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Hydrate formation of metronidazole benzoate in aqueous suspensions

Annie Hoelgaard and Niels Møller

Royal Danish School of Pharmacy, Department of Pharmaceutics, 2 Universitetsparken, DK-2100 Copenhagen (Denmark)

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Summary

Metronidazole benzoate is shown to be capable of existing in a monohydrate crystalline form having different solubility properties than the commercially available anhydrous form. The monohydrate is the thermodynamically stable form in water below 38°C. The enthalpy and entropy changes for the conversion of anhydrate to monohydrate were determined to be −1200 cal/mol and −3.7 cal/mol deg., respectively. Furthermore, the anhydrous form is shown to exhibit polymorphism. The phase transition of the anhydrous form to the hydrate in water is followed by a drastic increase in particle size causing physical instability of metronidazole benzoate in oral suspensions. These findings imply that a possible variation in bioavailability of the drug supplied as the hydrate or as the anhydrate can be ascribed to change in particle size and not to differences in thermodynamic activity at body temperature for the two forms.

Introduction

Formulation of oral preparations of metronidazole benzoate in the form of aqueous suspensions implies physical stability problems which may lead to an increase of the particle size compromising the intended effect of the substance in the treatment of aerobic bacterial infections. Growth of particles in suspensions occurs for several reasons. Two main mechanisms are: (1) dissolution and recrystallization caused by temperature fluctuations and/or presence of large quantities of sub-micron particles being more soluble than larger ones; and (2) interconversions of different crystal modifications due to polymorphism and solvation–desolvation.

Mechanism 1 leads normally to a slow increase of particle size often combined with caking phenomena whereas mechanism 2 abruptly may lead to immense changes of particle size ranging from micronized to several hundred microns. The particle growth problems of metronidazole benzoate considered in the present study are related to mechanism 2.

In literature no information about polymorphism or solvate formation of metronidazole benzoate is given. The present investigations deal with the crystalline behaviour of the drug in aqueous suspension. Specifically physical–chemical properties of the isolated crystalline modifications were associated with formulation and storage conditions of metronidazole benzoate suspensions.

Materials and methods

Materials

Metronidazole benzoate was obtained from Dumex, Copenhagen.

Differential scanning calorimetry (DSC)

DSC measurements were performed on a Perkin-Elmer instrument, type DSC-1B, equipped with an effluent gas detector. Dry nitrogen at 30 ml/min was used as carrier gas. Calibration was effected by means of an indium standard. Heating rate was 8°C/min.

Determination of water content

The mass loss of the crystals was determined after exposure to air at 60°C for 2 h.

The content of carbon, nitrogen and hydrogen was determined by elemental analysis (Perkin-Elmer Type 240).

Solubility determinations

An excess of drug in the appropriate form was dispersed in 30 ml of distilled water and placed on a magnetic stirring in a constant temperature incubator (± 0.1°C). Wetting of the metronidazole benzoate anhydrate powder was performed by ultrasonic treatment with a Branson S75 for 3 min. The mixtures were filtered after 48 h and the concentrations of the saturated solutions were determined spectrophotometrically at 320 nm using a Beckmann DU8. The half-life for the hydrolysis of metronidazole benzoate has been found to be 1000 h in phosphate buffer (pH 7.4) at 37°C (Bundgaard et al., 1982); therefore, the conversion of the ester to metronidazole was neglected in the assay. The solubilities were determined at temperatures in the range of 2–30°C and carried out in duplicate at each temperature.

Results and discussion

An immense increase in particle size of metronidazole benzoate has been registered in oral suspensions stored at 4°C for about 3 months whereas the same

suspension stored at 22°C did not show any change in particle size. The solid phases of such suspensions were isolated, washed with water and carefully dried before DSC recording. Fig. 1 shows that both samples have a well-defined endothermic

