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The assay and stability of chlorpropamide in solid dispersion with urea

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Thin layer chromatography followed by reflectance densitometry has been used to evaluate the stability of chlorpropamide-urea during the fusion process. Urea was found to decompose to biuret and chlorpropamide to *p*-chlorobenzenesulphonamide: several other unidentified decomposition products were detected. The energy for decomposition of chlorpropamide was 57·1 kJmol^{-1} for melts containing 15 and 30% chlorpropamide. Decomposition followed apparent first order kinetics.

Stability studies on solid dispersed systems have rarely been reported. Allen (1972) showed that an amber discolouration of solid dispersions of steroids in sugars was caused by decomposition of the dextrose and sucrose. Chiou & Riegelman (1971) suggested that decomposition of digitoxin dispersed in polyethylene glycol 6000 may be reduced by lowering the fusion temperature, decreasing the proportion of digitoxin in the solid dispersion or by using the solvent method. A composition-dependent decomposition of primidone-citric acid melts has similarly been shown (Summers & Enever 1976).

Urea has often been used as the water soluble carrier in solid dispersions. Melts containing more than 80% w/w sulphathiazole dispersed in urea were discoloured, soft and sticky after fusion because of thermal decomposition (Chiou & Niazi 1971). The decomposition of urea to biuret when fused with salicylic acid was predicted by Collett et al (1976) whilst El-Banna et al (1978) showed degradation in coprecipitates of aspirin and urea, and postulated that the partial decomposition of urea to ammonia increased the pH of residual water and thus increased the decay of aspirin.

Recently Ford & Rubinstein (1977a,b) studied urea-chlorpropamide solid dispersions and showed the existence of a eutectic containing 89% w/w chlorpropamide and of a solid solution of urea in chlorpropamide. Melts containing 30% w/w chlorpropamide possessed an intrinsic dissolution rate 930 times greater than that of the pure drug, although no stability studies on the system were reported. This paper now details the stability of such a system.

MATERIALS AND METHODS

Chlorpropamide (Berk Pharmaceuticals) and Urea (Analar) were used without further purification.

The solvents used for thin layer chromatography were chloroform (G.P.R. Grade A), methanol and absolute ethanol (both Analytical reagents) and ammonia solution (specific gravity 0·88; Analar). Biuret (Analytical reagent) and *p*-chlorobenzenesulphonamide (Aldrich Chemical Co., Inc.) were used as t.l.c. standards. Precoated t.l.c. plates, with fluorescing agent (silica gel 60F_{254}) and without fluorescing agent (silica gel 60) 20 × 20 cm, 0·25 mm absorbent (Merck) were used without activation. Iodine and ninhydrin were the locating reagents.

Solid dispersion preparation

Chlorpropamide and urea were powdered (sub 60 mesh sieve) and mixed thoroughly in the desired proportions by trituration. Weighed quantities of the various mixes, equivalent to 150 mg chlorpropamide, were placed in test tubes and used for stability studies.

For qualitative t.l.c. samples containing 10 and 90% chlorpropamide were used. The samples were heated to 98° or 135–140 °C for 10 min; these temperatures correspond respectively to just above the eutectic temperature and the temperature of complete melt (Ford & Rubinstein 1977a). After cooling on ice, the samples were dissolved in approximately 25 ml of ethanol before spotting on the t.l.c. plates.

For quantitative t.l.c. samples containing 15, 30, 80 and 100% w/w chlorpropamide were prepared. Samples were incubated at 70, 98, 111 and 132 °C for up to 4 h. Tubes were removed hourly and immediately cooled in iced-water to prevent further degradation. The samples were dissolved in ethanol, and the volume was made up to 100 ml before assay.

Qualitative t.l.c.

The plates were inserted in suitable tanks equilibrated with chloroform–methanol–ammonia solution (80:20:1). Plates were spotted with the melt solu-

* Correspondence.

tions, together with standard solutions of chlorpropamide and urea plus the breakdown products *p*-chlorobenzenesulphonamide and biuret. U.v. absorbing compounds were detected as dark spots on a green fluorescent background (silica gel F_{254} plates) when irradiated with short wave u.v. light. Plates were sprayed with iodine vapour (Brante 1949) or 0·5% alcoholic ninhydrin solution (Kaistha 1969) and heated at 110 °C (30 min) to visualize other components on both types of plates.

Quantitative t.l.c.

Plates without fluorescent indicator were used with the same solvent mixture as above. Solvent fronts were run for approximately 15 cm and the plates air dried to remove ammonia. Nine 5 μl samples were applied to each plate using a Tenimo microsyringe (M5–O5).

