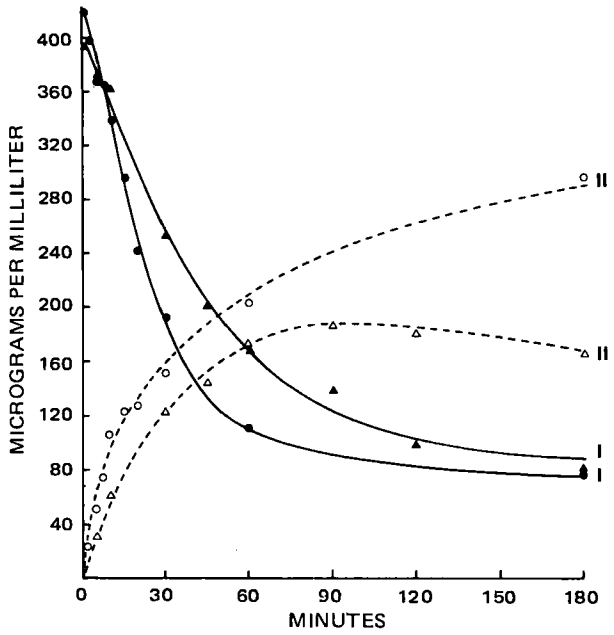

Figure 9—*Concentration* versus *time for two concentrations of prostaglandin 15-dehydrogenase enzyme. Key:* Δ, ▲, *25 μl;* ○, ●, *50 μl;* —, *dinoprostone (I); and* - - -, *15-keto derivative (II). Total incubation volume = 5.2 ml; samples assayed = 0.5 ml.*

CONCLUSIONS

An HPLC method was developed, allowing the simultaneous determination of substrate and metabolic conversion products from *in vitro* incubations with prostaglandin 15-dehydrogenase. The specific nature of the assay provides vital information for a complete characterization of enzyme quality and activity. Preliminary results pointed toward differences in *in vitro* enzyme kinetics for different substrates. Dinoprost, while showing the lowest initial reaction rate (*cf.*, Figs. 5–7), is completely consumed while prostaglandin E_1 and dinoprostone reach an asymptote with time.

The method may be useful as a: (*a*) screen for prostaglandin 15-dehydrogenase inhibitors, (*b*) reference method for questionable results from other screening assays, and (*c*) control method to ensure that the inhibitor screening assay is employing a satisfactorily specific and active enzyme.

If reasonably high activities can be obtained, then HPLC probably may be used to characterize other prostaglandin-metabolizing enzymes. With the availability of superfused organ techniques, it also may be possible to compare, by an accurate physical–chemical technique, the effect of inhibitors on the enzyme in this system and *in vitro* enzymes to distinguish differences.

REFERENCES

(1) H. Vonkeman, D. H. Nugteren, and D. A. van Dorp, *Biochim. Biophys. Acta*, **187**, 581(1969).

(2) S. A. Saeed and A. C. Roy, *Biochem. Biophys. Res. Commun.*, **47**, 96(1972).

(3) H. S. Hansen, *Prostaglandins*, **8**, 95(1974).

(4) C. J. Limas and J. N. Cohn, *Proc. Soc. Exp. Biol. Med.*, **142**, 1230(1973).

(5) H. H. Tai, C. L. Tai, and C. S. Hollander, *Biochem. Biophys. Res. Commun.*, **57**, 457(1974).

(6) J. Jarabak, *Proc. Nat. Acad. Sci. USA*, **69**, 533(1972).

(7) W. Schlegel, L. M. Demers, H. E. Hildebrandt-Stark, H. R. Behrman, and R. O. Greep, *Prostaglandins*, **5**, 417(1974).

(8) S. C. Lee and L. Levine, *J. Biol. Chem.*, **250**, 548(1975).

(9) L. J. Lerner, P. Carminali, F. Luzzani, and A. Soffientini, presented at the International Conference on Prostaglandins, Florence, Italy, 1975, Session 13.

(10) C. Pace-Asciak, *J. Biol. Chem.*, **250**, 2789(1975).

(11) W. Morozowich and S. Douglas, *Prostaglandins*, **10**, 19(1975).

