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Pharmaceutical Studies on the Polymorphism of Hydrochlorothiazide

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Abstract □ Four polymorphic forms (I, II, III and IV) of hydrochlorothiazide have been characterized on the basis of x-ray diffractometry and differential thermal analysis. Form I was obtained by crystallization from N,N-dimethylformamide and Form II was crystallized from hot methanol. Form III was precipitated from sodium hydroxide aqueous solution by treatment with hydrochloric acid and Form IV was crystallized from 50% methanol. The metastable form I was a most stable form among four polymorphs, which was stable more than ten months at room temperature. The thermodynamic parameters such as heat of solution, enthalpy, entropy, free energy difference and transition temperature were determined by the measurement of intrinsic dissolution rate. The transition temperature and the heat of transition between the metastable Form I and Form II were determined to be 299.15°K and 5.03 Kcal/mole, respectively and free energy difference(ΔF) was 302.13 cal/mole. Diuretic action of these four polymorphic forms was also evaluated by monitoring the difference in urinary excretion of sodium, potassium and magnesium in rats.

Keywords □ Hydrochlorothiazide, Polymorphs, Differential thermal analysis, x-Ray powder diffractometry, Transition temperature, Heat of transition.

A polymorph is a solid crystalline phase of a compound resulting from the possibility of at least two different arrangement of the molecules of that compound. The crystals of two polymorphs of a drug may show different melting points, x-ray diffraction patterns,solubilities and stabilities, even though the liquid forms of these polymorphs are chemically identical.[1] In the case of slightly soluble drugs, this may affect the rate of dissolution. As a result, one polymorph may be more active therapeutically than another polymorph of the same drug.[3] As a corollary to the importance of polymorphic forms on drug availability, it is necessary to consider hydrates and solvates of relatively insoluble drugs.[4-7] The anhydrous forms of theophylline, cholesterol, caffeine, glutathimide and succinylsulfathiazole dissolve more rapidly in water than the hydrated forms.[8] Poole and Bahal reported that the greater thermodynamic activity of the anhydrous form of ampicillin correlated with the enhanced biological activity over of the trihydrate.[9]

Many organic compounds have been reported to exist in more than a single crystalline form and differences in crystalline form have been described in terms of the thermodynamic properties such as heat of solution, and the entropy and enthalpy change in going from one form to another. Of biopharmaceutical interest is the difference in solubility commonly exhibited by crytstal modifications.[10-12] Enhanced therapeutic activity may be achieved with the use of the higher energetic form.[13-14] When the difference in free energy in each crystalline forms is large, it may affect the absorption profiles. The different crystalline forms exhibited by one substance may result from a variation in the crystallization temperature or a change of solvent or rate of cooling.[1,8]

In this study, hydrochlorothiazide, (6 chloro-3, 4-dihydro-7 sulfamoyl-2H-1, 2, 4-benzothiadiazine 1, 1-dioxide), was used as a model compound of slightly water soluble drugs. This compound has been used extensively as a diuretic and antihypertensive agent for about 20 years. In spite of their wide usage, little information exists on the bioavailability and stability of hydrochlorothiazide formulations. Attempts, therfore, were made in this study to prepare the drug in a crystalline forms using four different solvent systems, and to determine the characters of four distinct crystalline forms of hydrochlorothiazide, dissolution rate of each crystalline forms and their relative diuretic action following oral administration in rats.

EXPERIMENTAL METHODS

Materials

Hydrochlorothiazide(U.S.P)was selected as a model compound for this study. The other solvents used were of analytical reagent grade.

Crystallization Procedures

Form I-Hydrochlorothiazide was dissolved in N,N-dimethylformamide. The solvent was evaporated and dried in vacuo.

Form II-Hydrochlorothiazide was dissolved in hot methanol. The solvent was evaporated and dried in vacuo.

Form III-Hydrochlorothiazide was dissolved in 0.1 N sodium hydroxide, and then 0.1 N hydrochloric acid was added dropwise. The resulting solution permitted to crystallize at room temperature. The crystals were filtered, washed and dried in vacuo.

Form IV-Hydrochlorothiazide was dissolved in 50% methanol. The solvent was evaporated and dried in vacuo.

Characterization of the Crystalline Forms

Differential thermal analysis(D.T.A), x-ray powder diffractometry, and solubility and dissolution rate measurement were applied to evaluate the crystal forms of hydrochlorothiazide.

