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Molecular Behavior and Dissolution Characteristics of Uracil in Ground Mixtures

Kazuhiko BABA,*[a] Yoh'ichiro TAKEICHI,[a] and Yoshinobu NAKAI[b]

Pharmaceutical Research Laboratory, Taiho Pharmaceutical Co., Ltd.,[a] Kawauchi-cho, Tokushima 771–01, Japan and Faculty of Pharmaceutical Science, Chiba University,[b] Yayoi-cho, Chiba 260, Japan.

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Ground mixtures containing uracill were prepared using various additives such as celluloses, proteins, cyclodextrins, enteric coating agents and inorganic compounds in a planetary ball mill. The amorphous state of uracil was observed in the X-ray diffraction patterns of some of the ground mixtures. The results of infrared spectral analysis indicated deprotonation of uracil after 30 h of (30-h) grinding with sodium polyglutamate. All ground mixtures showed the transient supersaturation of uracil in dissolution studies. The initial amount of uracil dissolved from the 30-h ground mixtures with sodium benzoate derivatives, ethylcellulose, hydroxypropylmethylcellulose acetate succinate and proteins was 2.5 to 9-times that dissolved from intact uracil. The crystallinity and solubility of uracil in the ground mixtures were affected by the mixing ratio, grinding time and moisture content of the additive.

Keywords uracil; grinding; X-ray diffraction; amorphous; crystallinity; infrared; dissolution; solubility

Mechanochemical studies have been attracting considerable attention in pharmaceutical fields, since many powdery drugs have been shown to induce various mechanochemical effects during pharmaceutical operation. The mix-grinding procedure is of particular interest as an influence on the physicochemical properties of organic compounds. When some drugs were ground with such an additive as microcrystalline cellulose or β-cyclodextrin, both ingredients became amorphous, and the ground mixture showed features such as formation of an inclusion complex, facilitated chemical decomposition, accelerated dissolution and enhanced *in vivo* absorption.[1–5]

However, the amorphous state of organic compounds self-associated by hydrogen-bonding, *e.g.* amobarbital or hexobarbital, is difficult to bring about.[6–8] Uracil molecules strongly self-associate not only by hydrogen-bonding laterally but also by stacking vertically, and form layered crystal structures.[9,10] The extremely stable structure of uracil is mainly responsible for its marked high-melting point (*ca.* 338 °C)[9] and poor solublity in all solvents.[11] There has been extensive investigation of the mix-grinding effect on many kinds of compounds, but no studies have been reported for compounds whose melting points exceed 300 °C. It is considered significant to elucidate the effects of grinding with additives on the physicochemical properties of structurally stable compounds such as uracil.

In this study, we prepared ground mixtures of uracil and various additives using a planetary ball mill with considerably high grinding efficiency. Microcrystalline cellulose, cyclodextrins, enteric coating agents,[12] proteins,[13] polyvinylpyrrolidone,[13] chitin or chitosan,[14] inorganic compounds[6] and some bio-compatible polymers were used as additives. Sodium benzoate, sodium 2,6-dihydroxybenzoate, nicotinamide and urea, solubilizing agents for pyrimidine derivatives and/or purine derivatives, were also used.[15–18] The molecular behavior and dissolution properties of uracil in the ground mixtures were studied.

