water if necessary allow the transformation of remaining crystals of C into B. Several batches of pure form B were obtained without problems.

Quantitative analysis

Taking into account the kinetic aspects, DSC is not suitable for quantitative analysis of polymorphism. X-ray diffraction is the method of choice. As demonstrated in Fig. 7, it is possible to determine less than 1% modification A or modification C in modification B. The detection limit for modification D is about 2%.

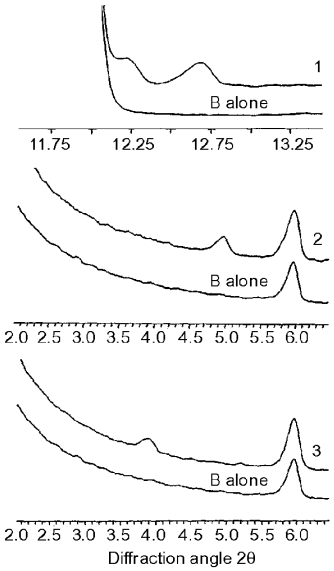

Fig. 7 Quantitative determination of forms A, C and D in form B by X-ray diffraction. Enlarged X-ray diffraction relevant parts. Comparison of X-ray diffraction pattern of form B and mixtures of form B with forms A, C and D in the limit of quantitation area: 1 – 1% form A in B, 2 – 1% form C in B and 3 – 2% form D in B

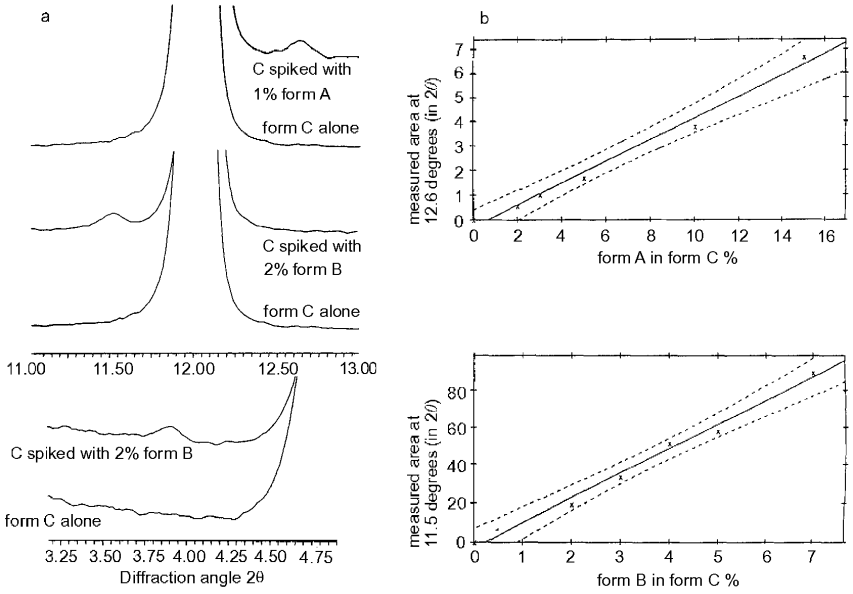

Fig. 8 Quantitative determination of forms A, C and D in form B by X-ray diffraction. a – Enlarged X-ray diffraction relevant parts. Comparison of X-ray diffraction pattern of form C and mixtures of form C with forms A, B and D in the limit of quantitation area: 1% form A in C, 2% form B in C and 2% form D in C; b – Linearity of the X-ray diffraction signal in area *vs.* the amount of the form A and the form B in the form C

The quantitation of form B in C (limit 2%) or form A in C (limit 1%) or form D in C (limit 2%) is also possible as demonstrated in Fig. 8.

Influence of amorphous state

The results of the polymorphic study showed a tendency of the substance to be partially amorphous. The drug substance was micronized. Since it is known that milling induces amorphicity, it was a need to quantify it. Two methods were developed, X-ray diffraction and microcalorimetry. The microcalorimetric method is based upon the measurement of the crystallization energy of the amorphous form into the crystalline form. The glass transition is lowered by the presence of moisture. This method applied to MKS 492 has been published [9] and the results expressed in % amorphous content. X-ray diffraction was performed at the end of the microcalorimetric experiment. Form B was always obtained. For the X-ray diffraction, the measurements are based upon the relation between amorphous background and crystalline area and the results are expressed in % crystallinity.