Differential thermal analysis-The tracor R.L. stone differential analyzer equipped with a stand cell was employed to detect the transition temperature and melting point of each crystalline forms of hydrochlorothiazide. In all determinations, the heating rate was 10° per min. αAl_2O_3 was used to calibrate the calorimeter cell. Approximately 3 mg of hydrochlorothiazide was used to make a differential thermograms. The instrument was set as follows: ΔT gain 150 μv, 500° C/FS, sample holder NIZ, nitrogen atmospheres, gas flow rate 0.075 SCFH.

x-Ray diffraction-x-ray diffractograms were obtained using a Rigaku 2037 diffractometer. The powder which passed through 200mesh sieve was packed into a planchet 1 mm deep, 20 mm long and 10 mm wide. The instrument variables were set as follows: scanning speed 4°/min., chart speed 40 mm/min., time constant 1 sec., range 2000 cps., CuKα radiation, 30 Kv, 15 mA, infiltered wavelength 1.5405Å.

Solubility and dissolution rate measurement-Solubility and dissolution rate measurement for the determination of the equilibrium solubility, an excess quantity of the crystals was shaken with 100 ml of the distilled water in an Erlenmyer flask immersed in water bath thermostated. No attempt was made to control the particle size of the crystals. 5 ml of the solution was withdrawn with a pipet fitted with a glass wool plug at proper time intervals. Aliqwots of 1 ml were withdrawn from the filtered solution and appropriately diluted, and the absorbance was measured spectrophtometically at 272 nm.

The apparatus used in the dissolution rate determanation was similar to that described by wood et al.[15] Approximately 500 mg of the crystals was compressed into a 1.3 cm diameter disk at 3,000 lb for 10 seconds. The flat disk,

with one face of the compressed disk exposed and the other open end of the disk closed with paraffin wax, was placed in a plastic holder. The disk was rotated in 600 ml of 0.1 N HCl solution at 150 rpm. Samples were removed at suitable time intervals and analyzed spectrophotometrically at 272 nm for hydrochlorothiazide. Dissolution of each crystalline forms was studied at several different temperatures. Intrinsic dissolution rates were calculated from the initial slope of an amount dissolved versus time curve divided by the surface area of the compressed disk.

Diuretic Action Study in Rats

Male Sprague Dawley rats weighing 100~200g were used for the diuretic action study. The animals were permitted free access to water and were fasted for 18 hrs prior to oral administration. Each crystalline forms was administered to 5 rats in each group perorally in a single dose of 1 mg/100g. Urine samples were collected at 0~2, 2~4, 4~6 and 6~24 hrs after oral administration. Urinary volume was measured on each sample, and then urine samples were stored at 4°C while awaiting analysis. Urine samples were analyzed for sodium, potassium and magnesium by means of atomic absorption spectrophotometry using a Perkin-Elmer 306 atomic absorption spectrophotometer.[16-18]

RESULTS AND DISCUSSION

Four crystalline forms of hydrochlorothiazide were identified from differential thermograms and x-ray diffractograms. Fig. 1 shows the DTA thermogram patterns of crystalline form I, II, III and IV. The thermograms of form I and II gave an essentially straight base line with a single endothermic peak corresponding to fusion with melting points at 268°C and 273°C, respectively. Crystalline form III and IV showed

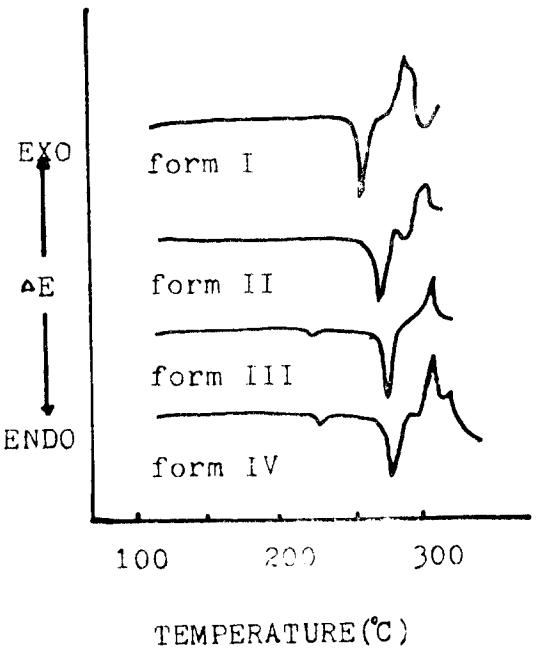

Fig. 1: Differential thermograms of various forms of hydrochlorothiazide.

two endothermic peaks. Form III exhibited a transition peak at 224°C, whereas form IV had a transition peak at 226°C. After 10 months storage period, the occurrence of four crystalline forms was ascertained, and each forms showed a reasonably high stabllity.