Experimental

Materials Uracil was purchased from Kyowa Hakko Co. and used as received. The additives used were: microcrystalline cellulose (MCC, Avicel-301 type, Asahikasei Ind. Co.), ethyl cellulose (STD-7cps, STD-100cps, MED-50cps type, Nisshinkasei Co.), low-substituted hydroxypropylcellulose (L-HPC, LH-31 type, Shin-Etsu Chem. Ind., Co.), carboxymethylcellulose (CMC, NS-300 type, Gotoku Pharmaceutical Co.), corn starch (Nihon Shokuhin Kako Co.), pregelatinized starch (Asahikasei Ind., Co.), soluble starch (Wako Pure Chem. Co.), dextran (M.W. $=4\times10^4$ and 2×10^5—3×10^5, Tokyo Kasei Ind., Co.), dextrin (Wako Pure Chem. Co.), α-cyclodextrin (Tokyo Kasei Ind., Co.), β-cyclodextrin (Sanraku Inc.), γ-cyclodextrin (Sanraku Inc.), pullulan (20pf type, Hayashibara Co.), sodium chondroitinsulfate (Tokyo Kasei Ind., Co.), chitin (Asahi Glass Co.), chitosan (Katakura Chikkarin Co.), hydroxypropylmethylcellulose acetate succinate (HPMCAS, AS-LF, AS-MF, AS-HF type, Shin-Etsu Chem. Ind., Co.), methacrylic acid copolymer (Eudragit L-100, Rohm Pharma GmbH, West Germany), hydroxypropylmethylcellulose phthalate (HPMCP, HP-55 type, Shin-Etsu Chem. Ind., Co.), carboxymethylethylcellulose (CMEC, Freund Ind. Co.), egg albumin (Wako Pure Chem. Co.), bovine serum albumin (BSA, crystallized and lyophilized type, Sigma Chem. Co., U.S.A.), human serum albumin (HSA, crystallized and lyophilized type, Sigma Chemical Co., U.S.A.), collagen peptide A (Funakoshi Co.), bromelain (Funakoshi Co.), papain (Wako Pure Chem. Co.), powdered acacia (Nippon Funmatsu Yakuhin Co.), polyethyleneglycol-6000 (PEG-6000, Nippon Oil & Fats Co.), polyvinylpyrrolidone (PVP, insoluble type, Tokyo Kasei Ind. Co.), vinylpyrrolidone–vinylacetate copolymer (VP–VA copolymer, BASF Japan), stylene–maleic anhydride copolymer (ST–MA copolymer, Polyscience Inc., U.S.A.), poly(*l*-lactic acid) (Mitsui Toatsu Chemical Inc.), sodium polyglutamate (NaPG, Ajinomoto Co.), sodium polystylenesulfate (Aldrich Chemical Co.), aminoalkyl methacrylate copolymer L (Eudragit RS, Rohm Pharma GmbH, West Germany), magnesium oxide (Wako Pure Chem. Co.), silicic anhydride (Wako Pure Chem. Co.), light anhydrous silicic and (Aerosil, Watanabe Yakuhin Shokai), aluminium oxide (Wako Pure Chem. Co.), magnesium chloride (anhydrous type, Wako Pure Chem. Co.), sodium benzoate (Yamaguchi Yakuhin Shokai), nicotinamide (Kawazu Sangyo Co.), sodium gluconate (Wako Pure Chem. Co.), sodium nicotinate (Tokyo Kasei Ind., Co.), fructose (Wako Pure Chem. Co.), urea (Wako Pure Chem. Co.), adenine (Wako Pure Chem. Co.), L-arginine hydrochloride (Wako Pure Chem. Co.). 2,6-Dihydroxybenzoic acid (Lancaster Synthesis, UK) was used as sodium salt. The other materials were of a reagent garde.

The additives were dried prior to grinding under the following conditions: ethylcellulose, HPMCAS , HPMCP, CMEC and sodium polystylene sulfate (105 °C, 1 h); NS-300, Eudragit RS and Aerosil (105 °C, 4h); corn starch (105 °C, 6 h); pullulan (85 °C, reduced pressure, 5 h); egg albumin, BSA, HSA, bromelain, papain, nicotinamide, sodium nicotinate and fructose (room temperature, reduced pressure, 8 h); silicic anhydride (850 °C, 3 h); magnesium oxide and aluminium oxide (900 °C, 3 h); magnesium chloride (112 °C, 3 h); PEG-6000, sodium gluconate, urea and arginine hydrochloride (not dried); and the other additives (105 °C, 3 h).