Absorbance was determined by scanning the plates along their lines of development with a Carl Zeiss Chromatogram Spectrophotometer, coupled to a chart recorder and using the relectance mode. Preliminary studies indicated that the maximum absorbance of chlorpropamide occurred at 232 nm. The test area measured 1 cm × 0·5 mm. A scanning rate of 12 cm min^{-1} was used, and peak areas were determined with a planimeter. Ethanolic solutions of chlorpropamide were used for calibration; the limit of detection was 0·05 μg. The estimated error in the assay was ±5%. Preliminary studies indicated that breakdown products did not interfere with the assay, although the spot areas of chlorpropamide and biuret were not always completely separated.

RESULTS AND DISCUSSION

Qualitative t.l.c.

Table 1 summarizes the t.l.c. details of chlorpropamide, urea and their breakdown products. Biuret, *p*-chlorobenzenesulphonamide and 6 unidentified products (A–F) were characterized: the day to day variation in their R_F values is reflected in the ranges given. The u.v. absorbing compounds were always well-separated from chlorpropamide and their separation formed the basis of the densitometric assay.

The number of breakdown products identified under the fusion conditions depended on the temperature used, more being detected at the higher temperature of fusion although *p*-chlorobenzenesulphonamide was detected in all fused samples. Biuret was detected only at the higher fusion temperature: products A, B and F were detected at 135–140 °C whilst product E was detected in samples fused only at 98 °C. Product F was detected in samples containing 90% chlorpropamide only, whereas product A was in samples containing either 90% or 10% chlorpropamide. Breakdown products B to E were identified only in melts containing 10% chlorpropamide.

Table 1. Summary of qualitative t.l.c. results.

Material		u.v.	Colour with ninhydrin reagent*	Reaction with iodine vapour	R_F range ×100
Chlorpropamide		+	Pink	+	13–26
Urea		−	White	+	5–16
Biuret		−	White	−	10–23
p-Chlorobenzene sulphonamide		+	Pink	+	51–69
Break down Product	A	−	White	+	31–52
	B	−	—	+	14–23
	C	−	—	+	6–12
	D	+	White	+	3–8
	E	−	Pink	−	9–20
	F	+	Pink	−	4–10

* After development the whole plate appeared pale brown.

Formation of biuret from urea is not unexpected during fusion. Sengupta & Varma (1972) showed that hydrolysis of urea occurs at elevated temperatures in the presence of trace quantities of water. Similarly *p*-chlorobenzenesulphonamide is a recognized breakdown product of chlorpropamide (Kaistha 1969). The presence of n-propylamine has also been shown (Kaistha 1969; Kuniki et al 1974), and under the t.l.c. conditions used should have a R_F value lower than chlorpropamide with production of a purple colour with ninhydrin (Kuniki et al 1974). However, the use of fusion temperatures of 98 °C or greater, would volatalize the formed propylamine.

Quantitative t.l.c.

The u.v. assay of chlorpropamide (Toolan & Wagner 1959; Ford & Rubinstein 1977b) is not specific if a u.v. absorbing compound such as *p*-chlorobenzene-sulphonamide is present in the sample (Kaistha 1969). The method of the British Pharmacopoeia (1973) is unspecific if other titratable break-down products are present and the B.P. (1968) method which involves determination of nitrogen, could not be used due to the presence of urea. The selective t.l.c. assay for chlorpropamide was success-

ful because of prior separation from other products.

Typical calibration peaks of chlorpropamide were symmetrical and a linear relationship between the square root of the area under each peak and the $\log_{10}$ concentration of chlorpropamide applied was obtained. Calibration was linear between 1 and 11 μg and amounts as low as 0·05 μg could be detected, although each plate had to be calibrated with known quantities of chlorpropamide. The concentrations of unknowns were determined using linear regression of the calibrations for each plate.

Fig. 1 shows typical recordings of a calibration peak T, and a test peak U both of chlorpropamide, in the presence of decomposition products labelled V (unidentified) and W (*p*-chlorobenzenesulphonamide). The densitometric assay was used to study the degradation kinetics of chlorpropamide in melts with urea.

Chlorpropamide-Urea solid dispersion stability

Accelerated stability tests on chlorpropamide alone show that degradation to *p*-chlorobenzenesulphonamide occurred at about its melting point. The decomposition at 132 °C followed 1st order kinetics with an estimated t50% of approximately 502 min (Table 2). The instability was not reported by Burger (1975), who prepared polymorphic modifications of chlorpropamide from the vitreous state.

Figs 2 to 4 show that the presence of urea in the melt of chlorpropamide accelerated this degradation, the decomposition following apparent first order kinetics. In the solid state (70 °C) below the eutectic temperature, no decomposition of chlorpropamide occurred. However, at and above the eutectic temperature the liquid solution of chlorpropamide and urea showed great instability. All the compositions used (15, 30 and 80% chlorpropamide) correspond to those on the excess urea side of the eutectic (Ford & Rubinstein 1977a), thus all the chlorpropamide present would be in the liquid state. The indicated t50% values (the time for 50% of chlorpropamide to degrade) and decomposition rate constants, are given in Table 2. Arrhenius plots of these (Martin et al 1970) lead to the derived energy of decomposition of chlorpropamide- 57·1 kJmol^{-1} for melts containing 15 and 30% chlorpropamide. Similar plots for the decomposition of melts containing 80% chlorpropamide did not show a linear relationship and therefore no estimation of a decomposition energy could be made.