(12) F. F. Sun, S. B. Armour, and V. R. Bockstanz, presented at the International Conference on Prostaglandins, Florence, Italy, 1975, Session 10.

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Preparation and Properties of Solid Dispersion System Containing Citric Acid and Primidone

M. P. SUMMERS and R. P. ENEVER ˣ

Abstract □ Solid dispersions containing 1–32% (w/w) primidone were prepared by fusing the drug with citric acid and rapidly cooling the melt. The solidified dispersions were clear glasses which devitrified on aging or when stored at 60° for up to 3 days. The phase diagram of the devitrified system indicated that the drug may exist as a solid solution at 1–3% (w/w) concentrations but that a eutectic mixture is formed at higher concentrations. The solubility of primidone increased in the presence of citric acid. Preliminary dissolution studies showed that the dissolution rate from the solid dispersion was greater than that of the pure drug or the physical mixture.

Keyphrases □ Solid dispersion systems—primidone with citric acid, effect of concentration, aging, and temperature □ Primidone—solid dispersion with citric acid, effect of concentration, aging, and temperature □ Citric acid—solid dispersion with primidone, effect of concentration, aging, and temperature □ Anticonvulsant agents—primidone, solid dispersion with citric acid, effect of concentration, aging, and temperature

Sekiguchi and Obi (1) first demonstrated the use of a eutectic mixture of a sparingly soluble drug and a water-soluble compound in increasing the rates of dissolution and absorption of the drug. This type of formulation, a "solid dispersion system," has undergone much investigation and has been extended to include

solid solutions, such as those believed to be formed at low drug concentrations with the high molecular weight polyethylene glycols (2–5), and the amorphous or glassy dispersions formed with povidone (3, 6–8). The theories and pharmaceutical applications of such solid dispersions were reviewed extensively (3).

Chiou and Riegelman (2) proposed the use of organic glass-forming compounds to form binary glass systems with water-insoluble drugs and demonstrated the increased dissolution rate of griseofulvin in a glass of citric acid. The glass system gave faster drug release than other solid dispersion systems studied.

In the present investigation, the applicability of the glass system for the formulation of primidone was investigated. This drug is relatively water insoluble (1 in 2000 at 20°) and melts (with decomposition) at the relatively high temperature of 295°. It appears to be a suitable candidate for such formulation since binary systems can be prepared containing low drug concentrations at temperatures below which the drug decomposes. Furthermore, primidone exists in two polymorphic forms (9), and one (Form II) can be prepared from a melt.

It was, therefore, of interest to determine whether this method of formulation could cause a change in the polymorphic form of a drug. Chiou and Niazi (10) showed that different polymorphic forms of a drug can give different phase diagrams and that crystalline changes may occur in such solid dispersion systems on aging.

EXPERIMENTAL

Preparation of Solid Dispersions—Primidone[1] was intimately mixed in a pestle and mortar with citric acid monohydrate[2]. Mixtures containing 1–32% (w/w) primidone were prepared. The mixtures were transferred to Pyrex tubes and gently heated over a flame until complete melting occurred. Heating was continued to remove any water of crystallization condensed on the tube walls.

Gentle heating had to be used to prevent decomposition of citric acid. Mixtures containing more than 32% (w/w) primidone could not be prepared because primidone decomposition occurred on melting. Subsequently, the melt was poured into a glass petri dish and immediately placed in a desiccator to prevent moisture absorption.

Chiou and Riegelman (3) found that the griseofulvin–anhydrous citric acid glass became brittle and transparent after storage at 37° for a few days. In this system, however, devitrification of the glasses occurred. Since high temperatures aided devitrification, the glasses were stored at 60° until complete devitrification occurred, usually in less than 3 days.

Construction of Phase Diagram—The phase diagram of the devitrified system was constructed by determining the onset and completion of melting of a 125–180-μm size fraction of each dispersion. Hot-stage microscopy[3] and differential scanning calorimetry[4] were used to determine the melting points. A heating rate of 2°/min was used.

Hot-stage microscopy was performed using scattered particles on a glass slide, with a field of view containing at least eight particles.