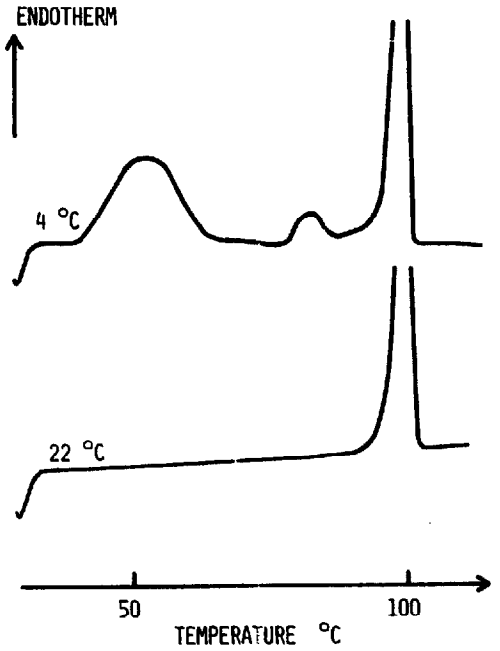

Fig. 1. DSC curves of metronidazole benzoate crystals isolated from an oral suspension stored for 3 months at 4°C and 22°C.

peak at 98°C corresponding to the melting of metronidazole benzoate. However, the sample from the cooled suspension produced a broad endothermic peak at about 40°C as well. Metronidazole benzoate dispersed by ultrasonic treatment in pure water and kept at 8°C for 3 weeks exhibited a similar pattern in the DSC curves (Fig. 2). Simultaneous effluent gas recording showed a peak in connection with the first endotherm, indicating the escape of a volatile gas. This behaviour must be due to hydrate formation when metronidazole benzoate is in contact with water at 8°C. Fig. 2 shows also the DSC curve of the original anhydrate powder, which corresponds to the commercial available product. Besides, Fig. 2 shows the detection of another modification of the anhydrate (anhydrate II) which occasionally crystallizes from isopropanol–water mixtures at room temperature. The DSC curve of this anhydrate modification shows an endothermic peak followed by an exothermic peak indicating a crystalline transformation at 77–78°C. This polymorphic transformation was verified by hot stage microscopy where the uniform needle-shaped crystals of the anhydrate II were identified (Fig. 3) and seen to transform into heterogeneous square crystals.

Fig. 3 shows microphotographs of the different crystals identified: (a) is the commercial powder on anhydrous form with particles in the sub-sieve range used for formulation of the suspensions; (b) is the hydrate appearing after storage of (a) in

water at 4°C — the hydrate is especially sensitive to photochemical surface reactions and quickly turns yellow when exposed to light; (c) is the same crystals as (b) after dehydration in dry air — after escape of water the crystals appear lustreless but

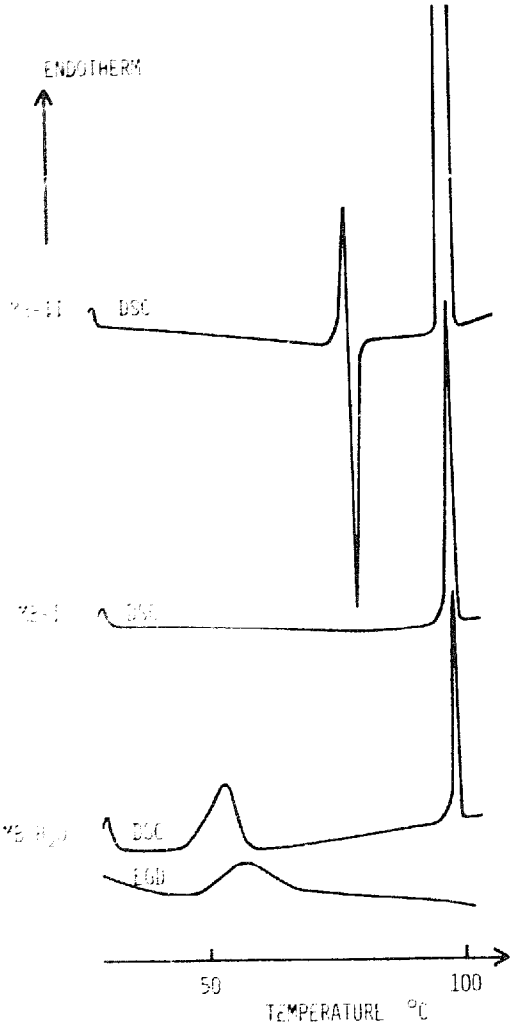

Fig. 2. DSC curves of metronidazole benzoate modifications: MB-H_2O, hydrate; MB-I, anhydrate I (commercial product); and MB-II, anhydrate II.

keep their shape; and (d) is anhydrate II crystallized from isopropanol–water mixture.

In Table 1 data are shown for quantitative determination of the water of crystallization of the hydrate. The 6.1% mass loss on drying corresponds to a 1 : 1 molar ratio of metronidazole benzoate and water. The result from the elemental analysis based on the hydrogen and oxygen content of the sample also indicates that the substance is a monohydrate.

