Horvath, G., Benko, G. (1984) Acta Physiol. Hung. 63: 21–27

Levin, R. I., Jaffe, E. A., Weksler, B. B., Tack-Goldman, K. (1981) J. Clin. Invest. 67: 762–769

Mayeux, P. R., Ignarro, L. J., Hyman, A. L., Kadowitz, P. J., McNamara, D. B. (1985) Fed. Proc. 44: 1469

Morcillo, E. (1980) J. Pharm. Pharmacol. 32: 340–343

Morcillo, E., Reid, P. R., Dubin, N., Ghodgaonkar, R., Pitt, B. (1980) Am. J. Cardiol. 45: 53–57

Nakabayashi, S., Uyama, O., Nagatsuka, K., Uehara, A., Wanaka, A., Yoneda, S., Kimura, K. Kamata, T. (1985) Res. Commun. Chem. Pathol. Pharmacol. 47: 323–332

Neichi, T., Tomisawa, S., Kubodera, N., Uchida, Y. (1980) Prostaglandins 19: 557–586

Nugent, W. C., Nieminen, M. T., Liapis, C. D., Watkins, W. D., Kong, D. L., Peterson, M. B., Philbin, D. M., Levine, P. M. (1982) J. Surg. Res. 33: 225–232

Pace-Asciak, C. R., Carrara, M. C. (1979) Biochim. Biophys. Acta 574: 177–181

Panganamala, R. V., Hanumaiah, B., Merola, A. J. (1981) Prostaglandins Med. 6: 233—235

Schror, K., Grodzinska, L., Darius, H. (1981) Thromb. Res. 23: 59–67

Simonetti, I., De Caterina, R., Marzilli, M., De Nes, M., L'Abbate, A. (1983) Z. Kardiol. 72 (suppl. 3) 40–45

Thadani, V., Kellerman, D. (1983) Z. Kardiol. 72 (suppl. 3): 35–39

Unger, W. G. Stamford, I. F., Bennett, A. (1971) Nature 233: 336–337

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Surface area and crystallinity of Form A of chloramphenicol palmitic and stearic esters: which one is the limiting factor in the enzymatic hydrolysis?

F. Forni, V. Iannuccelli, R. Cameroni*, *Department of Pharmaceutical Sciences, University of Modena, 19 Via S. Eufemia, 41100 Modena, Italy*

Chloramphenicol stearic and palmitic esters in the polymorphic Form A, when ground for 85 h showed an in-vitro enzymatic hydrolysis rate constant (K_{hydr}), the value of which was the same as that of a commercial Form B. The increase in the rate of the enzymatic hydrolysis was not related to the specific surface area as shown by the fact that the micronized Form A, having a higher specific surface area value than ground Form A, showed the same K_{hydr} as the unground Form A. The K_{hydr} value of the ground Form A could be the result of an increase in the crystalline disorder brought about by the grinding process.

The therapeutic activity of chloramphenicol (CAP) palmitic (Glazko et al 1952) and stearic (Pauletta 1952) esters is due to their in-vivo enzymatic hydrolysis to free chloramphenicol by pancreatic lipase.

Glazko et al (1958) reported that the in-vitro enzymatic hydrolysis rate of chloramphenicol palmitate depended on the particle surface area. Almirante et al (1960) stated that blood levels reached with the CAP stearic ester were dependent on the polymorphic form used: either a mostly amorphous form that is hydrolysed by enzymes in the gastrointestinal tract or a crystalline form that does not yield useful blood levels of CAP.

Tamura & Kuwano (1961) found two crystalline forms and one amorphous form of CAP palmitic ester; Aguiar et al (1967) found that suspensions containing only Form B gave higher blood levels than those with Form A; those authors also specified that the increase of particle size from 5 to 25 μm had a limited influence on the bioavailability of the drug.

Kelbaek & Ulrich (1969) found that the hydrolysis rate decreased with increasing particle size.

There are different opinions about the mechanism of enzymatic hydrolysis of the CAP esters, but the existence of a biologically active Form B and of a biologically inactive Form A has been proved (Almirante et al 1960; Aguiar et al 1967). The bioactivity differences between the CAP ester polymorphs have been explained by the relatively large free energy difference (Andersgaard et al 1974) and by the different surface properties of the crystals (Kaliszan 1986).

The present work aimed for more information about the factors involved in the enzymatic hydrolysis of the biologically inactive Form A of CAP palmitic and stearic esters.

Materials and methods

As chloramphenicol palmitate (CAP-P) (F.U. IX) and stearate (CAP-S) (Codex Erba) (Carlo Erba), named 'non polimorfo A' by the manufacturer, correspond to the biologically active Form B of both esters, they were used in the present work without purification as commercial CAP-P and CAP-S samples. To prepare the polymorph A, these products were used after repeated crystallizations from toluene–n-hexane and from methanol–water according to Miyamoto et al (1973).