At least two determinations were performed on each dispersion, and at least two batches were prepared and tested at each primidone concentration.

X-Ray Diffraction—X-ray diffraction[5] was used to assess the nature of the solid dispersion system before and after devitrification and to characterize the two polymorphic forms of primidone. Powdered samples of a mixed size range were mounted on adhesive tape, and the X-ray diffraction pattern was determined using CuKα radiation.

Powdered samples of devitrified glasses were compared with physical mixtures of the same concentration. Physical mixtures were prepared using citric acid monohydrate and anhydrous citric acid to establish the nature of the citric acid in the devitrified system. Furthermore, physical mixtures were prepared using both polymorphic forms of primidone to determine the polymorphic form in the devitrified system.

Solubility Determinations—Solution concentration–time profiles ("dynamic solubility" determinations) of the two polymorphic forms of primidone (prepared using the method in Ref. 9) were determined by shaking suspensions of the polymorphs in distilled water (pH 5.5) in a water bath at 37°. At 5-min intervals, 5-ml samples were removed with a pipet fitted with a grade 3 sintered-glass filter stick. The solutions were assayed at 257 nm using a UV spectrophotometer[6]. Saturation concentration was usually achieved within 1 hr, but samples were withdrawn for periods up to 12 hr to check this fact.

Equilibrium solubility values of the two polymorphs and the commercial sample of primidone were determined by equilibrating aqueous suspensions at 37° in the same apparatus for 12 hr. The commercial sample was also equilibrated with citric acid solutions of various concentrations for the same period. At the end of this time, 5-ml samples were removed and assayed in the manner already described.

Aqueous suspensions of the two polymorphic forms were also equilibrated at 37° for 21 days. Subsequently, the crystals were removed and analyzed by IR spectroscopy[7].

Dissolution Studies of Powdered Materials—The experiments were conducted in a 1-liter, flat-bottom, glass reaction vessel held in a water bath maintained at 37°. The dissolution medium was stirred by a T-shaped glass stirrer inserted through a gland in the lid of the vessel and connected to a constant-speed motor *via* a belt drive. The stirrer was positioned 1.2 cm from the base of the vessel, and a stirring speed of 110 rpm was used.

Stainless steel stirrers could not be used because a reaction took place between steel and citric acid. Pitting and discoloration were observed, and the soluble products of the reaction interfered with the UV assay.

All dissolution studies were performed using a 150–250-μm size fraction of powdered material. When physical mixtures of primidone and citric acid were tested, a 150–250-μm size fraction of both powders was used.

The powder was placed on the vessel base at the beginning of the dissolution experiment. The vessel top and stirrer were replaced and, after the stirrer motor was started, 400 ml of distilled water (pH 5.5), previously equilibrated at 37°, was added. Then 5-ml samples were withdrawn at frequent intervals using a pipet fitted with a grade 3 sintered-glass filter stick. Sampling was continued for 20 min. The volume withdrawn was not replaced with fresh dissolution medium. Samples were assayed at 257 nm[6].

The studies were performed with the following systems: (*a*) 200-mg quantities of both powdered devitrified glasses and physical mixtures containing either 16 or 32% (w/w) primidone (equivalent to 32 or 64 mg of primidone, respectively), and (*b*) amounts of powdered devitrified glasses equivalent to a primidone content of 32 mg [200 mg of 16% (w/w) or 100 mg of 32% (w/w) concentrations].

Rotating-Disk Dissolution Studies—Disks of each polymorphic form of primidone were prepared by compressing powdered samples at 5000 kg in a 1.2-cm diameter die using a hydraulic press[8]. Preliminary experiments established that a minimum force of 4500 kg was necessary to obtain constant dissolution rate data.

The experiments were carried out using the apparatus described under *Dissolution Studies of Powdered Materials*, except that the glass stirrer was replaced by a stainless steel shaft bearing a disk support. The disks were attached to the support with microcrystalline wax[9], and their edges were sealed with the wax to ensure that only one surface was exposed to the dissolution medium.

