ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 1069–1076

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Influence of the presence of trace amounts of metals on the polymorphism of tolbutamide[1]

A.I. Olives[a], M.A. Martin[a], B. del Castillo[a,*], C. Barba[b]

[a]*Laboratorio de Técnicas Instrumentales, Sección Departamental de Química Analítica, Facultad de Farmacia, Universidad Complutense de Madrid, 28040 Madrid, Spain*
[b]*Centro de Microscopia Electrónica, Universidad Complutense de Madrid, 28040 Madrid, Spain*

Received for review 30 January 1996

Abstract

The polymorphism phenomenon causes an important problem in the preparation of many pharmaceutical dosage forms, including oral suspensions, tablets, creams and suppositories. In this work the influence of the presence of certain metallic elements (Ca(II), Fe(III), Mn(II), Zn(II), Cd(II), Ni(II) and Co(II)) in trace amounts on the polymorphism of tolbutamide is described. For these studies, known amounts (5–500 ppm) of different cations were added to ethanol–water solutions of the pharmacologically active polymorph A of tolbutamide prior to its crystallization. These cations were chosen as being among those that commonly appear in drug solutions as a consequence of elution from glass containers. IR studies show that the presence of cations prevents the appearance of the polymorph B of the tolbutamide. Thus, the peaks at 2980, 2940, 2890, 905, 851, 816 and 727 cm^{-1} (polymorph A) appeared but not the ones at 1045 and 847 cm^{-1} corresponding to polymorph B. Scanning electron microscopy studies reveal that, in the presence of Ca^{2+}, a third kind of crystal different from polymorphs A and B appears. This form of olbutamide cannot be identified with "polymorph III" of Al-Saieq and Rileys [S.S. Al-Saieq and G.S. Riley, Pharm. Acta. Helv., 56 (1981) 125–129] due to their different IR data. Dissolution rate tests and X-ray diffractometry were also employed in this study. All these results showed that divalent cations partially avoid the transformation of polymorph A into polymorph B.

Keywords: Polymorphism; Tolbutamide; Trace metals

1. Introduction

Polymorphism is an important problem which affects many pharmaceutical formulations. Thus, certain drugs exhibit different crystallographic unit cells and space groups, and consequently show different physical and chemical properties. Although different polymorphic forms of a dissolved compound produce identical solutions, they differ in their thermodynamic actvities, equilibrium solubilities and rates of dissolution and, therefore, significant absorption and bioavailabil-

* Corresponding author.

[1] Presented at the Fifth International Symposium on Drug Analysis, September 1995, Leuven, Belgium.

PII S0731-7085(96)01784-0

ity variations can occur because of the existence of different polymorphic forms [1]. Phase transformations of polymorphs can be facilitated in oral suspensions [2], where the crystalline form of a drug coexists with dissolved drug, tablets [3], creams [4] and suppositories [5].

During the last few years research efforts of several groups have been devoted to the study of the importance of the containers on the safety of pharmaceutical products. Thus midazolam, diazepam and carmustine can be adsorbed on PVC containers [6–8]. Injections can be contaminated as a consequence of leaching of antiozonants and antioxidants from elastomers [9,10]. Mineral trace elements can be eluted to the injectable solutions and oral suspensions from the different glass containers [11] and therefore can affect the stability of dissolved pharmaceutical products.

Trace amounts of metals can favour chemical degradation of several pharmaceutical compounds due to their ability to catalyze oxidation reactions in different pharmaceutical dosage forms [3].

Several sensitive analytical instrumental techniques allow the detection of the existence of polymorphic forms. Among them, the sophisticated techniques of X-ray diffractometry or scanning electron microscopy may be employed. However, more easily available laboratory techniques such as IR spectroscopy or thermal analysis also provide important information on the changes produced in the crystalline forms.

In the present work the influence of mineral trace elements on the change of polymorphic forms of tolbutamide is described using different instrumental techniques such as IR spectroscopy, X-ray diffractometry and scanning electron microscopy as well as the conventional dissolution rate test.

2. Experimental

All reagents and solvents were analytical grade and they were used without further purification. Doubly-distilled, deionized (Milli-Q) water was used. The solutions of trace elements were prepared from the corresponding standard solution for each element purchased from Merck and Panreac (atomic absorption quality).