Pancreatin, FIP-lipase 7·5 u mg^{-1} (Merck), was used as received from the manufacturer. All solvents were of analytical grade (Carlo Erba).

Preparation of polymorph A

Unground Form A (A). Form A of CAP-P and CAP-S was obtained according to Borka (1970) by crystallization from chloroform of the commercial purified CAP-P and CAP-S.

Micronized Form A (A_m). The Form A obtained from CAP-S was micronized using a JM-80 (Fryma).

* Correspondence.

Ground Form A (A_g). Form A was ground for 85 h using a Pulverisette O grinder equipped with agate mortar and ball (Fritsch).

IR spectroscopy. An IR spectrophotometer (model 681, Perkin-Elmer) was used to record the spectra in Nujol mull according to Borka & Backe-Hansen (1968). The IR spectra of polymorph A samples (A, A_g and A_m) and of commercial samples (Form B) agree with those reported by Borka & Backe-Hansen (1968) for CAP-P and by Almirante et al (1960) for CAP-S.

Differential scanning calorimetry. The thermograms were recorded on a DSC-4 differential scanning calorimeter equipped with a computerized data station (Perkin-Elmer). Indium (99·99%, Perkin-Elmer) and phenanthrene (RS, Carlo Erba) were used to check instrument calibration. All samples (3–4 mg) were heated at a scanning rate of 10 °C min^{-1} using nitrogen as the effluent gas (30 mL min^{-1}). Each melting-point was assumed to be the temperature at which the transition was first detected (onset). The determinations of the transition temperature and heat were made by a computerized procedure (Perkin-Elmer). The melting-points of the commercial samples (Form B) were 86·2 °C for CAP-P and 88·2 °C for CAP-S.

X-ray diffractometry. X-ray diffraction patterns were recorded using a PW 1050 Powder Diffractometer (Philips). Experimental settings: Ni-filtered Cu radiation (λ = 1·5418 Å); tube settings 40 kV, 20 mA; angular speed 1° (2θ) min^{-1}; 1-0.1-1 slits. All X-ray diffraction analyses were carried out on totally randomized powder samples to avoid the (0 k 0) preferred orientation effects (Brown & Brindley 1980).

Surface area determination. The specific surface area was determined using a mercury porosimeter (model 225, Carlo Erba) with the technique described by Carli & Motta (1984).

Determination of in-vitro enzymatic hydrolysis. The in-vitro enzymatic hydrolysis was carried out using the apparatus and the methods of Cameroni et al (1976).

Determination of polymorph A in the commercial samples. The amount of polymorph A in the commercial samples (Form B) was calculated following the spectrophotometric method described by Borka & Backe-Hansen (1968). This showed no polymorph A in the commercial samples within the detection limits.

Identification of decomposition products. Purity of the samples was checked by thin-layer chromatography according to Knabe & Kräuter (1963); no degradation products were shown.

Determination of free chloramphenicol. Free chloramphenicol in each sample was calculated following the methods of Andersgaard et al (1974). The procedure showed that CAP esters contained not more than 0·03% free chloramphenicol.

Results and discussion

The ground Form A of both esters shows differences both in the thermogram and in the X-ray diffraction pattern compared with unground and micronized Form A.

From the differential scanning thermograms, a small lowering of about 0·5–0·7 °C in the melting-point temperature and of about a 0·5 kcal (21 kJ) mol^{-1} in the heat of fusion value were noted for the ground Form A compared with unground and micronized Form A (Table 1).

Table 1. Melting point (°C) and heat of fusion (kcal mol^{-1}) values of chloramphenicol palmitic (CAP-P) and stearic (CAP-S) esters.

	CAP-S		CAP-P	
Preparation	Melting point (°C)	Heat of fusion (kcal mol^{-1})	Melting point (°C)	Heat of fusion (kcal mol^{-1})
A	93·7	16·99	91·0	15·59
A_m	93·8	17·09	—	—
A_g	93·2	16·35	90·3	14·97

Neither grinding or micronization produced a phase change. The peak angular values in the X-ray diffraction patterns (Fig. 1) are the same values reported by Aguiar

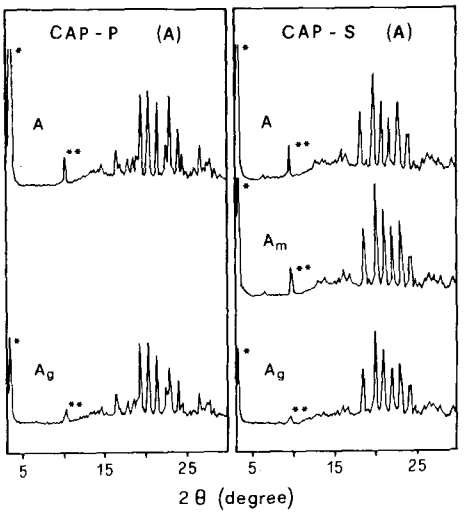

FIG. 1. X-ray diffraction patterns of Form A of chloramphenicol palmitic (CAP-P) and stearic (CAP-S) esters. Key: (*) (0 2 0) reflection, (**) (0 6 0) reflection.