The dissolution medium was distilled water (pH 5.5), which had previously been boiled to remove air and subsequently equilibrated at 37° before use. At 30-min intervals, 5-ml samples were withdrawn

[1] Batch No. ADM 16619/73, I.C.I. Pharmaceuticals Ltd., Macclesfield, England.
[2] Analar Grade, B.D.H. Chemicals Ltd., Poole, England.
[3] Mettler FP2, Mettler Instrument Corp, Switzerland.
[4] D.S.C. 1B, Perkin-Elmer, Beaconsfield, United Kingdom.
[5] Nonius Mk. 2 self-focusing Guinier diffractometer.
[6] SP 1800, Pye-Unicam, Cambridge, United Kingdom.
[7] 175 G spectrophotometer, Perkin-Elmer, Norwalk, Conn.
[8] Apex hydraulic press, Apex Construction Ltd., United Kingdom.
[9] B.D.H. Chemicals Ltd., Poole, England.

and assayed as described previously. Sections from the disk were subjected, before and after dissolution, to IR spectroscopy to determine the effect of compression and dissolution on the polymorphic form of the drug.

RESULTS AND DISCUSSION

Preparation and Devitrification of Glass System—Chiou and Riegelman (3) suggested the use of citric acid monohydrate for preparing solid dispersions of this type, because the lower melting point of the monohydrate would be advantageous in preventing decomposition of citric acid and the drug. Hot-stage microscopy showed that the monohydrate lost water of crystallization at approximately 70° before finally melting at the melting point of the anhydrous material (153°).

It was confirmed, however, that the monohydrate was the better form to use, became decomposition of primidone occurred at lower concentrations of primidone in anhydrous citric acid. It is possible that the released water of crystallization in the restricted environment of the test tube caused the formation of a citric acid solution which then acted to dissolve the primidone.

The glasses were unstable and devitrified after preparation. This devitrification was concentration dependent; glasses containing low concentrations of primidone devitrified faster than those of higher concentration. A 1% glass started to devitrify within 15 min, whereas a glass containing 30% primidone remained clear for longer than 6 months.

Although no quantitative data are available, the glasses containing low concentrations of primidone were less viscous than those of greater concentrations. The glass containing 30% (w/w) primidone was hard, brittle, and very hygroscopic. Glasses of concentrations lower than about 28% (w/w) were softer and did not harden until devitrified. When vitreous, these glasses were also hygroscopic.

The time taken for the onset of devitrification reflects this increase in viscosity with an increased concentration of primidone. It is thought that the decreased viscosity of the lower concentrations permits the ordering of the molecules necessary for crystallization (11).

Moreover, the increasing viscosity of the glass with an increasing primidone concentration is probably due to the extent of hydrogen bond formation between the citric acid and primidone molecules and the strength of these bonds. If the bonds formed between citric acid and primidone are stronger than those between citric acid molecules, the cooling melt is composed of a network of noncrystallizing molecules. These molecules are responsible for the increased viscosity of the melt and the glass that subsequently forms.

Phase Diagram of Devitrified System—A portion of the phase diagram of the devitrified solid dispersion system is shown in Fig. 1. The upper line shows the liquidus line obtained from hot-stage microscopy. The lower portion is the phase diagram found by differential scanning calorimetry. The two methods gave similar results, but the diagrams were displaced by approximately 2°. The calibration of both instruments had been checked before use, and the discrepancy is probably due to the errors inherent in the hot-stage technique when observing a binary system without a sharp melting point. The liquidus line obtained from hot-stage microscopy ceases at the boiling point of the melt.

The primidone concentration is expressed as a percent (w/w) in anhydrous citric acid, because X-ray diffraction indicated that the citric acid in the devitrified system was anhydrous.

The phase diagram of the system indicates that a solid solution of primidone in citric acid exists at very low concentrations of primidone (up to 3%) and that a eutectic mixture is formed at higher primidone concentrations. Because the phase diagram in Fig. 1 is not complete, it is not possible to define the nature of the eutectic mixture. It may consist of a mixture of the solid solution of primidone in citric acid with primidone. Alternatively, if a solid solution of citric acid in primidone is formed at high primidone concentrations, the eutectic consists of a mixture of the solid solutions of primidone in citric acid and citric acid in primidone. The eutectic contains 20% (w/w) primidone, and the melting point of this eutectic is 141° by differential scanning calorimetry.