Fig. 2 shows the powder diffractograms of each crystalline forms. Each are distinguished by peak differences in the region of 2 values of 15 to 30. Distinct differences are apparent and are attributed to differences in the arrangements of the molecules in the crystal lattices.

The equilibrium solubilities of each crystalline forms(I, II, III, IV) are 0.88 mg/ml, 0.73 mg/ml, 0.80 mg/ml and 0.80 mg/ml, respectively. The equilibrium solubility of form I is higher than others. This difference could not be due to the difference in the crystal size, but is attributed to the higher free energy state of form I. Because form I had a lower melting point and a higher solubility than the other

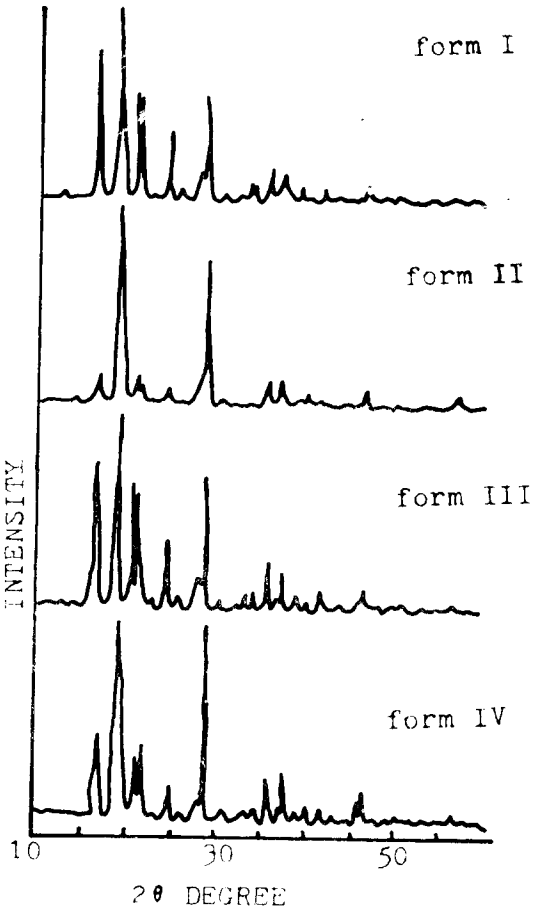

Fig. 2: x-Ray diffractograms of various forms of hydrochlorothiazide.

polymorphic forms, it was very corresponded with the fact that the metastable crystal form have a lower melting point and a higher solubility.[15,19]

The dissolution profiles for four crystalline forms at different temperatures were made respectively. Cumulative amounts of dissolved drug were plotted as a function of time and then initial dissolution rates of each form were calculated. The logarithm of the initial dissolution rate was plotted as a function of the reciprocal of absolute temperature. Then, the heat of dissolution for each solid form, ΔH_{diss}, was calculated from the slope of the lines. For each experimental pair the point of intersection represents the transition temperature at which the two forms exhibit identical dissolution rates. The difference between the heats of dissolution of the components of each pair represents the enthalpy of transition, ΔH_{trans}. Under the conditions of constant temperature and pressure, the difference in free energy of the two forms of each pair is given as follows:[20]

$$\Delta F_T = RT \ln \frac{\text{dissolution rate (unstable form)}}{\text{dissolution rate (stable form)}}$$

The knowledge of ΔF_T and $\Delta Hu,s$ permits computation of the entropy difference, ΔS_T, for each pair as follows:

$$\Delta S_T = \frac{\Delta Hus - \Delta F_T}{T}$$

where subscripts u and s stand for unstable and stable forms, respectively. The values of H_{diss}, F_T, S_T, $H_{u,s}$ and transition temperature for the conversions of form I to II, form III to II and form IV to II are summarized in Table I.

Poole and Bahal pointed out conversion speed of higher energetic form to lower one to be an important factor in determining the difference in *in vivo* performance.[9] This, together with

Table I: Thermodynamic values for crystalline forms of hydrochlorothiazide.