Tolbutamide was a generous gift from Hoechst Laboratories and was used without further purification. The analysis reveals that commercial tolbutamide corresponds to polymorphic form A [12,13] or I [14,15]. The identity test and the qualitative analysis described in the British Pharmacopoeia [16] were positive.

Form B was prepared by recrystallization of form A from ethanol/water as described by Simmons et al. [12].

The mixture of A and B forms was prepared using a mixed procedure consisting of slow crystallization for 24 h followed by evaporation to dryness under reduced pressure; this approach avoids the total transformation of A to B forms.

Small amounts of trace elements (Ca^{2+}, Fe^{3+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+} and Co^{2+}) were added to tolbutamide samples in order to study the influence of the presence of metals on the polymorphism of tolbutamide. The concentrations of the different metals were selected in a range corresponding to the metals eluted from glasses [11]. An appropriate amount of each metal was dissolved in water and aliquots of these aqueous solutions were independently added to an ethanolic solution of tolbutamide according to the procedure mentioned above for preparing the polymorph B [12]. The final concentrations of the metals present in these samples are presented in Table 1 [11]. In another experiment, all metals assayed were added to the tolbutamide solution.

IR spectra were recorded with a Beckman IR Acculab 4 spectrophotometer. Tolbutamide was ground with KBr (4% w/w) and each sample was compressed for 10 min under a pressure of 15 kp cm^{-2} (1470 kPa). In order to discount polymorphic conversion of tolbutamide due to grinding and compression during the preparation of KBr disks, these operations were carried out on mixtures of polymorph A and all metallic salts assayed. In all cases, the spectra obtained matched that of polymorph A.

Powder X-ray diffraction patterns were obtained with a Phillips X-ray diffractometer model X'pert MPD (copper anticathode). The samples

Table 1
Amounts of metals found in the different samples. The values in parentheses are those for the sample prepared with all the metals together

Metal	Detection limit (μg)	Concentration range[a] (mg l^{-1})	Amount added per g tolbutamide (μg)	Amount found per g tolbutamide (μg) ($n=6$)
Ca	400	1.2×10^4–11.3×10^4	1000	1032.3 ± 261
			(1000)	(1085.2 ± 202)
Fe	250	<500	500	ND[b]
			(125)	(ND)
Mn	75	<500	500	130.4 ± 23
			(500)	(103.6 ± 17)
Zn	75	<500	500	ND
			(125)	(87.4 ± 6.6)
Cd	10	<100	100	17.3 ± 8.9
			(100)	(22.3 ± 1.9)
Ni	30	<100	100	31.5 ± 7.9
			(100)	(ND)
Co	40	<5	5	ND
			(5)	(ND)

[a] Concentration range of metals eluted from glasses in pharmaceutical preparations [11].
[b] ND = not detectable

were placed directly on glass wafers using the "backload" procedure.

Concentrations and amounts of the different trace elements were measured with a Perkin-Elmer 403 atomic absorption spectrophotometer using the hollow cathode lamp corresponding to each element. The samples containing tolbutamide and trace elements were dissolved in a small volume of ethanol (1 or 5 ml), diluted 50, 25 or 10 times and directly measured using a flame atomizer.

The shape and surface topography of the crystals were observed by scanning electron microscopy (Jeol, JSM 6400 microscope).

The dissolution rate assays were performed in duplicate and quadraplicate as described by Al-Saieq and Riley [17]. 500 mg of each form of tolbutamide was compressed for 10 min at 15 kp cm^{-2} (1470 kPa) in a 13 mm IR punch. The disk was stirred (100 rev min^{-1}) with a stainless-steel propeller placed 3 cm from the bottom of the dissolution flask. The dissolution medium was Sörensen buffer (pH 7.6) and was kept at 37 ± 0.5°C. 10 ml aliquots from this medium were taken every 15 minutes. 10 ml volumes of Sörensen buffer were added to the medium in order to maintain a constant volume. The aliquots taken from the medium were filtered on a Millipore syringe system using a 0.45 μm pore size filter and the solution thus obtained was spectrophotometrically measured at $\lambda_{max}=228$ nm. The concentration of the tolbutamide was then calculated using calibration curves. Blank experiments were simultaneously performed in each case for the different polymorphic forms of tolbutamide in the absence of trace elements.