X-ray diffraction patterns (Fig. 2) show that a physical mixture containing 15% (w/w) primidone in anhydrous citric acid resembles the diffraction pattern of the devitrified glass containing 15% (w/w) primidone. This result is typical of devitrified glasses containing 5–30% (w/w) primidone. There was no change in citric acid lattice

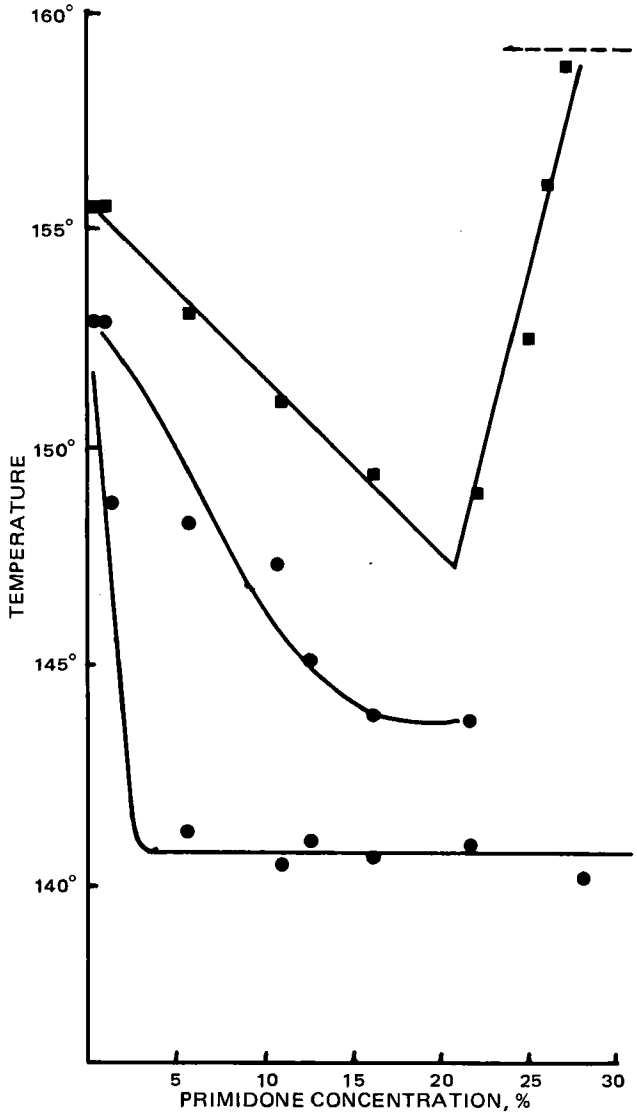

Figure 1—*Phase diagram of the primidone–citric acid system. Key: ■, hot-stage microscope; ●, differential scanning calorimetry; and - - -, boiling point of citric acid–primidone melt.*

parameters with increasing concentration of primidone. Such changes would indicate solid solution formation. The presence of typical primidone lines at all concentrations in which it could be detected suggests that a simple eutectic mixture is formed and that the solid solubility of primidone in citric acid is very small.

The apparent discrepancy in the X-ray diffraction data and the differential scanning calorimetry–hot-stage phase diagram may be attributed to the fact that the latter data relate to the solubility of primidone in citric acid at the temperature of the solid–liquid equilibrium. If this solubility is greater than the room temperature solubility of primidone in citric acid, precipitation of primidone from the solid solution under ambient conditions would account for the simple eutectic mixture observed with X-ray diffraction studies.

The results of dispersions containing up to 3% (w/w) primidone were inconclusive; at these low concentrations, X-ray diffraction failed to detect primidone in the physical mixtures.

The results also show that primidone existed in the devitrified system as the Form II polymorph, whereas the starting material (*i.e.*, the commercial sample) was the Form I polymorph.

The diffraction pattern of the 30% (w/w) vitreous glass exhibits only very broad lines typical of the backing tape, suggesting that primidone is molecularly dispersed in the glassy state throughout the citric acid glass or exists in the amorphous form. This behavior is likely to be observed with all glasses prepared.