Preparation of Sodium Uracilate Uracil was dissolved in a stoichiometrically equal NaOH solution. The sodium salt was obtained by evaporation of the solvent.

Ground Mixtures Ground mixtures were prepared using a planetary ball mill (P-5, Fritsch Japan) made of agate. Four balls 25 mm in diameter, six balls 20 mm in diameter, and about 80 ml of a sample were put into a 250-ml capacity bowl. The rotating velocities of the bowl and supporting disk were about 570 and 270 rpm, respectively. The mixting ratio of uracil to additive was 1 : 4 by weight, but 1 : 2 by moles in the case of low-molecular additives.

X-Ray Diffractometry Powder X-ray diffraction patterns were measured with a diffractometer (Geigerflex 2028, Rigaku Denki). Conditions: target Cu, voltage 50 kV, current 25 mA, time constant 0.5 s, scanning speed 2°/min, chart speed 20 mm/min.

Infrared (IR) Spectra IR spectra were obtained with a spectrophotometer (IR-425, Shimadzu) by the KBr disk method.

Dissolution Study A powder sample containing 600 mg of uracil equivalent (unless stated otherwise in Table I) was put into a 30 ml beaker containing 15 ml of a dissolution medium maintained at 37 °C and stirred with a magnetic stirrer at about 300 rpm. Deionized water was used as the dissolution medium in all cases. At specified times, about 1 ml of each solution was pipetted through a membrane filter (0.45 μm pore size, Millipore). The concentration of uracil was determined by the liquid chromatograph method as shown below.

Determination of Uracil Concentration The sample solutions were analyzed using an high performance liquid chromatography (HPLC) (LC-6A, Shimadzu) equipped with a column (μ-BondapakC18, Waters). The mobile phase was sodium acetate 20 mM/PIC-A 5 mM aqueous solution, operated at a flow rate of 1.0 ml/min. The column effluent was monitored at 254 nm.

Results

Powder X-ray diffraction patterns of intact uracil and 30-h ground uracil without additives are shown in Fig. 1. Ground uracil showed apparent diffraction peaks. The amorphous state of uracil was difficult to obtain by grinding without additives.

Figure 2 shows the X-ray diffraction patterns of the ground mixtures with dried and un-dried corn starch. The crystallinity of uracil in ground mixtures prepared with a dried additive was less than that with an un-dried additive. All ground mixtures were thus prepared using dried additives.

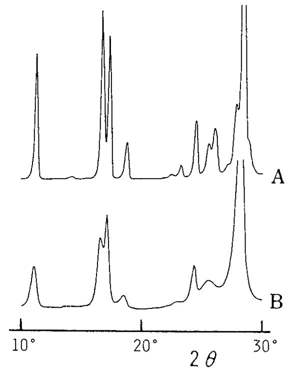

Fig. 1. X-Ray Diffraction Patterns of Intact Uracil and 30-h Ground Uracil

A, intact uracil; B, 30-h ground uracil.

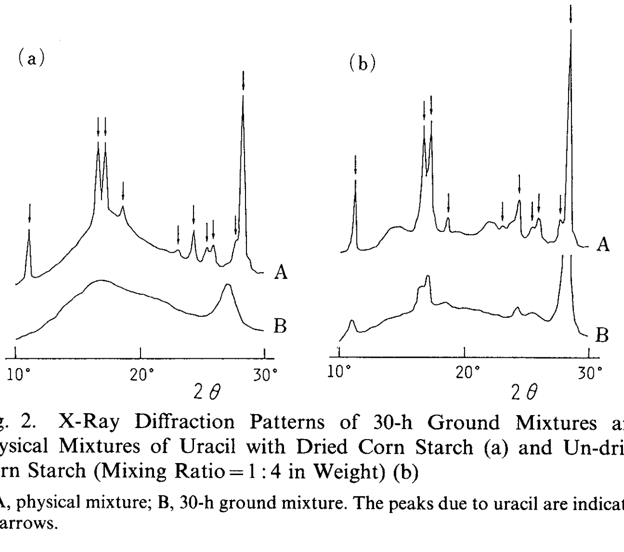

Fig. 2. X-Ray Diffraction Patterns of 30-h Ground Mixtures and Physical Mixtures of Uracil with Dried Corn Starch (a) and Un-dried Corn Starch (Mixing Ratio = 1 : 4 in Weight) (b)

A, physical mixture; B, 30-h ground mixture. The peaks due to uracil are indicated by arrows.

