The Concentration of Spermine, Spermidine, and Putrescine in Rat Liver

The concentration of polyamines in the liver of two rats was determined by our method. As shown in Table II, the duplicability of the method was quite good. The mean values of spermine, spermidine, and putrescine per gram of liver were 0.69, 0.52, and 0.06 in the first rat, and 0.68, 0.56, and 0.04 in the second rat, respectively. These data are similar to those reported by other workers[6] using electrophoretic analysis. The present method has proved to be rapid and reliable for the estimation of polyamine content in *E. coli* also.

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Solvent Effects on Comparative Dissolution of Pharmaceutical Solvates[1]

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Two potential applications of organic solvates in pharmaceutical formulation have been suggested, *viz.*: (i) as intermediates to achieve particle size reduction and (ii) as pharmaceutical derivatives to promote dissolution of poorly soluble drugs. While there are evidences which testify to the success of the first application to drugs such as griseofulvin[3] and chloramphenicol,[4] conflicting data exist in the literature about the general ability of solvates to show enhanced dissolution rates over their non-solvated compounds. Thus, while some solvates[5,6] showed higher *in vitro* dissolution rates when compared to their non-solvated forms, others[7,8] demonstrated the reverse behavior. This confusing picture is further complicated by the lack of standardization of dissolution techniques used to study solvate dissolution. In particular, in the dissolution studies of water insoluble drugs, it is often expedient to employ mixed aqueous organic solvent systems to facilitate data collection.[5] This practice does not necessarily lead to ready extrapolation of comparative solvate dissolution data in these media to those in aqueous solutions. This is primarily due to the probable difference in the rate or extent of dissociation of the solvate in these solvent systems, which in turn provides a dif-

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ferent thermodynamic driving force for dissolution. In this brief report, we show that it is quite possible to draw erroneous conclusions on the usefulness of particular solvates to give higher dissolution rates based on their comparative dissolution behavior in mixed solvents. As examples, we used the chloroform solvate of griseofulvin and the *t*-butylamine (TBA) disolvate of fluprednisolone, both of which have been shown to exhibit slower dissolution rates than their anhydrous parent compounds in aqueous solutions.

Griseofulvin

Mayersohn and Gibaldi[7] showed that the particulate dissolution rate of griseofulvin chloroform solvate in water was slower than that of microcrystalline griseofulvin. Under our experimental conditions of constant surface area, we also found no significant enhancement in dissolution rate of the solvate over that of the anhydrous non-microcrystalline form, either in the presence or absence of a small percentage (0.005% w/v) of Tween 80. However, when a water miscible organic solvent was added to the dissolution medium, the solvate became the faster dissolving species. For example, when the alcohol content was 50% v/v, the solvate dissolved about three times faster than the non-solvated form (Fig. 1). Table I tabulates the effects of solvent on the initial dissolution rates of griseofulvin and its chloroform solvate. The rates were obtained for dissolution runs in the first twenty minutes during which time the dissolution curves were linear for systems containing twenty or more percent v/v organic solvent and approximately linear for those systems of a lower organic solvent concentration. It can be readily seen that as the concentration of the non-aqueous solvent increased in the dissolution medium, the relative enhancement in initial dissolution rate of the solvate over the anhydrous form also increased. Preliminary data showed that the solvate was also the faster dissolving species in pure polar organic solvents, such as methanol and acetone, but not in cyclohexane (Table I).