et al (1967) for CAP-P Form A and by Almirante et al (1960) for CAP-S Form A. The X-ray diffraction pattern of ground CAP-P and CAP-S Form A shows a remarkable reduction in the intensities of (0 k 0) reflections, chiefly of the (0 2 0) and (0 6 0) reflections. As the experimental method used for the X-ray diffractometry avoids any preferential orientation effect on the reflection intensity, the reduction in the intensities of (0 k 0) reflections suggests an increase of the crystalline disorder. Micronized Form A shows no change in the X-ray diffraction intensities.

The grinding and the micronization processes increase the specific surface area (Table 2).

Table 2. Specific surface area ($m^2 g^{-1}$) and in-vitro enzymatic hydrolysis rate constant (K_{hydr}) (h^{-1}) values of chloramphenicol palmitic (CAP-P) and stearic (CAP-S) esters.

	CAP-S		CAP-P
	Specific surface area ($m^2 g^{-1}$)	K_{hydr} (h^{-1})	K_{hydr} (h^{-1})
A	2·033	0·046	0·103
A_m	4·627	0·048	—
A_g	3·376	0·394	0·398
Commercial sample (Form B)	2·702	0·246	0·987

The in-vitro enzymatic hydrolysis rate constant (K_{hydr}) value of the ground Form A increases as the specific surface area increases. However, the specific surface area value of the micronized CAP-S Form A are the highest one, whereas its K_{hydr} value is the same as the one of the unground CAP-S Form A. Therefore, the increase of the specific surface area obtained by micronization does not give an increase of the K_{hydr} value (Table 2). Should the surface area play a key role in the rate of enzymatic hydrolysis, the K_{hydr} value of the micronized Form A also had to increase.

Different authors (Glazko et al 1958; Aguiar et al 1967; Kelbaeck & Ulrich 1969) stated that the in-vitro enzymatic hydrolysis test could be used to predict CAP blood levels. Therefore, from Table 2 it is also clear that the ground Form A, (Ag), should give at least the same in-vivo enzymatic hydrolysis as the CAP-S commercial sample (Form B). Hence the results obtained seem to allow the conclusion to be drawn that the ground Form A may be biologically active.

In our opinion, the increase in the rate of enzymatic hydrolysis is related to the reduction in the intensities of (0 k 0) reflections. In fact the mere increase of the surface area does not affect the K_{hydr} value of the micronized Form A of chloramphenicol stearate.

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REFERENCES

Aguiar, A. J., Krc, J., Kinkel, A. W., Samyn, J. C. (1967) J. Pharm. Sci. 56: 847–853

Almirante, L., De Carneri, I., Coppi, G. (1960) Il Farmaco, Ed. Pr. 15: 471–482

Andersgaard, H., Finholt, P., Gjermundsen, R., Hoyland, T. (1974) Acta Pharm. Suec. 11: 239–248

Borka, L. (1970) Ibid. 7: 1–6

Borka, L., Backe-Hansen, K. (1968) Ibid. 5: 271–278

Brown, G., Brindley, G. W. (1980) in: Brindley, G. W. Brown, G. (eds) Crystal Structures of Clay Minerals and their X-ray Identification, Mineralogical Society, London, pp 310–311

Cameroni, R., Coppi, G., Gamberini, G., Forni, F. (1976) Il Farmaco, Ed. Pr. 31: 615–624

Carli, F., Motta, A. (1984) J. Pharm. Sci. 73: 197–203

Glazko, A. J., Edgerton, W. H., Dill, W. A., Lenz, W. R. (1952) Antibiot. Chemother. 2: 234–242

Glazko, A. J., Dill, W. A., Kazenko, A., Wolf, L. M., Carnes, H. E. (1958) Ibid. 8: 516–527

Kaliszan, R. (1986) J. Pharm. Sci. 75: 187–189

Kelbaek, A., Ulrich, K. (1969) Arch. Pharm. Chem. 76: 1089–1098

Knabe, J., Kräuter, R. (1963) Arch. Pharm. 296: 190–205

Miyamoto, M. A., Kiyotaki, T., Kisoh, N. A., Mitsunaga, T., Maeda, T. (1973) Chem. Pharm. Bull. 21: 1857–1867

Pauletta, G. (1952) Il Farmaco, Ed. Sc. 7: 3–10

Tamura, C., Kuwano, H. (1961) Yakugaku Zasshi 81: 755–759