Figure 3 shows the time course of X-ray diffraction patterns of the ground mixtures with different additives. In most cases, the diffraction peaks of uracil decreased and broadened with grinding time. The peaks were not observed after 5 h of (5-h) grinding of the mixtures with Aerosil, magnesium oxide and magnesium chloride. By contrast, the peaks showed only a slight change even after 30 h of (30-h) grinding of the mixtures with PEG-6000 and nicotinamide.

Figure 4 shows the X-ray diffraction patterns of 30-h ground mixtures with HSA and HPMCAS using three different mixing ratios. Uracil became more amorphous as the concentration of the additives increased, as was also the case using other additives.

The dissolution profiles of uracil from the ground mixtures with MCC and Aerosil are shown in Fig. 5. Supersaturation of uracil was observed with all ground mixtures. Dissolution patterns from the ground mixtures showed a negative curvature with time, due to the recrystallization of uracil.

Figure 6 shows the effects of grinding time on the dissolution of uracil from the ground mixtures with HPMCAS and ethylcellulose. The amount of uracil dissolved from these 30-h ground mixtures was considerably greater than the amount dissolved from the corresponding 5-h ground mixtures. In the X-ray diffraction study, however, a further decrease in the diffraction intensity of uracil was not observed by grinding for more than 5 h (data

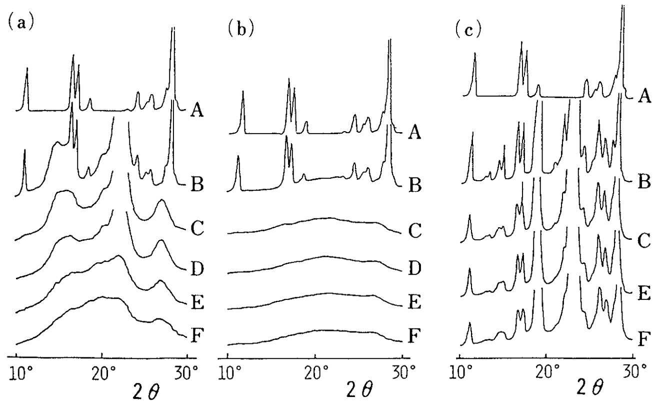

Fig. 3. X-Ray Diffraction Patterns of the Ground Mixtures of Uracil with MCC (a), Aerosil (b) and PEG-6000 (c) as a Function of Grinding Time

A, uracil; B, physical mixture; C, ground mixture (ground for 5 h); D, ground mixture (ground for 10 h); E, ground mixture (ground for 20 h); F, ground mixture (ground for 30 h).

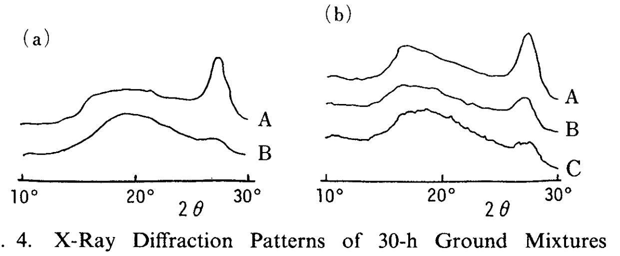

Fig. 4. X-Ray Diffraction Patterns of 30-h Ground Mixtures of Uracil : HSA (a) and Uracil : HPMCAS (AS-MF) (b) in Different Mixing Ratios

Mixing ratio in weight; 1 : 2 (A), 1 : 4 (B). 1 : 8 (C).