Fluprednisolone

It has been shown previously[8] that the TBA disolvate did not exhibit any enhancement in dissolution in water over the anhydrous form. In this study, the dissolution rates of the disolvate and the non-solvated form (Form I) were found to be essentially the same also in 0.1 N hydrochloric acid. The lack of enhancement did not appear to be due to a lack of wetting of the solvate, nor was it diffusion layer limited; since it was found that neither the addition of Tween 80 nor an increase in the stirring rate from 60 to 250 rpm caused significant en-

Table I. Initial Dissolution Rates and Rate Ratios for Griseofulvin and Its Chloroform Solvate in Various Dissolution Media

Dissolution media	Rate (anhydrous) ($M \cdot min^{-1}$)	Rate (solvate) ($M \cdot min^{-1}$)	Rate (solvate) / Rate (anhydrous)
Water[a]	(1.38×10^{-2})[b]	(1.38×10^{-2})[b]	1.00
Tetrahydrofuran[c]			
5%	1.15×10^{-7}	1.50×10^{-7}	1.30
10%	2.23×10^{-7}	3.02×10^{-7}	1.35
20%	1.98×10^{-6}	2.96×10^{-6}	1.50
40%	1.04×10^{-5}	2.40×10^{-5}	2.31
Ethanol[a]			
25%	2.78×10^{-7}	4.70×10^{-7}	1.69
50%	0.35×10^{-6}	1.00×10^{-6}	2.84
100%	0.87×10^{-5}	1.01×10^{-5}	1.16
Methanol[d]	7.05×10^{-6}	8.67×10^{-6}	1.23
Acetone[d]	0.74×10^{-5}	1.48×10^{-5}	2.00
Cyclohexane[a]	(1.53×10^{-2})[b]	(1.06×10^{-2})[b]	0.70

a) stirring rate: 750 rpm *b*) absorbance: units/hr *c*) 2000 rpm *d*) 60 rpm

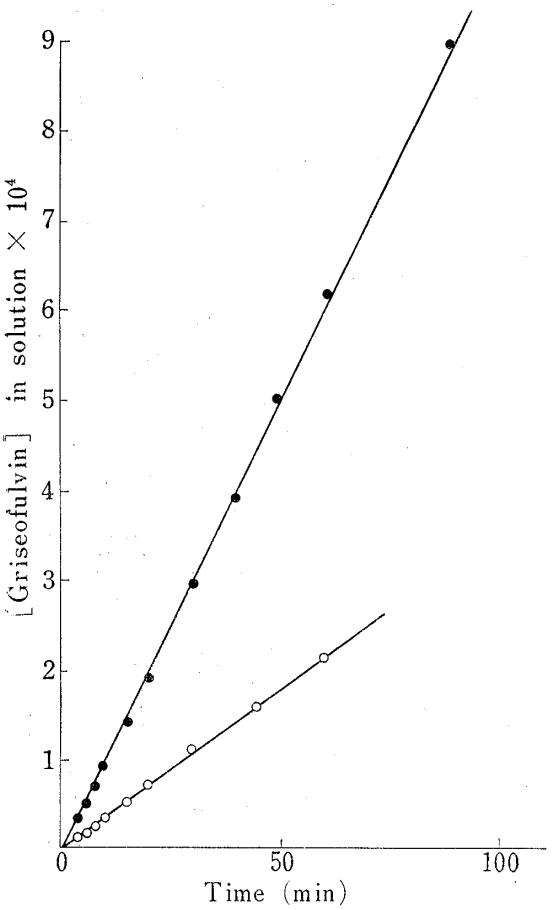

Fig. 1. Initial Dissolution Curves of Griseofulvin (○) and Its Chloroform Solvate (●) in 50% v/v Ethanol

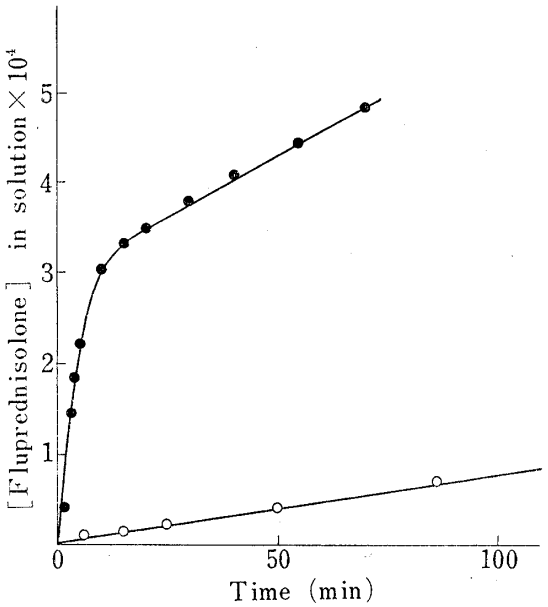

Fig. 2. Initial Dissolution Curves of Fluprednisolone Form I (○) and Its TBA Disolvate (●) in 25% v/v Ethanol