Solubility Studies—The polymorphic forms of primidone have

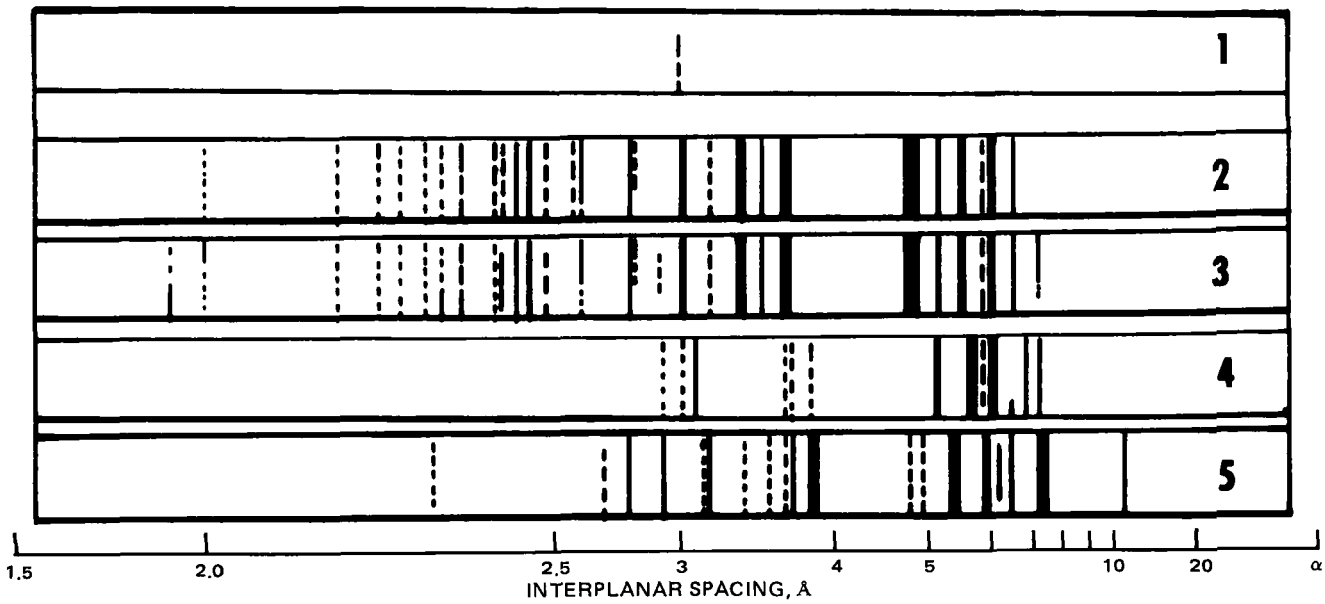

Figure 2—*X-ray powder diffraction pattern of (1) 30% vitreous glass, (2) 15% devitrified glass, (3) 15% physical mixture of anhydrous citric acid and primidone Form II polymorph, (4) primidone Form II polymorph, and (5) primidone Form I polymorph. Dotted lines indicate bands of weaker intensity.*

solubilities of 56.4 mg/100 ml (Form I) and 56.5 mg/100 ml (Form II) at 37°. There was no evidence of a phase change during the dynamic solubility determinations, and IR spectroscopy confirmed that there was no change in aqueous suspensions equilibrated for 21 days.

Figure 3 shows that citric acid increased the solubility of primidone. A solution containing 500 mg/ml of citric acid increased the solubility of primidone from 56.4 to 172.2 mg/100 ml. The effect of citric acid on the solubility of primidone would be expected to influence the dissolution rate of the drug from the solid dispersion system.

Dissolution Studies—Figure 4 shows the results of the rotating-disk dissolution study of the two primidone polymorphs. There was no difference in the dissolution rates of the polymorphs at 37°. Neither compression of powder into disks nor the process of dissolution caused any change in polymorphic form. Thus, the results of both the dissolution and solubility studies suggest that the dissolution rate of primidone will not be altered by precipitation of the Form II polymorph in the devitrified system.