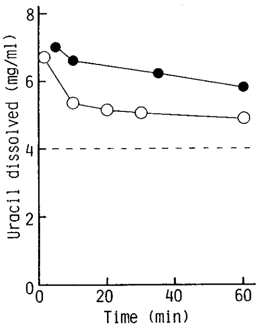

Fig. 5. Dissolution Profiles of Uracil from 30-h Ground Mixtures with MCC and Aerosil (Mixing Ratio = 1 : 4 in Weight)

Ground mixture with MCC (●), ground mixture with Aerosil (○). The dotted line shows the solubility of uracil.

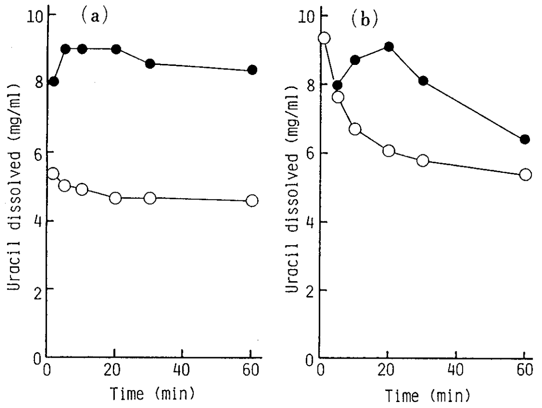

Fig. 6. Effect of Grinding Time on Dissolution Profiles of Uracil from Ground Mixtures with HPMCAS (AS-LF) (a) and Ethylcellulose (STD, 7 cps) (b) (Mixing Ratio = 1 : 4 in Weight)

Grinding time; 5 h (○), 30 h (●).

not shown).

Figure 7 shows the dissolution patterns of uracil from the ground mixtures with HSA and HPMCAS in different mixing ratios. The amount of uracil dissolved increased with the concentrations of the additives. However, the amount dissolved from the ground mixtures with HPMCAS in the initial period decreased with an increase in HPMCAS concentration.

The crystallinity and solubility of uracil for ground mixtures and physical mixtures are summarized in Table I. The X-ray diffraction patterns of some of the ground mixtures indicated an amorphus state of uracil. The amount of uracil dissolved from ground mixtures with sodium benzoate derivatives, proteins, HPMCAS (AS-MF) and ethylcellulose (STD-100 cps) was 2.5 to 9-times greater than that from intact uracil in the initial period.

The IR spectra of 30-h ground mixtures and physical mixtures of uracil with NaPG and MCC are shown in Fig. 8. Each absorption band at 1415, 1502, 1670 and 1716 cm^{-1} in the spectrum of uracil (curve A) was assigned to N(3)–H bending, N(1)–H bending, C(4) = O stretching and C(2) = O stretching, respectively.[19] Three bands (1400, 1568 and 1660 cm^{-1}) in the spectrum of NaPG (curve B) were attributed to carbonyl stretching of the peptide bond and the carboxylic group. The N(3)–H band of uracil was observed in the IR pattern of the physical mixture with NaPG (curve C), but not in the spectrum of the ground mixture (curve D). The C(2) = O stretching band of uracil obviously shifted to a lower wavenumber following grinding with NaPG. The band at 1665 cm^{-1}, attributable to both C(4) = O stretching of uracil and carbonyl stretching of NaPG, did not change in its peak wavenumber but it did broaden, probably due to a shift of the C(4) = O stretching band to a lower wavenumber. The N(3)–H band showed no change in its wavenumber after 30-h grinding with MCC (curve F), although a slight shift of the C(2) = O band was observed.