3. Results and discussion

Mineral elements in trace amounts which can be eluted from glass containers can effect the change between different polymorphic forms of a drug. This work is a model study of the changes in the polymorphism of tolbutamide as a consequence of the presence of these mineral elements in solution. Tolbutamide was selected for this study because polymorphism of sulfonamides is well established [18]. The differences in crystal packing are mainly responsible for polymorphism. Commercial tolbutamide corresponds to polymorph A [12,13] or form I [14,15]. This was

confirmed in these experiments by IR spectroscopy, microphotography, dissolution rate test and X-ray diffractometry.

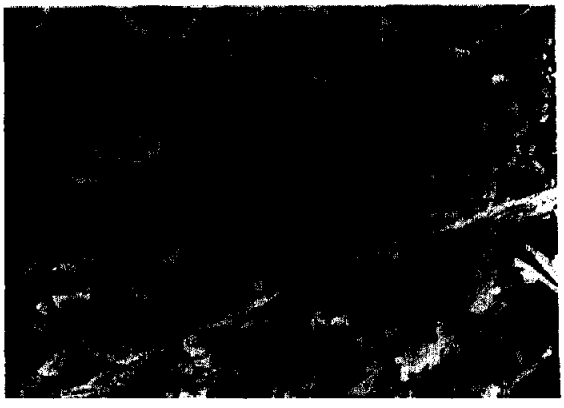
Table 1 shows the amounts of metals found in the samples of tolbutamide evaluated by atomic absorption spectroscopy. The metals were added independently and all together with tolbutamide and the different concentrations of the metals were calculated. The concentrations were calculated from the calibration curve after six replicates of each sample.

All assays were carried out in order to study the influence of the presence of metals on the change of the commercial form of tolbutamide (polymorph A) into the B form.

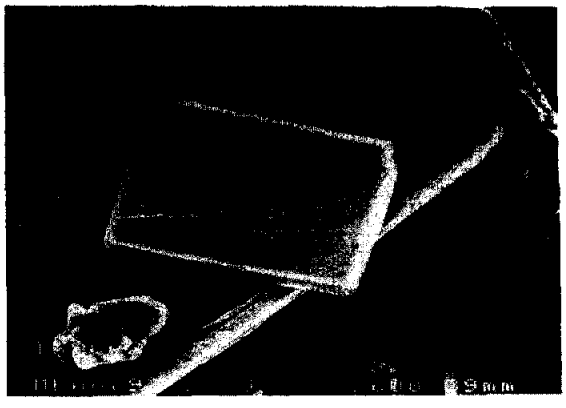
Transformation of form A of tolbutamide into form B was performed using the procedure described by Simmons et al. [12], either in the absence or in the presence of metals in the amounts shown in Table 1. The morphology of the crystals thus obtained was studied by scanning electron microscopy.

Microphotographs show the existence of different types of crystal cell depending on the polymorphic form. Thus, form A corresponds to orthorhombic prisms which have a symmetric end terminating in an angle (Fig. 1a). Form B presents a clearly different structure (Fig. 1b) with needles coexisting with plates as described by Simmons et al. [12]. When Ca(II) is added, the crystal (Fig. 1c) changes and appears as thin rectangular prisms together with isolated orthorhombic prisms or twins corresponding to form A (in the left corner of Fig. 1c). Nevertheless the latter predominate over the thin rectangular prisms. Fig. 1d and 1e show two examples of the twins (form A, as can be assigned by the orthorhombic prisms) obtained in the presence of Mn(II) and Ni(II). All metals produce the same kind of twins except for Co(II), where only the isolated crystals of form A can be observed. When all metals are present in the medium, the crystals correspond to isolated form A and twins, as well as isolated form B and the thin rectangular prisms which appear in the presence of Ca(II), both (form B and rectangular prisms) being present in smaller amounts than form A. Electron microscopy is a powerful tool for studying the surface properties and structural imperfections of the crystals. It is more useful than optical microscopy, since the crystal size can be easily determined using it [1]. The appearance of the thin rectangular prisms which is not de-

(a)

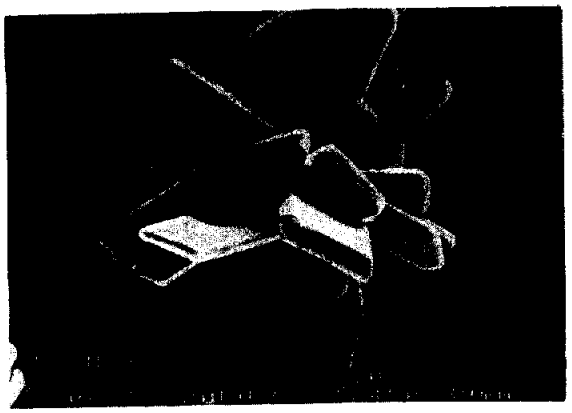


(b)