hancement in the relative dissolution rate of the solvate. A change of the dissolution medium to a 0.1N NaOH solution also had little effect on the dissolution of the solvate.

Preliminary data on aqueous alcoholic solvents showed that addition of a small amount of alcohol (10%) caused only a slight increase in dissolution, but when the alcohol content was increased to 25% v/v (Fig. 2), a tremendous difference resulted (Table II). The dissolution rate of the solvate was now very much enhanced. At 5 minutes, the concentration of the drug in solution was more than 50 times higher for the dissolution of the solvate than for the anhydrous form.

Table II. Initial Dissolution Rates and Rate Ratios for Fluprednisolone and Its TBA Disolvate in Various Dissolution Media

Dissolution media[a]	Rate (anhydrous) ($M \cdot min^{-1}$)	Rate (solvate) ($M \cdot min^{-1}$)	Rate (solvate) / Rate (anhydrous)
Water	—	(0.9×10^{-3})[b]	—
0.1N HCl	(1.2×10^{-2})[b]	(1.1×10^{-2})[b]	0.9
0.1N NaOH	—	(1.0×10^{-2})[b]	—
Ethanol 10%	5.6×10^{-7}	6.3×10^{-7}	1.1
25%	0.8×10^{-6}	$\sim 4.5 \times 10^{-5}$[c]	55

a) stirring rate: 60 rpm b) absorbance units/min c) initial rate (0—5 min)

These results demonstrated the profound importance of solvent effects in solvate dissolution. We submit, therefore, that the choice of solvent systems in the study of *in vitro* dis-

solution of solvates may be extremely critical, since studies in aqueous organic solvent systems may not accurately reflect the dissolution behavior of these solvates in purely aqueous system, much less the *in vivo* systems in which the dissociation of the solvate is unpredictable.

Further, the data presented here also provided some interesting information with regard to the mechanism of solvate dissolution. In the case of the griseofulvin solvate, the results appeared to be consistent with the dissociation model[5] for solvate dissolution: *i.e.*, the lack of enhancement in dissolution of the solvate in water was apparently due to a lack of favorable molecular interaction between the complexing solvent (chloroform) and water, so that the dissociation of the solvate and its accompanying "dissolution driving force" could not be readily attained. When an interacting solvent such as ethanol, acetone and tetrahydrofuran was provided to solvate the chloroform, dissociation of the solvate became favorable, and enhancement in dissolution rate resulted. Further indication that these observed enhancements in mixed aqueous organic solvents were related to a specific solvation mechanism and not to a decrease in the dielectric constant of the medium was provided by the dissolution data in cyclohexane. In this solvent, no enhancement was observed apparently because cyclohexane could not solvate chloroform favorably and dissociation was, therefore, not promoted. The above conclusions, of course, could be substantiated by measuring the dissociation constant of the solvate and also the rate at which dissociation equilibrium is obtained in each solvent under the same experimental conditions.