Uracil exists in a diketo form in solid state and neutral media,[9,20] but behaves as a weak acid in aqueous alkaline media (pK_a = 9.45), with deprotonation occurring at N(3)

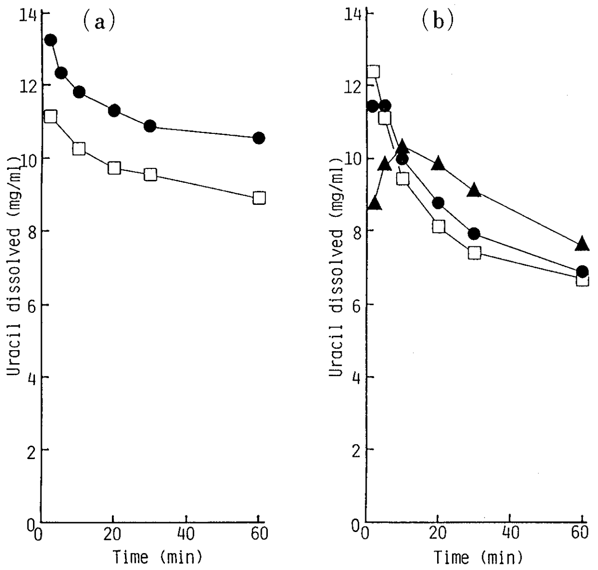

Fig. 7. Effect of Mixing Ratio on Dissolution Profiles of Uracil from 30-h Ground Mixtures with HSA (a) and HPMCAS (AS-MF) (b)

Mixing ratio in weight; 1 : 2 (□), 1 : 4 (●), 1 : 8 (▲).

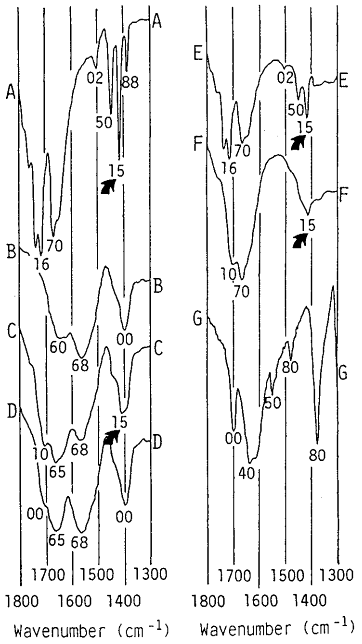

Fig. 8. IR Spectra of 30-h Ground Mixtures and Physical Mixtures of Uracil with NaPG and MCC (Mixing Ratio = 1 : 4 in Weight)

A, uracil; B, NaPG; C, physical mixture with NaPG; D, ground mixture with NaPG; E, physical mixture with MCC; F, ground mixture with MCC; G, sodium uracilate.

TABLE I. Crystallinity and Solubility of Uracil in Ground Mixtures with Various Additives (Mixing Ratio = 1 : 4 in Weight, Grinding Time = 30 h)