(c)

Fig. 1 (a, b and c)



(d)

(e)

Fig. 1. Microphotographs of the different polymorphs of tolbutamide as well as the forms obtained after adding different metals: form A (a), form B (b), tolbutamide + Ca (c), tolbutamide + Mn (d); tolbutamide + Ni (e).

scribed in the literature, could be ascribed to other polymorphs of tolbutamide different from forms A and B. The verification of the existence of this form was attempted employing other techniques.

Figs. 2 and 3 show the IR absorption spectra of forms A and B as well as the corresponding spectra obtained in the presence of trace metals. Differences between forms A and B can be clearly appreciated in the 2900 cm^{-1} region. Thus, form A exhibits three peaks with similar intensities at 2980, 2940 and 2890 cm^{-1}, while in the case of form B only a broad band is observed with a maximum at 2950 cm^{-1}. Other significant differences can be detected in the intensity and shape of

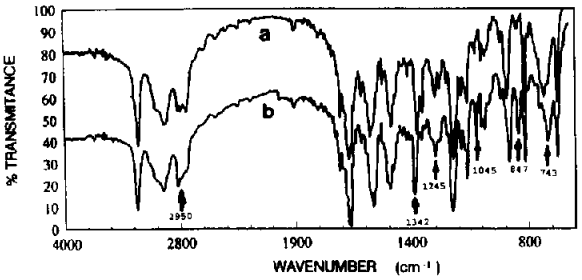


Fig. 2. IR absorption spectra of forms A (a) and B (b). (The spectra are shifted in order to avoid overlapping among the different bands. Real transmittance for (a) is 65% and for (b) 51%.)

the spectra in the region 900–700 cm^{-1}. Thus, form A exhibits maxima at 905, 851, 816 and 727 cm^{-1} which are shifted to 883, 847, 816 and 743 cm^{-1} in the case of form B; the intensity and shape of the peaks are also changed. At the same time, IR spectra of form B present a new intense and sharp peak at 1045 cm^{-1}, which does not appear in the case of form A, and an intense peak at 847 cm^{-1} which is not well defined in the case of form A. This spectral behaviour is in agreement with that described by Simmons et al. [12] and Leary et al. [13]. According to Al-Saieq and Riley [17], form B [12,13] corresponds to their form I. However, the current results produce an IR spectrum for form B [12,13] which corresponds to form II [17] and an IR spectrum for form A which agrees with Al-Saieq and Riley's form III [17].

The inclusion of metals in the crystalline structure causes changes in the IR spectra, particularly

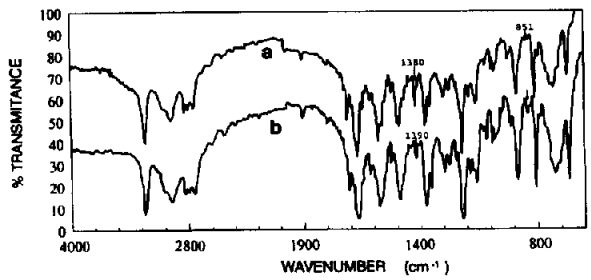


Fig. 3. IR absorption spectra of forms tolbutamide + Ca (a) and tolbutamide + Mn (b). (The spectra are shifted in order to avoid overlapping among the different bands. Real transmittance for (a) is 74% and for (b) 48%).