Form	Transition temperature (°C)	△Hdiss Kcal/mole.	$\triangle F_{310}$ cal/mole.	$\triangle S_{310}$ eu	△Strans eu	△Hu→s* cal/mole
I	26.0	8.47	−302.1	−15.25	−17.29	−5.03
II		13.50				
III	43.0	11.81	− 42.3	− 5.32	− 5.35	−1.69
IV	52.2	10.89	− 38.5	− 8.30	− 8.03	−2.61

* u : unstable, s : stable

Table II: Urinary electrolyte excretion in reponse to crystalline forms of hydrochlorothiazide in rats.

Form	Variable	Interval(hrs) 0~2	2~4	4~6	6~24	Na^+/K^+*
I	Sodium[a]	9034.6±332.1	4421.5±175.8	965.1± 62.9	5584.0±134.9	1.46
	Potassium[a]	6195.4±113.9	5256.9±182.3	2142.5±130.5	20110.5±783.3	
	Magnesium[a]	138.5± 35.5	65.1± 22.6	20.1± 7.1	364.8± 64.8	
	Volume[b]	2.7± 0.9	1.8± 0.7	0.5± 0.2	2.2± 0.5	
II	Sodium	7793.1±216.5	6578.9±106.7	1350.6±151.7	8265.6±191.9	0.94
	Potassium	8286.6±203.0	5779.5± 80.9	1486.5±161.9	15208.3±460.1	
	Magnesium	129.5± 30.9	89.6± 20.7	17.8± 7.0	260.6± 82.5	
	Volume	2.6± 0.7	1.9± 0.3	0.4± 0.2	2.3± 0.3	
III	Sodium	6551.0±233.5	4952.5±189.7	1125.1± 44.0	6048.3±223.1	1.32
	Potassium	4951.8±180.1	5537.3±193.8	2438.9±109.8	16293.1±260.1	
	Magnesium	116.5± 16.0	97.5± 26.6	29.0± 8.2	479.0± 52.4	
	Volume	2.1± 0.7	2.2± 0.5	0.5± 0.2	2.5± 0.4	
IV	Sodium	7679.3±316.4	5075.1±211.0	1202.5± 86.7	8161.0±424.8	0.79
	Potassium	9741.5±271.3	5977.3±180.3	3591.3±252.0	21682.2±479.7	
	Magnesium	170.6± 24.2	84.0± 24.2	42.9± 10.9	292.6± 39.9	
	Volume	2.5± 0.5	1.8± 0.4	0.6± 0.3	2.4± 0.5	
Control	Sodium	2229.8±143.4	1713.2±131.0	648±107.4	8851±236.8	0.61
	Potassium	3661.8±249.2	3434.6±209.0	1137.5±178.8	24355.7±319.7	
	Magnesium	180.2± 28.2	117.3± 22.1	40.8± 7.6	563.1± 47.1	
	Volume	1.1± 0.6	0.9± 0.4	0.3± 0.2	2.6± 0.6	

6 rats in each group.

All animals were fasted for 18hrs prior to the administration and administered perorally in a single dose of 1mg/100g.

Accumulated values for each fractioning collection period and each data given as Mean±S.E. (a : mcg, b : ml)

Statistically significant for all pairs ($p<0.05$)

* Na^+/K^+ ratio during the first 2hrs after administration.

the solubility difference, account for the *in vivo* difference between the two forms. In this study form I, metastable form, showed a reasonablely high stability for at least 48 hrs in water.

Since hydrochlotiazide is extensively eliminated from the body as unchanged drug in the urine, bioavailability studies of this drug have been usually focused on the determination of the drug concentration in urine. Also, it is possible to evaluate the bioavaility of hydrochlorothiazide products by monitoring the change in sodium levels in serum and/or urinary excretion of sodium. Volume of urine, and concentration of sodium, potassium and magnesium in urine after oral administration with a single dose of 1 mg/100g were summarized in Table II. Urinary volume changes for 24 hrs after administration of each crystalline forms were measured. Diuretic activity of form I after 2 hrs of administration was higher than other forms. None of these values were changed significantly, when it passed 24 hrs after single oral dose. Table II also shows the results of the values of natriuresis and kaliuresis. The use of urine Na/K ratio has been documented as an accurate indication of aldosterone activity. In the diuretics, increase in natriuresis without a significant increase in kaliuresis clinically important. As shown in Table II, the change in Na/K ratio after single dose administration of form I was more increased than those in the other forms. Certain diuretics such as benzothiadiazine

are frequently quoted as a cause of symptomatic magnesium deficiency.[17] In this study. concentration of magnesium in urine after oral administration of each crystalline forms were decreased more than those in the control.