Additive	Apparent crystallinity[a]	Solubility at 37 °C (mg/ml)			Ref.
		Ground mixt.		Physical mixt. 30 min[c]	
		Max[b]	30 min[c]		
None	+ +	4.6	4.5	4.0	
(Polysaccharides)					
Microcrystalline cellulose	±	7.0	6.2	4.5	
Ethylcellulose (STD, 7 cps)	+	9.1	8.1	4.4	
Ethylcellulose (STD, 100 cps)	+	10.0	5.3	4.1	
Ethylcellulose (MED, 50 cps)	+	9.4	9.3	4.2	
L-HPC	±	6.8	6.2	4.2	
Carboxymethylcellulose	+	6.9	5.4	4.4	
Corn starch	+	4.7	N.M.[f]	4.1	
Pregelatinized starch	+	4.7	4.0	4.1	
Soluble starch	+	5.4	5.3	4.8	
Dextran (M.W. = 4×10^4)	+	5.0	4.8	4.6	
Dextran (M.W. = 2×10^5—3×10^5)	+	4.6	4.1	3.2	
Dextrin	±	5.5	5.2	4.9	
α-Cyclodextrin	− (10 h)	6.3	5.6	5.3	
β-Cyclodextrin	− (10 h)	6.2	5.0	4.2	
γ-Cyclodextrin	− (10 h)	5.5	4.9	4.2	
Pullulan	+	5.7	5.4	5.0	*d)*
Sodium chondroitinsulfate	− (30 h)	6.3	5.4	4.8	
Chitin	±	6.8	6.3	4.8	
Chitosan	+	6.6	6.0	N.M.[f]	*d)*
(Entetic coating agents)					
HPMCAS (AS-LF)	+	9.0	8.6	4.3	
HPMCAS (AS-MF)	+	11.4	8.0	4.5	
HPMCAS (AS-HF)	+	9.4	9.0	4.3	
Eudragit L-100	±	9.7	7.1	4.6	
HPMCP	±	8.4	6.6	4.8	
CMEC	+	8.8	5.9	4.6	
(Proteins)					
Egg albumin	±	10.5	9.3	4.8	*e)*
Bovine serum albumin	±	11.1	10.9	4.8	*e)*
Human serum albumin	±	13.3	10.9	4.6	*e)*
Collagen peptide A	±	8.6	6.8	5.2	
Bromelain	±	13.1	10.8	5.2	
Papain	+	10.8	8.9	4.7	
(Other polymers)					
Powdered Acacia	− (30 h)	8.2	6.4	4.5	
PEG-6000	+ +	4.6	4.5	4.8	
PVP	− (20 h)	7.8	6.9	3.8	*d)*
VP–VA copolymer	±	6.3	5.8	4.7	
ST–MA copolymer	±	8.0	5.5	4.9	
Poly(*l*-lactic acid)	+	5.8	5.8	4.3	
NaPG	− (10 h)	8.1	7.3	4.4	
Na polystylenesulfate	±	5.8	5.7	5.6	
Eudragit RS	+	7.5	5.2	4.2	
(Inorganic compounds)					
Magnesium oxide	− (5 h)	34.6[g]	31.8[g]	18.3[g]	
Silicic anhydride	±	9.0	5.3	4.0	
Aerosil	− (5 h)	6.7	5.1	3.6	
Aluminium oxide	±	7.1	6.6	4.1	
Magnesium chloride	− (5 h)	7.0	5.2	4.4	*h)*
(Low-molecular organic compounds)					
Na 2,6-dihydroxybenzoate	− (20 h)	35.4	16.2	13.6	*h)*
Na benzoate	− (20 h)	11.2	8.6	7.9	*h)*
Nicotinamide	+ +	7.9	7.2	7.1	*h)*
Na gluconate	+	4.9	4.5	4.2	*h)*
Na nicotinate	+ +	8.7	7.4	7.0	*h)*
Fructose	+	5.6	4.9	4.4	*h)*
Urea	−	7.6	5.7	5.2	*h, i)*
Adenine	±	7.0	5.5	4.4	*h, i)*
L-Arginine hydrochloride	±	5.7	5.4	5.3	*h, i)*

a) Evaluated by the intensity of the peak around 27—28.5° in X-ray diffraction patterns of final ground mixtures. Sharp (+ +), weak (+), slight (±), no distinct (−) peak. The time when the peak was not distinct is shown in parentheses. *b)* The maximum value in dissolution study. *c)* The value at 30 min in dissolution study. *d)* or *e)* The sample containing 300 or 400 mg of uracil equivalent was put into 15 ml of water in the dissolution study, respectively. *f)* Not measured. *g)* The pH of the solution (*ca.* 10.0) exceeding pK_a of uracil (9.45) probably contributed to its high solubility. *h)* Mixing ratio = 1 : 2 in moles. *i)* Grinding time = 5 h.