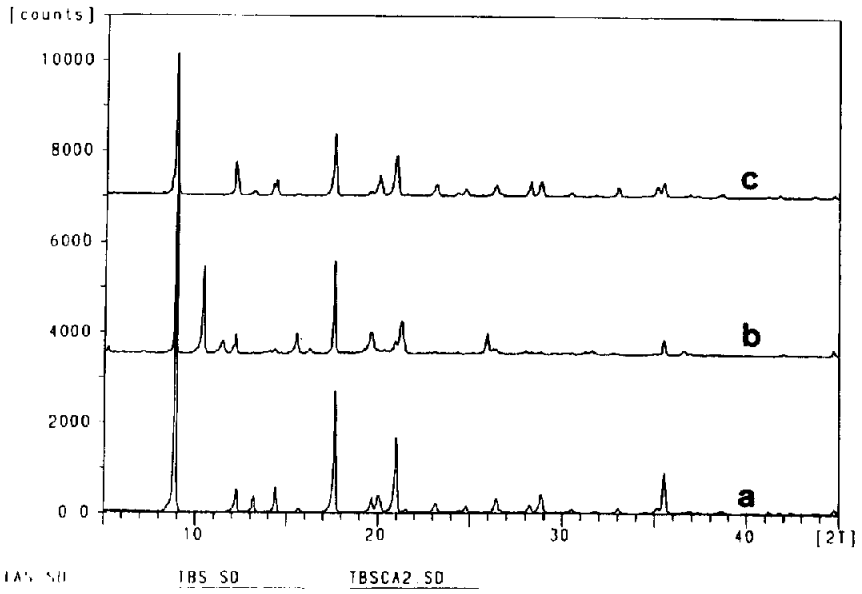


Fig. 4. X-ray diffractogram patterns of forms A (a), B (b) and tolbutamide + Ca (c).

in the case of Ca. The shape of the spectra is reminiscent of those due to form A, with slight displacements. However, the peak at 1390 cm^{-1} in form A has a weak intensity and in the presence of Ca(II) it appears at 1380 cm^{-1} with a significant intensity. In the lower frequency region, the peak at 851 cm^{-1} is weak for form A and decreases notably in the case of tolbutamide–Ca(II). This form cannot be ascribed to forms A or B [12,13] or I–IV [14,15], although it resembles form III [17].

From these spectral data it can be deduced that the presence of calcium prevents the appearance of form B of tolbutamide. The differences with the spectrum of pure form A can be attributed to a third minority structure observed in the microphotographs as rectangular prisms, which are clearly differentiated from the orthorhombic prisms due to form A. The low concentration of the new species and the relatively low sensitivity of IR spectroscopy explain the fact that only small modifications are observed in the spectra. IR spectra obtained after addition of other metals (Zn^{2+}, Fe^{3+}, Mn^{2+}) were very similar. In the case of the other metals, the IR spectra also correspond to form A, although for Mn, Fe, Zn and Ni a weak increase in the intensity of the band at 1390 cm^{-1} is observed. Thus, in the presence of these metals polymorph B was not obtained. Simultaneous addition of all metals caused the same effect on IR spectra as addition of calcium alone, suggesting that the form crystallizing as rectangular prisms is related to the concentration of metallic ions rather than to their nature. In separate experiments it was proved that grinding and pressing of KBr disks did not cause polymorph conversion.

Figs. 4 and 5 show the X-ray diffractogram patterns of the polymorphs A and B, together with those obtained in the presence of metals. It can be appreciated that both forms A and B exhibit intense diffraction peaks at $2\theta = 8.77°$ (A), 8.82° (B), and 8.82° (Ca(II)) and $2\theta = 17.62°$ (A), 17.57° (B) and 17.52° (Ca(II)). In spite of the fact that 2θ values for more intense peaks in the case of Ca(II) are very close to those observed for form B, the shape of the diffractogram resembles the one found for form A. In the presence of the different metals, the diffractogram patterns present shapes similar to those observed for form A since the characteristic peaks for form B at $2\theta = 10.36°$, 11.45° and 12.15° do not appear in these cases, while the peaks at $2\theta = 12.25°$, 13.21° and 14.39° present for form A also exist in the