In conclusion, the metastable crystal form, form I, have a lower melting point and a higher solubility. Higher diuretic activity of form I may be due to the higher solubility of it. As a result of this study, form I might be a choice of crystalline form for medicinal use.

LITERATURE CITED

1) Martin, A., Swarbrick, J. and Cammarata, A.: *Physical Pharmacy*, 3rd Ed., Lea & Febiger, 72 (1983).
2) Aguiar, A.J., Kinkel, A.W. and Samyn, J.C.: Effect of polymorphism on the absorption of chloramphenicol from chloramphenicol palmitate. *J. Pharm. Sci.* **56**, 847(1967).
3) Aguiar, A.J. and Zelmer, J.E.: Dissolution behavior of polymorphs of chloramphenicol palmitate and mefenamic acid. *J. Pharm. Sci.* **58**, 983 (1969).
4) Mullins, J.D. and Macek, T.J.: Some pharmaceutical properties of novobiocin. *J. Amer. Pharm. Assoc.* **49**, 245(1960).
5) Simmons, D.L., Ranz, R.J. and Gyanchandani, N.D., Polymorphism in pharmaceuticals III: Chloropropamide. *Can. J. Pharm. Sci.* **8**, 125 (1973).
6) Had, A., Mezösi, J., Kedvessy, G. and Morvay, J.: Biopharmaceutical studies on sulphadimidine modifications. *Pharmazie* **32**, 791(1977).
7) Kato, Y. and Watanabe, F.: Relationship between polymorphism and kioavailobility of phenobarbital. *Yakugaku Zasshi* **98**, 639(1978).
8) Martin, A., Swarbrick, J. and Cammarata, A.: *Physical Pharmacy*, 3rd Ed., Lea & Febiger, 575, (1983).
9) Poole, J.W. and Bahal, C.K.: Dissolution behavior and solubility of anhydrous and trihydrate forms of ampicillin. *J. Pharm. Sci.* **57**, 1945(1968).
10) Clements, J.A. and Popli, S.D.: The preparation and properties of crystal modification of meprobamate. *Can. J. Pharm. Sci.* **8**, 89(1973).
11) Jamali, F. andMitchell, A.G., The recrystallization and dissolution of acetylsalicylic acid. *Acta Pharm. Suecica.* **10**, 343(1973).
12) Florence, A.T. and Salole, E.G.: Changes in crystallinity and solubility on comminution of digoxin and observations on spironolactone and oestradiol. *J. Pharm. Pharmac.* 28, 637(1975).
13) Ghielmetti, G., Bruzzess, T., Bianchi, C. and Recusani, F.: Relationship between acute toxicity in mice and polymorphic forms of polyene antibiotics, *J. Pharm. Sci.* **65**, 905(1976).
14) Thakkar, A.L., Hirsch, C.A. and Page, J.G.: Solid dispersion approach for overcoming bioavailability problems due to polymorphism of nabilone, a cannabinoid derivative. *J. Pharm. Pharmac.* **29**, 783(1977).
15) Lachman, L., Lieberman, H.A. and Kanig, J.L.: *The Theory and Proctice of Industrial Pharmacy.* Lea & Febiger, Philadelphia (1976).
16) Leary, W.P., Asmal, A.C. and Reyes, A.J.: Time-course of the diuretic, natriuretic and kaliuretic effects of xipamide. *Curr. Ther. Res.* **27**, 16(1980).
17) McLeod, P.L., Ogilvie, R.I. and Ruedy, J.: Effects of large and small doses of hydrochlorothiazide in hypertensive patients. *Clin. Pharm. Thera.* **11**, 733(1970).
18) Sutton, R.A.L. and Walker, V.R.: Responses to hydrochlorothiazide and acetazolamide in patients with calcium stones. *New Engl. J. Med.* **302**, 709(1980).
19) Carstensen, J.T.: *Theory of Pharmaceutical Systems*, Vol. II, Academic Press (1973).
20) Wadke, D.A. and Reier, G.E.: Use of intrinsic dissolution rates to determine thermodynamic parameters associated with transition. *J. Pharm. Sci.* **61**, 868(1972).