Different mixtures of form B and amorphous MKS 492 were manufactured and analyzed by X-ray diffraction and by microcalorimetry. Figure 9 shows the relation between the theoretical amounts and the values found by X-ray diffraction between 0 and 100% crystallinity. The slope is practically 1 and the correlation coefficient r is 0.999. For values lower than 10% of amorphous fraction, microcalorimetry is bet-

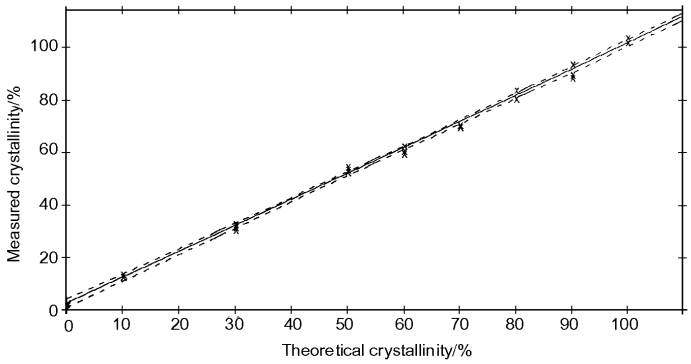

Fig. 9 X-ray diffraction: relation between theoretical crystallinity and measured crystallinity between 0 and 100%. Slope: 0.99, *r*=0.999, *n*=27

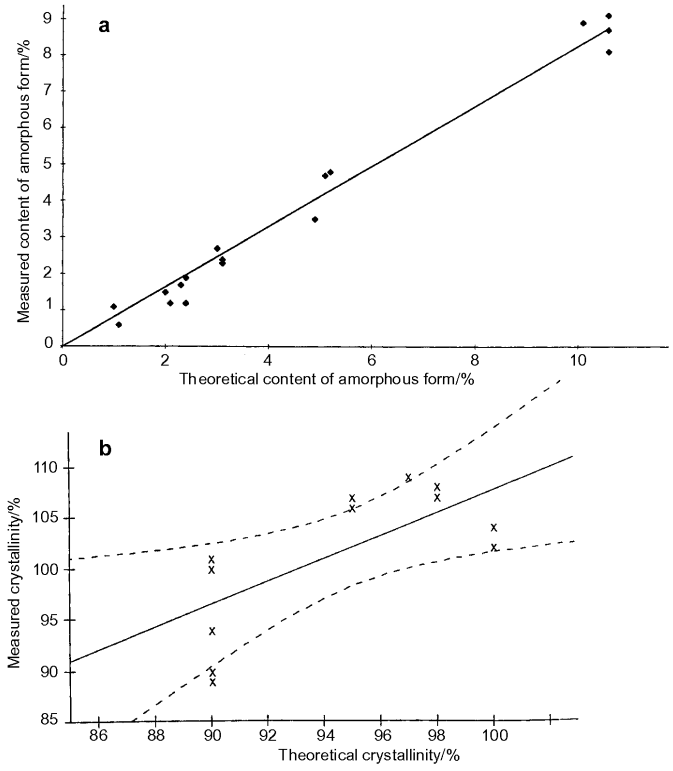

Fig. 10 Comparison of the accuracy of microcalorimetry and X-ray diffraction for low amounts of amorphous form <10% (or crystallinity >90%).
a – Microcalorimetry: measured content of amorphous part in % *vs*. the theoretical content of amorphous part in %. Slope=0.8, *r*=0.99, *n*=17.
b – X-ray diffraction: measured crystallinity in % *vs*. the theoretical crystallinity in %. Slope=1.1, *r*=0.69, *n*=14

ter than X-ray diffraction as demonstrated in Fig. 10. The slope is 0.8 and the correlation coefficient 0.99 for microcalorimetry. The correlation coefficient is only 0.69 for X-ray diffraction and the values are strongly dispersed. The limit of detection is about 1% for microcalorimetry [9]. For higher amounts, the X-ray method is preferred because it is faster and easier performed.

Conclusions

Polymorphism problems in pharmaceutical development areas have to be solved very early. The example of MKS 492 shows that several forms may appear and that thermodynamic and kinetic aspects have to be taken into consideration. An amorphous form and five crystalline modifications have been characterized. Four of them are monotrop to the stable form which is enantiotrop to the higher melting form B'. Depending of the batches of form B and the heating rates for the DSC measurements, the enantiotropic transition endotherm lies in the range of the melting temperatures of two metastable forms A and C. Only in applying equilibration of suspensions in solvents and combined techniques such as DSC/TG and temperature resolved X-ray diffraction enable an elucidation of the relationships between the polymorphs.

By this outlined procedure a careful selection of the most stable polymorph at room temperature and at the temperature range of production and storage of the drug substance and the drug product is possible. Quantitative determinations of the polymorphs or of the amorphous form may be performed by using several techniques. Microcalorimetry is an emerging technique successfully applied for low amounts of amorphous forms with limits of detection of approximately 1%.

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