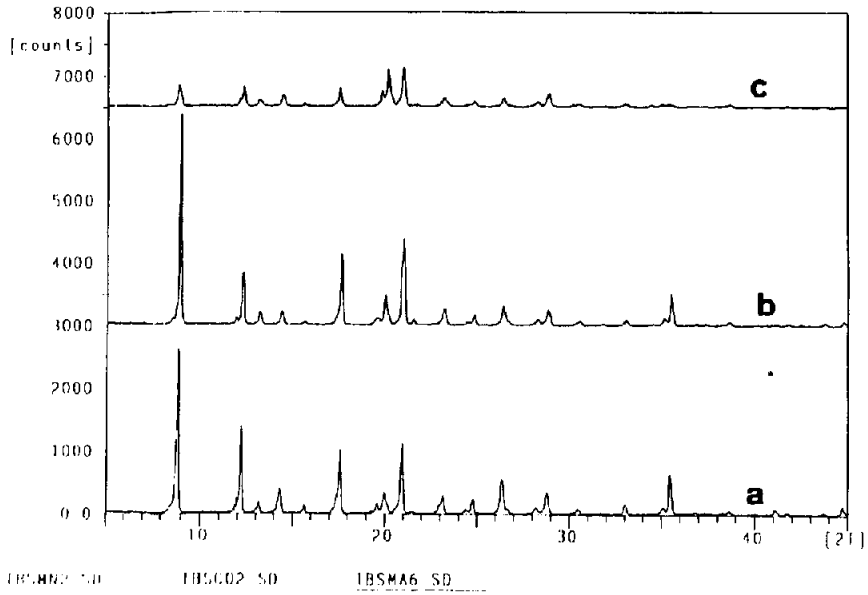


Fig. 5. X-ray diffractogram patterns of forms tolbutamide + Mn (a), tolbutamide + Co (b) and tolbutamide + all metals (c).

presence of the different metals, although with different intensities.

The profiles of the diffraction patterns obtained in the presence of Ca, Mn and Co are similar to that of form A, although the intensities are different. No signals due to form B ($2\theta = 10–11°$) were observed, proving again that the presence of metals prevents its formation. Intensity changes can be attributed to variations in the distances between the crystal planes as a consequence of ion inclusion.

Dissolution rate tests were carried out for the samples of tolbutamide (forms A and B) and also for the corresponding samples containing different metals. Polymorphic forms of tolbutamide (A and B) show a dissolution rate (Fig. 6) with the same behaviour described in the literature [17,19] and thus form B is more soluble and consequently this form is metastable.

The presence of Mn, Co or Ni (Fig. 7) lowers the dissolution rate of tolbutamide, since more time ($\approx$210–240 min) is needed to reach the same

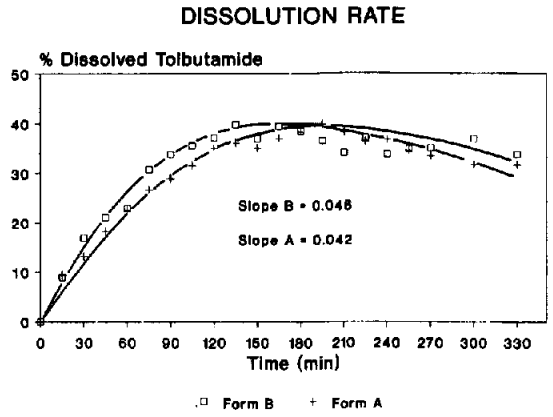


Fig. 6. Dissolution rates of forms A and B. +, Form A; □, Form B.

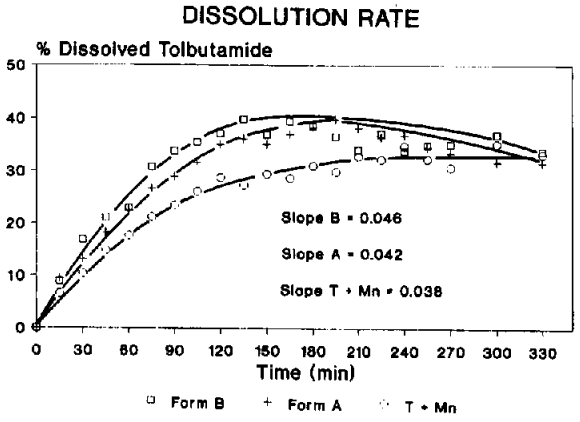


Fig. 7. Dissolution rates of forms A and B and tolbutamide + Mn. +, Form A; □, Form B; ○, T + Mn.

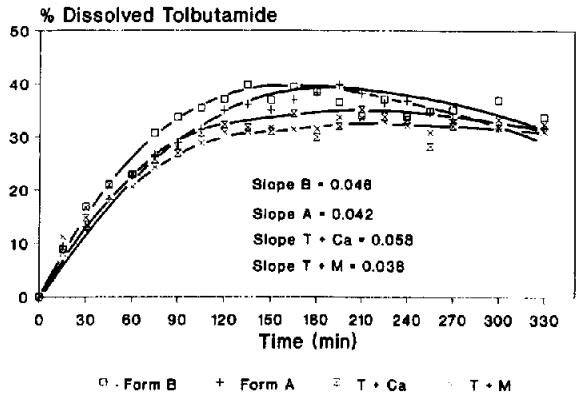


Fig. 8. Dissolution rates of forms A and B and tolbutamide + Ca and tolbutamide + all metals. +, Form A; □, Form B; ⊠, T + Ca; ×, T + M.