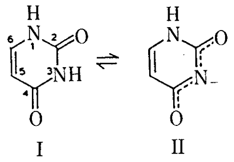

Fig. 9. Dissociation of Uracil in Alkaline Media

as shown in Fig. 9.[21)] Curve G in Fig. 8 shows the IR spectrum of sodium uracilate. The N(3)–H band ceased to be evident, and both carbonyl stretching bands appeared to have shifted to lower wavenumbers, owing possibly to the resonance effect.

Discussion

The crystallinity and solubility of uracil in ground mixtures differed according to the additives and also to the mixing ratio, grinding time and moisture content of the additive. An increase both in the concentration of the additives and in grinding time resulted in enhanced uracil solubility. This is consistent with previous reports.[13,22)] However, the amount of uracil dissolved from ground mixtures with HPMCAS in the initial period decreased with an increase in HPMCAS concentration (Fig. 7). Law and Kayes[23)] reported hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC) and hydroxypropylmethylcellulose (HPMC) to be adsorbed on the solid-water interface in suspensions of ibuprofen and polystryrene latex and they reported the occurence of multilayer adsorption at higher concentrations of these polymers. If HPMCAS adsorbed on the surface of uracil in a similar fashion, it may possibly have hindered rapid dissolution.

It is difficult to clarify the molecular interactions between uracil and the additives in the ground mixtures, but perhaps they can be surmised from relevant data on several points. Differences in the molecular state of uracil for two ground mixture, with NaPG and MCC, were examined by infrared spectral analysis (Fig. 8). The disappearance of the N(3)–H bending band and the shifts of carbonyl stretching bands were observed in the spectrum of the ground mixture with NaPG, but not in the spectrum of the ground mixture with MCC. This is considered an indication that 30-h grinding with NaPG caused uracil deprotonation at N(3) in a manner similar to that for dissociation in an alkaline media, but grinding with MCC did not affect the chemical structure of uracil. The mixture with NaPG showed an amorphous state of uracil after 10-h grinding, but this did not occur with MCC even after 30-h grinding (Table I). Differences in the crystallinity of the mixtures may be related to the molecular state of uracil in them.

Of all the additives used, sodium 2,6-dihydroxybenzoate enhanced uracil solubility most effectively. Higuchi and Connors[15)] reported that the 2,6-dihydroxybenzoate ion showed a strong solubilizing effect on theophylline and suggested charge-transfer complexing. Poochikian and Cradock[24)] pointed out that the two hydroxyl moieties in 2,6-dihydroxybenzoate are capable of forming two simultaneous intramolecular hydrogen bonds with the carboxyl anion, resulting in a great planar π-cloud area. Uracil is a π-exessive compound. Such a large π-cloud area of 2,6-dihydroxybenzoate may also intensify the π–π interaction with uracil and contribute to the great increase in its solubility.

Ground mixtures with some inorganic additives were found to take on color during grinding (magnesium oxide: pale yellow, magnesium chloride: pale purple, aluminium oxide: pale brown). The color of the ground mixture with magnesium oxide disappeared as soon as the mixture was put into water. Wu *et al.*[25)] investigated the interactions of isoniazid and magnesium oxide in the solid state by diffuse reflectance spectroscopy. Major spectral differences were observed between the triturated mixture and the physical mixture. They pointed out the possibility that the π-electron of isoniazid was transferred to the low-lying antibonding orbital of magnesium oxide instead of to its own higher energy antibonding orbital. They considered that strong adsorption of isoniazid to magnesium oxide in the triturated process may possibly be essential for the overlapping of molecular orbitals. It is highly probable that similar interactions may occur between uracil and metallic-containing additives, giving rise to coloration as mentioned above.

Thus, the molecular interactions concerning the π-electron of uracil are considered to be an important factor affecting the physicochemical properties of ground mixtures. The major changes in the properties of uracil may result from strong π–π interactions with additives.

In conclusion, high-efficiency grinding with various additives affected the molecular behavior and dissolution characteristics of uracil. It should be noted that the physicochemical properties of uracil, which is an extremely stable compound, were modified as a result of mechanochemical interactions with the additives.

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