The interconversions of the 3 modifications are summarized in Fig. 4. The monohydrate powder is dehydrated in air of low humidity while no transformation

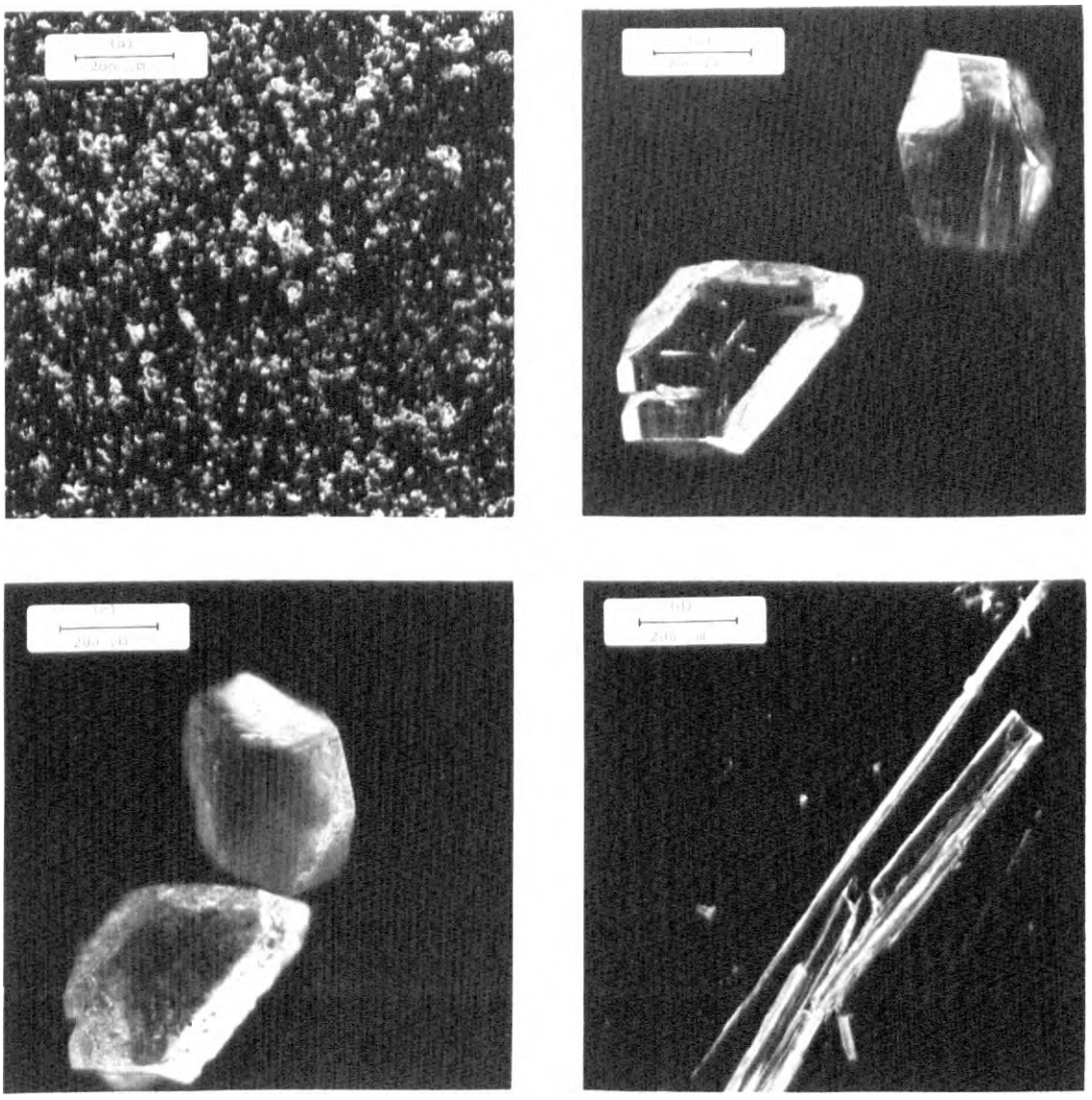

Fig. 3. Single crystals of metronidazole benzoate. (a) anhydrate I (commercial product); (b) monohydrate; (c) the (b)-crystals after dehydration; and (d) anhydrate II.

occurred in air of a 100% relative humidity when stored for at least 4 months. The anhydrates convert to monohydrate in cool water (8°C). No transformation of anhydrate I was registered in air of 100% relative humidity (stored for 4 months).