"maximum concentration" of the dissolved tolbutamide. However, polymorph A reaches the "maximum concentration" at 195 min and polymorph B at 135 min.

Small differences in the slopes of the dissolution curves of polymorphs A and B allow one to establish the existence of both forms. Similar differences among the slopes are observed in the presence of different metals. Nevertheless, the more significant difference in slope values was observed in the case of Ca (Fig. 8). This behaviour can be attributed to the presence of the rectangular prisms detected by microphotography together with the main orthorhombic prisms corresponding to form A, although the changes in solubility observed in the presence of metals can be explained by chelation reactions among the metals and the sulfonylurea group of tolbutamide.

In summary, studies on tolbutamide polymorphism due to the presence of metals reveal that they avoid the polymorphic transformation of form A into form B. Electron scanning microscopy shows the presence of crystals with different morphologies together with the characteristic crystals of form A, when metals are present in trace amounts. Therefore, small amounts of these metals, which can be eluted from glass containers, exert an important effect on the stability of tolbutamide.

Acknowledgement

A.I.O. thanks Glaxo for a research fellowship.

References

[1] S.R. Byrn, Solid State Chemistry of Drugs, Academic Press, New York, 1982, pp. 79–148.
[2] L. Lachman, H.A. Lieberman and J.L. Kanig, The Theory and Practice of Industrial Pharmacy, 3rd edn., Lea Febiger, Philadelphia, PA, 1986.
[3] H.A. Lieberman and L. Lachman, Pharmaceutical Dosage Forms: Tablets, Vol. 3, M. Dekker, New York, 1982.
[4] J.T. Carstensen, in G.S. Banker and C.T. Rhodes (Eds.), Modern Pharmaceutics, 2nd edn., M. Dekker, New York, 1990.
[5] J.T. Carstensen, Drug Stability: Principles and Practices, M. Dekker, New York, 1990.
[6] C. Bianchi, C.B. Airaudo and A. Gayte-Sorbier, J. Clin. Pharm. Ther., 17 (1992) 223–227.
[7] C.B. Airaudo, A. Gayte-Sorbier and C. Bianchi, J. Clin. Pharm. Ther., 18 (1993) 389–392.
[8] M.F. Hadji-Minaglou-Gonzalvez, A. Gayte-Sorbier, C.B. Airaudo and M. Verdier, Clin. Ther., 14 (1992) 821–824.
[9] C.B. Airaudo, A. Gayte-Sorbier, R. Momburg and P. Laurent, J. Chromatogr., 354 (1986) 341–354.
[10] C.B. Airaudo, A. Gayte-Sorbier, R. Momburg and P. Laurent, J. Biomater. Sci. Polym. Edn., 1 (1990) 231–241.
[11] D. Pradeau, J. Petiot, V. Bissery, M. Hamon, R. Grangeon, M. Lacaze and J. Chenebaux, Int. Pharm. J., 2 (1988) 209–215.
[12] S.L. Simmons, R.J. Ranz, N.D. Gyanchandani and P. Picotte, Can. J. Pharm. Sci., 7 (1972) 121–123.
[13] J.R. Leary, S.D. Ross and J.K. Thomas, Pharm. Weekbl. Sci. Edn., 3 (1981) 62–66.
[14] A. Burger, Sci. Pharm., 43 (1975) 161–168.
[15] E.L. Rowe and B.D. Anderson, J. Pharm. Sci., 73 (1984) 1673–1675.
[16] British Pharmacopoeia, Vol. I, HMSO, London, 1993.
[17] S.S. Al-Saieq and G.S. Riley, Pharm. Acta Helv., 56 (1981) 125–129.
[18] M. Kuhnert-Brandstutter, Thermomicroscopy in the Analysis of Pharmaceuticals, Pergamon, New York, 1971.
[19] H. Ueda, N. Nambu and T. Nagai, Chem. Pharm. Bull., 30 (1982) 2618–2620.