Dissolution studies of the devitrified glasses were initially carried out using the rotating-disk method, but this attempt failed. During dissolution from the disk of the solid dispersion, primidone precipitated on the disk surface and subsequently flaked off into the dissolution medium. Within 5 min, complete breakdown of the disk occurred because of this phenomenon.

Dissolution studies were finally performed using the powder method described. Vitreous glasses could not be tested, because they became very cohesive during comminution due to moisture absorption. A suitable size fraction could not be prepared.

Large particles of the glass were placed in the dissolution medium, however, and it was found that primidone was precipitated on the surface of the glass during dissolution. When devitrified glasses dissolve, a similar process may occur if a solution of primidone in citric acid is formed in the diffusion layer around the particle. Therefore, dissolving devitrified glasses may be covered with a layer of primidone crystals precipitated during devitrification and dissolution.

The results of the powder dissolution experiments are shown in Fig. 5. All results were corrected to a 400-ml volume of dissolution medium

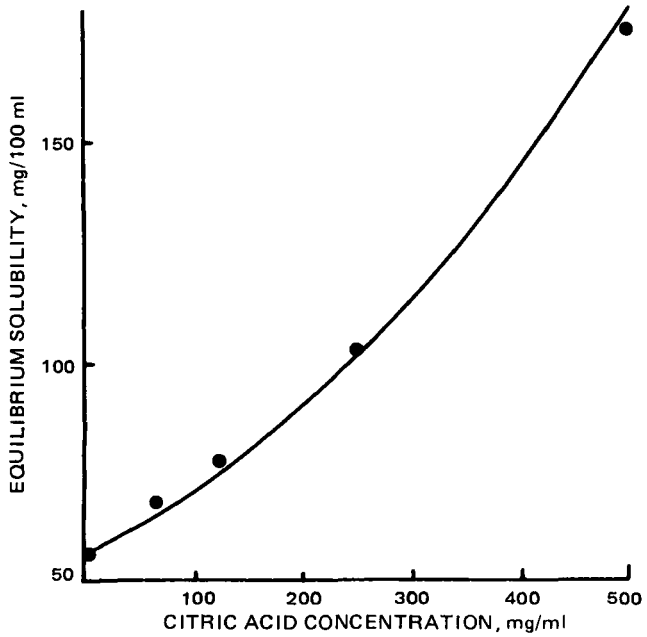

Figure 3—*Effect of citric acid on primidone solubility.*

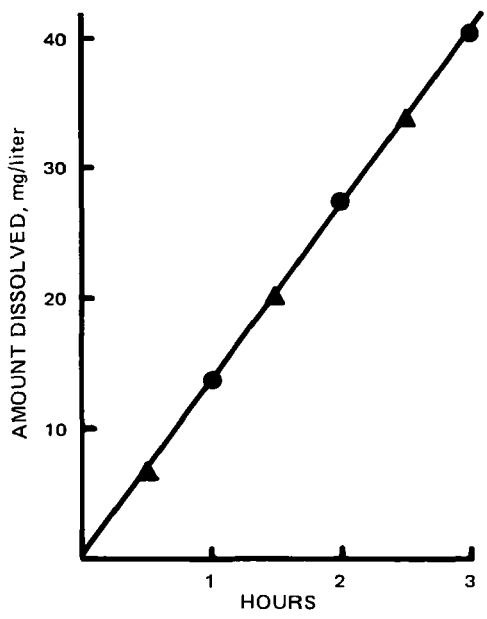

Figure 4—*Dissolution rate of two primidone polymorphs. Key: ▲, Form II; and ●, Form I.*