The mechanism of dissolution for the TBA disolvate of fluprednisolone is less clear. In the griseofulvin case the complexing solvent (chloroform) is very hydrophobic and may not be expected to dissociate from the drug in water because its interaction with water is not favorable. However, since TBA is very hydrophilic (it is freely miscible with water), its free energy of dilution should be readily available. Thus, based on the dissociation model, it is difficult to rationalize why enhancement of dissolution through this solvate in water is not achieved. One possible answer to this question may be found from the DSC data for the solvate. The enthalpy of desolvation was found to be 34 ± 2 kcal/mole of solvate for the fluprednisolone TBA disolvate and 8.2 ± 0.3 kcal/mole of solvate for the griseofulvin chloroform solvate respectively. It can be seen that approximately four times as much energy was required to completely desolvate the steroid solvate as compared to the griseofulvin solvate. Even after stoichiometric correction, liberation of a TBA molecule from the fluprednisolone disolvate would apparently require at least twice the amount of energy necessary for the release of chloroform from the griseofulvin solvate. It may then be speculated that the *t*-butylamine molecules in the solvate are so strongly bound to the steroid that a large amount of energy may be required to cause dissociation. Apparently this amount of energy cannot be supplied in water because solvation of the steroid and the base by water molecules does not compensate for the energy lost in breaking the solvate bonds. In mixed alcoholic solvents, possible solvation of the steroid may now lead to favorable dissociation of solvate, which is then accompanied by an observed enhancement in dissolution rate.

Interpretation of dissolution data of the TBA disolvate of fluprednisolone is further complicated by possible transformation during dissolution. Haleblian, *et al.*[8] reported that in water, this solvate rapidly transforms to its β-monohydrate and subsequently to its α-monohydrate. It was also stated that the Form I polymorph was converted to the α-monohydrate in water. However, on examination of their dissolution data, it was noted that, at least up to one hour, the dissolution rates of all these forms were significantly different. This tends to suggest that phase transition during initial dissolution is probably not extensive. Thus, the initial dissolution curves shown in Fig. 2 most likely represent predominantly those of the starting material. The abrupt change in slope after 10 minutes in the TBA dissolution curve might be due to gradual buildup of phase-transformed material or might represent a slowing of rate due to higher concentration of the steroid in the dissolution medium.

Experimental

Materials——Griseofulvin[9] and fluprednisolone[10] were obtained from their respective manufacturers and used as the anhydrous compounds without further recrystallization. The latter compound had the same X-ray diffraction pattern as that described for Form I of fluprednisolone.[11]

The chloroform solvate of griseofulvin was prepared by recrystallizing the anhydrous drug in chloroform.[3] Both weight loss and Beer's Law plot confirmed a 1 : 1 drug–solvent stoichiometry. Fluprednisolone TBA disolvate was obtained by recrystallizing the drug in *t*-butylamine. From a Beer's Law plot the solvate was found to contain two moles of TBA per mole of drug. X-ray powder diffraction patterns of both solvates were distinctly different from those of the anhydrous compounds. In the case of the TBA disolvate, the diffraction data agreed with the published data.[8] Since both solvates were found to desolvate on prolonged standing, dissolution runs were performed with freshly prepared solvates. Dissolution disks were prepared by compressing 500 mg of pure drug material, using a pressure of 1000 psi for 10 seconds. Tetrahydrofuran A.R. was distilled to remove the stabilizer before use.

Dissolution Studies——The rotating disk method of Levy and Sahli[12] was essentially adopted. Temperature was at $25 \pm 0.2°$. A stirring rate of 60 rpm was used for the fluprednisolone systems. In the case of griseofulvin, where dissolution was quite slow, higher stirring rates were employed for both the tetrahydrofuran systems (2000 rpm) and the alcohol systems (750 rpm). Similar quantitative data, however, were also obtained at low stirring rates. Concentrations were determined from ultraviolet (UV) absorbance readings at the appropriate wavelengths. Where they were determined, both solvates had the same molar absorptivities as the non-solvated forms. In water and cyclohexane systems, where preparation of Beer's Law plots was difficult, due to low solubility of the drug, dissolution rates were expressed in terms of absorbance units per unit time.

DSC Studies——Thermograms were obtained from uncrimped samples at a heating rate of 10° per minute with a Perkin-Elmer DSC-1B calorimeter, calibrated with indium (enthalpy of fusion = 6.8 cal/g). Enthalpies of desolvation were determined from areas of desolvation endotherms, as measured by a planimeter.

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