Knowledge of the transition temperature between the anhydrate I and the monohydrate is of great importance with respect to stability of metronidazole benzoate suspensions during storage. Table 2 shows the determined solubilities at different temperatures. By DSC runs of the excess solid phase it was confirmed that no phase transition took place during the 48 h equilibrium time. The apparent equilibrium solubilities observed over the temperature range 2–30°C when plotted in the van 't Hoff fashion give a reasonably good linear relationship (least-squares

TABLE 1

WATER CONTENT IN METRONIDAZOLE BENZOATE

	Loss on drying (%)	Elemental analysis	
		Hydrogen (%)	Oxygen (%)
Experimentally determined	6.1 [a]	5.0	27.9 [b]
Theoretical for monohydrate	6.1	5.1	27.3
Theoretical for anhydrate	0	4.7	23.3

[a] $s_{rel} = 8.2\%$.

[b] Calculated from the determined carbon (52.9%), hydrogen (5.0%) and nitrogen (14.2%) content.

analysis) for both forms as shown in Fig. 5. The transition temperature for the monohydrate–anhydrate system corresponds to the temperature at which the solubility of the two forms is equal. The transition temperature determined from the point of intersection of the two linear plots of ln C_s versus $1/T$ is calculated to be 38°C. This value was experimentally confirmed by incubation of the monohydrate for 4 days in water at different temperatures around 38°C. The transition of hydrate to anhydrate was observed at 37.5°C. Besides, Fig. 5 shows that the solubility of both forms increases with increasing temperatures.

The values of the heat of solution (ΔH^0) for each of the crystal forms are calculated from the slopes of the lines according to the van 't Hoff's equation:

$$\ln C_s = \frac{-\Delta H^0_{solution}}{RT} + \text{constant}$$

where C_s is the solubility, R is the gas constant and T is the absolute temperature. The ΔH^0 values become 7100 and 8300 cal/mol for the anhydrate and monohydrate, respectively (Table 3), i.e. the enthalpy of hydration ($\Delta H_{anhydrate \rightarrow hydrate}$)

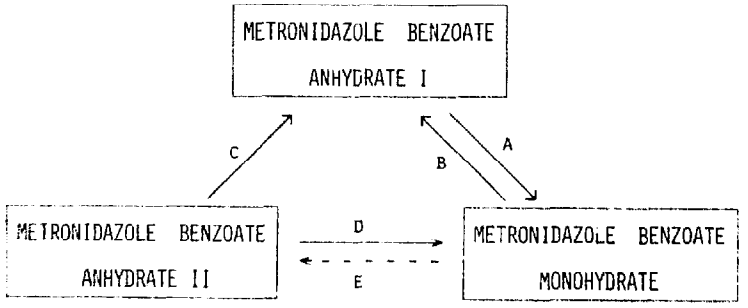

Fig. 4. Phase interconversion of metronidazole benzoate modifications. A and D: suspension in water below 8°C; B: heating above 40°C and in dry air; C heating to 77–78°C; (E) occurs occasionally by heating.

TABLE 2

AQUEOUS SOLUBILITIES OF ANHYDRATE AND MONOHYDRATE OF METRONIDAZOLE BENZOATE

Temperature (°C)	Solubility (mg/l)	
	Anhydrate I	Monohydrate
2.0	–	32.5
10.0	–	51.2
14.0	–	66.2
16.0	80.5	–
20.5	96.5	–
23.5	–	96.3
25.6	116.5	–
30.2	144.5	–

determined as the difference in heat of solutions becomes −1200 cal/mol. At the transition temperature (T_{trans}) of the anhydrate–monohydrate system the free energy change involved is equal to zero and the entropy change ($\Delta S_{A \rightarrow H}$) is calculated as:

$$\Delta S_{A \rightarrow H} = \frac{\Delta H_{A \rightarrow H}}{T_{trans}}$$

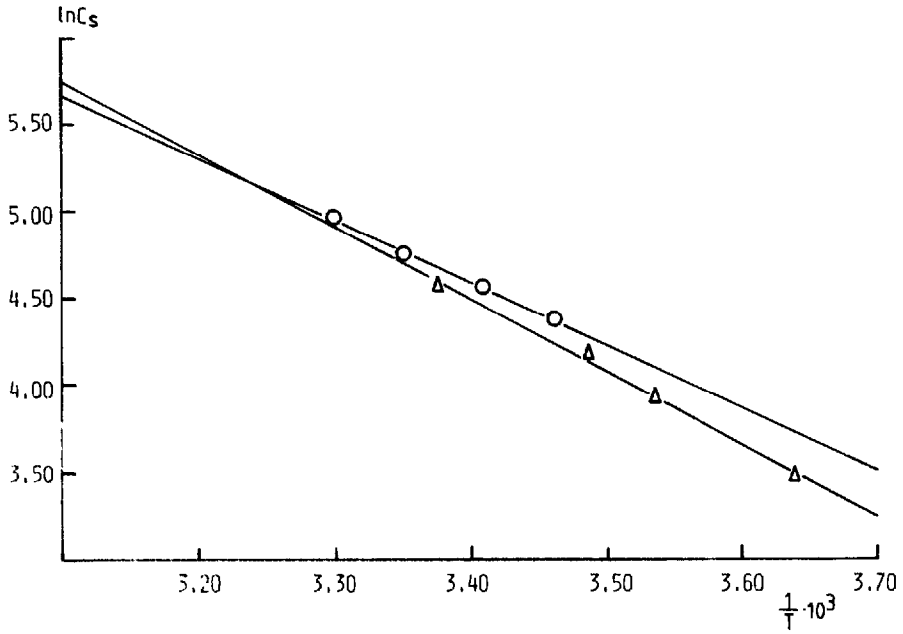

Fig. 5. Van 't Hoff plots of the aqueous solubility of metronidazole benzoate anhydrate (○) and monohydrate (△). (C_s in mg/l).

TABLE 3

THERMODYNAMIC VALUES CALCULATED FOR THE METRONIDAZOLE BENZOATE ANHYDRATE–MONOHYDRATE SYSTEM

Anhydrate, ΔH^0 (solution)	7100 cal/mol
Monohydrate, ΔH^0 (solution)	8300 cal/mol
$\Delta H_{A \rightarrow H}$	−1200 cal/mol
Transition temperature	38°C
$\Delta S_{A \rightarrow H}$	−3.7 cal/mol deg.
ΔF_T at 25°C	−49 cal/mol
ΔF_T at 8°C	−119 cal/mol

and becomes −3.7 cal/mol deg. This value is numerically considerably less than that noted by Poole and Bahal (1968) for the trihydrate–anhydrous system of ampicillin (−20.3 cal/mol deg.). However, the hydrated species in the present metronidazole benzoate system contain only one molecule of water where the possible intramolecular hydrogen bond formation between one associated water molecule may account for the relatively lower entropy change noted.

From the thermodynamic considerations a conversion of the more soluble anhydrous form to the less soluble monohydrate species would be expected. However, as mentioned earlier there was no evidence of this conversion at the temperatures utilized in the solubility studies of the anhydrate (16–30°C). At lower temperatures (below about 8°C) the conversion of anhydrate to monohydrate is relatively easy. The free energy change (ΔF_T) involved in the conversion can be calculated from:

$$\Delta F_T = RT \ln \frac{C_s(\text{monohydrate})}{C_s(\text{anhydrate})}$$

ΔF_T at 25°C and 8°C (corresponding to room- and refrigerator-temperature) are calculated to be −49 cal/mol and −199 cal/mol, respectively. Because the free energy change involved in the conversion increases as the temperature decreases the tendency of spontaneous conversion increases with lower temperatures. The thermodynamic values calculated for the anhydrate–monohydrate metronidazole benzoate system are summarized in Table 3.

The bioavailability of a drug is often seen related to its thermodynamic activity in a system of this type. But from the data presented in the present investigation no difference in biological activity between the anhydrate and the monohydrate form is to be expected because the transition temperature is equivalent to body temperature. Therefore, if anhydrate and monohydrate differ in bioavailability it is attributed to changes in particle size and surface area.

Conclusions

In the utilization of the anhydrous form of metronidazole benzoate for oral suspensions, the physical stability aspect must be considered. The system is only

apparently stable and when stored at low temperatures the metastable condition leads to hydrate formation accompanied by crystal growth. Suspensions containing the monohydrate form result in physical stable systems when stored at temperatures below the transition temperature at about 38°C.

Acknowledgement

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