Dissolution rate increases previously reported for melts of chlorpropamide and urea (Ford & Rubinstein 1977b) thus clearly referred to partially decomposed systems. Estimation of the percent of chlorpropamide lost during the fusion period (5 min) for melt preparation indicate a loss of 8·8% chlorprop-

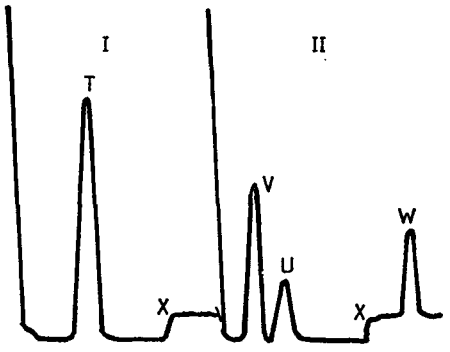

FIG. 1. Thin layer chromatogram of (I) Calibration peak (11 μg) and (II) Test mixture containing 15% chlorpropamide and 85% urea maintained at 111 °C for 3 h. T, Calibration chlorpropamide peak. U, Undecomposed chlorpropamide peak. V, Breakdown product. W, *p*-chlorobenzenesulphonamide. X, Developing solvent impurities.

Table 2. The effect of temperature and composition on the decomposition half lives (t50%: min) and first order decomposition rate constants (k h^{-1}) of various chlorpropamide-urea mixes.

	Temperature					
	98 °C		111 °C		132 °C	
% Chlorpropamide	t50%	k	t50%	k	t50%	k
15	184	0·226	86	0·484	38	1·104
30	176	0·236	90	0·462	38	1·097
80	211	0·197	—	—	110	0·376
100	—	—	—	—	502	0·083

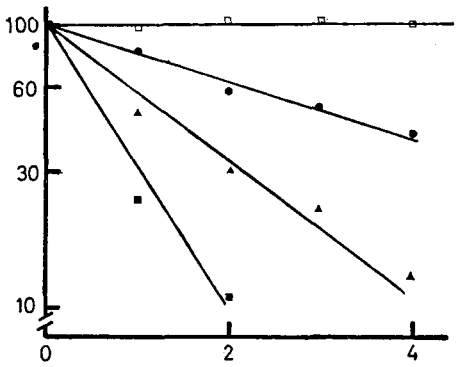

FIG. 2. Decomposition of melts containing 15% chlorpropamide and 85% urea. Temperatures of fusion: □70°, ●98°, ▲111° and ■132 °C. Ordinate: % chlorpropamide undecomposed. Abscissa: time (h).

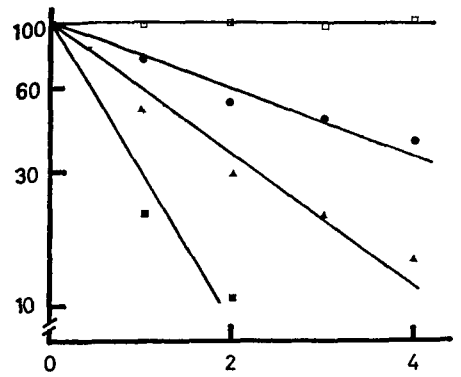

FIG. 3. Decomposition of melts containing 30% chlorpropamide and 70% urea. Temperatures of fusion: □70°, ●98°, ▲111° and ■132°C. Ordinate: % chlorpropamide undecomposed. Abscissa: time (h).

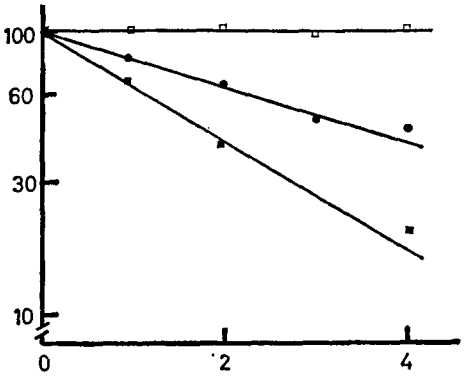

FIG. 4. Decomposition of melts containing 80% chlorpropamide and 20% urea. Temperatures of fusion: □70°, ●98° and ■132°C. Ordinate: % chlorpropamide undecomposed. Abscissa: time (h).

amide for melts containing 15 and 30% chlorpropamide, and 3·1% for melts containing 80% chlorpropamide at 132 °C. However, the fusion time previously reported (Ford & Rubinstein 1977a,b) included total heating times of the test tubes, and therefore the total decomposition would be much less. Any developed formulation of the chlorpropamide-urea solid dispersion would require an overage of chlorpropamide.

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