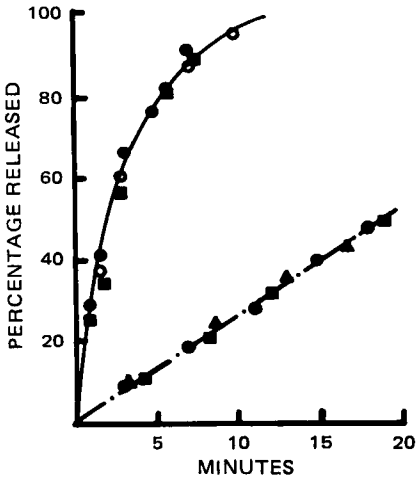

Figure 5—*Dissolution of primidone expressed as a percentage of the total drug content. Key: —, devitrified glass; —·—, physical mixtures; ●, 16% primidone (200 mg); ○, 32% primidone (100 mg); ■, 32% primidone (200 mg); and ▲, primidone Form II powder.*

at each sample time. The dissolution rates of the devitrified glasses were faster than those of the physical mixtures. This could be due to the solubility increase associated with the presence of citric acid or the small particle size of the primidone precipitated during devitrification and dissolution. The dissolution rates of the physical mixtures would not be expected to show any significant increase due to the presence of citric acid, because the acid concentration in the bulk of the dissolution medium is relatively low. This assumption is supported by the data in Fig. 5, which show that primidone Form II powder dissolves at the same rate as the physical mixtures.

When the glasses and devitrified glasses dissolve, however, citric acid forms a saturated solution in the diffusion layer around the particle, and this would be expected to aid dissolution because of the solubility increase. When this concentrated acidic solution is diluted during diffusion away from the particle, the primidone is present in a supersaturated solution, accounting for its precipitation from the glass during dissolution.

Because citric acid is very soluble (592 g in 1 liter at 20°), rapid depletion of the citric acid probably occurs and, in the later stage of dissolution, the rate depends upon the size of the precipitated primidone crystals. These crystals are smaller than the particles in the physical mixtures and, therefore, dissolution from the devitrified glasses would be expected to be greater than that from the physical mixtures even in the later stages.

The results in Fig. 5 are plotted as a percentage of the drug content and show that the dissolution rates of the two devitrified glasses are the same irrespective of sample size. This finding tends to indicate that the dissolution rates are dependent upon the concentration of drug in the devitrified glasses and that the particle-size distribution of primidone is similar in both during drug dissolution. One explanation of this phenomenon would be that rapid depletion of the citric acid causes the formation of a suspension of primidone particles that were precipitated during devitrification. These particles would control the rate of dissolution and would account for the dependence of the dissolution rate on primidone concentration.

CONCLUSIONS

Solid dispersions of primidone can be prepared by fusing the drug with citric acid to form a glass. The glass is unstable and devitrifies, and X-ray diffraction indicates that this devitrified state is a eutectic mixture of primidone and citric acid.

The devitrified glass has a faster dissolution rate than the pure drug and a physical mixture of the drug with citric acid. This increased dissolution rate is probably due to three factors:

1. Citric acid has been shown to increase the solubility of primidone and this may affect the solubility of primidone in the diffusion layer around the dissolving devitrified glass.
2. Primidone is precipitated as fine crystals during devitrification, and this will increase the effective surface area of the drug in the devitrified state.
3. The glass and the devitrified glass have superior wetting characteristics compared with the pure drug, and this will also affect the surface area available for dissolution. This aspect was discussed by Chiou and Riegelman (2).

REFERENCES

(1) K. Sekiguchi and N. Obi, *Chem. Pharm. Bull.*, **9**, 866 (1961).

(2) W. L. Chiou and S. Riegelman, *J. Pharm. Sci.*, **58**, 1505(1969).

(3) *Ibid.*, **60**, 1281(1971).

(4) W. L. Chiou and L. D. Smith, *J. Pharm. Sci.*, **60**, 125(1971).

(5) W. L. Chiou and S. Riegelman, *ibid.*, **60**, 1569(1971).

(6) M. Mayersohn and M. Gibaldi, *ibid.*, **55**, 1323(1966).

(7) A. P. Simonelli, S. C. Mehta, and W. I. Higuchi, *ibid.*, **58**, 538(1969).

(8) T. R. Bates, *J. Pharm. Pharmacol.*, **21**, 710(1969).

(9) R. D. Daley, in "Analytical Profiles of Drug Substances," vol. 2, K. Florey, Ed., Academic, New York, N.Y., 1973, p. 417.

(10) W. L. Chiou and S. Niazi, *J. Pharm. Sci.*, **60**, 1333(1971).

(11) G. O. Jones, "Glass," Wiley, New York, N.Y., 1958.

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