October, 1982] © 1982 The Chemical Society of Japan *Bull. Chem. Soc. Jpn.*, **55**, 3123—3128 (1982) 3123

The Solubility of Ball-milled Amobarbital and the Stability of Its Amorphous State

Akiko Ikekawa* and Sohachiro Hayakawa†
Faculty of Pharmaceutical Sciences, Showa University, Hatanodai, Shinagawa-ku, Tokyo 142
†*Department of Applied Physics, Faculty of Science, Tokyo Institute of Technology, Oh-okayama, Meguro-ku, Tokyo 152*

(Received February 18, 1982)

Amobarbital was ball-milled with methylcellulose (MC), microcrystalline cellulose, or dextran. The analysis of the IR spectra indicated that hydrogen bonds were formed between the C=O groups of amobarbital and the OH groups of the diluents upon ball-milling. It was also shown that formation of the bonds was more predominant with MC than with dextran. After suspensions of various kinds of samples in distilled water had been set aside for a week after the time of preparation, the solid state of the amobarbital remaining undissolved in the suspension was compared with the state before dissolution. There are three kinds of polymorphs for amobarbital: Crystals I, II, and III. Crystal I is given by heating Crystal II (at 150 °C), which has been recrystallized from a 25 v/v% aqueous ethanol solution. Crystal III is given by freeze-drying an aqueous solution. The above comparison suggests that Crystal III and the amorphous part are transformed into Crystal I in the absence of any diluent. It seems that the presence of dextran ball-milled with amobarbital prevents Crystal III from being transformed into Crystal I. The presence of MC ball-milled with amobarbital also seems to prevent both the amorphous part from crystallization and Crystal III from transformation into Crystal I. The solubility of amobarbital in distilled water increased remarkably upon ball-milling with diluents; this was attributed to the stabilization of the amorphous state by the interaction with diluents.

The solubility of amobarbital in distilled water increased upon ball-milling with such diluents as methylcellulose,[1] though the solubility increased only slightly without any diluent.[2] Previously, the effect of ball-milling, both with a diluent and without any diluent, on the solid state of amobarbital was investigated, and the relationship between the solid state and solubility was discussed.[1,2] Consequently, it was suggested that the interaction of amobarbital with diluents was one of the most important reasons for the increase in the solubility.[1] In this paper, the interaction of amobarbital with diluents resulting from ball-milling was investigated, and the influence of the interaction on the solid state was discussed.

Experimental

The materials used were amobarbital (5-ethyl-5-isopentylbarbituric acid), dextran 2000, microcrystalline cellulose (MCC), and methylcellulose (MC), all described in a previous paper.[3]

The following samples were ball-milled in the way described previously;[3] 10 g of commercial powders of amobarbital (AC) dextran or MCC; 5 g of MC; a mixture of 1 g of AC and 9 g of each of the diluents; a mixture of 3 g of AC and 7 g of dextran or MCC; a mixture of 2 g of AC and 3 g of MC. In this paper, the "mixture with dextran" contains 30 w/w% of amobarbital and the "mixture with MC" contains 40 w/w% of amobarbital, when no annotation is given.

Various kinds of samples were prepared as follows.

ACS: A suspension of 1 g of AC in 500 cm^3 of distilled water was stirred for 2 h, allowed to stand at 30 °C for a week, and then filtered. The residue was dried under reduced pressure at 70 °C.

AB: AC was ball-milled for 60 h.

ABS: ABS was prepared from AB in the same way as that used to obtain ACS from AC.

DexAB: A mixture with dextran was ball-milled for 200 h.

DexABS: A suspension of 3 g of DexAB in 500 cm^3 of distilled water was stirred for 2 h, allowed to stand at 30 °C for a week, and then filtered. The residue was washed with an aqueous saturated solution of amobarbital and dried under reduced pressure at 70 °C.

MCAB: A mixture with MC was ball-milled for 100 h.

MCABS: A suspension of 2 g of MCAB in 400 cm^3 of distilled water was stirred for 2 h, allowed to stand at 30 °C for a week, and then centrifuged. The precipitate was washed with an aqueous saturated solution of amobarbital and dried under reduced pressure at 70 °C.

The results of the elemental analysis for ACS, ABS, and DexABS were identical with the results for AC; this fact demonstrated that the amount of dextran contaminating DexABS was negligible. The nitrogen content in MCABS was a little smaller than the content in AC; this finding showed that MC mixed in MCABS because of the low solubility of MC in distilled water. The content of MC in MCABS was presumed to be 7.1 w/w% from the ratio of the content of nitrogen to that of carbon.

The IR spectra and X-ray diffraction diagrams were recorded in the way described previously.[3]

Results and Discussion

IR Spectra. Figures 1, 2, and 3 show the effect of ball-milling on the IR spectra of mixtures of amobarbital, as determined by a KBr method. The absorbances around the 3400, 1300—1450, and 1000—1120 cm^{-1} regions increased upon ball-milling. The bands from 1650—1800 cm^{-1}, corresponding to the stretching vibration of the C=O groups of amobarbital,[4] were also affected by ball-milling. The same trends were also found by a Nujol method. In the IR spectra of cellulose derivatives and dextran, the band around the 3400 cm^{-1} region was assigned to the stretching mode of hydrogen-bonded OH groups,[5] though this band was largely overlapped with the absorption of associated water.[6] Keighley *et al.* reported that the absorbance of the band at 2.92 μm (at 3420 cm^{-1}) was found to be higher by the use of a poly(tetrafluoroethylene) (PTFE) disk method than by the use of a KBr disk method or a nujol method. They suggested that this finding was associated with the formation of hydrogen bonds between the

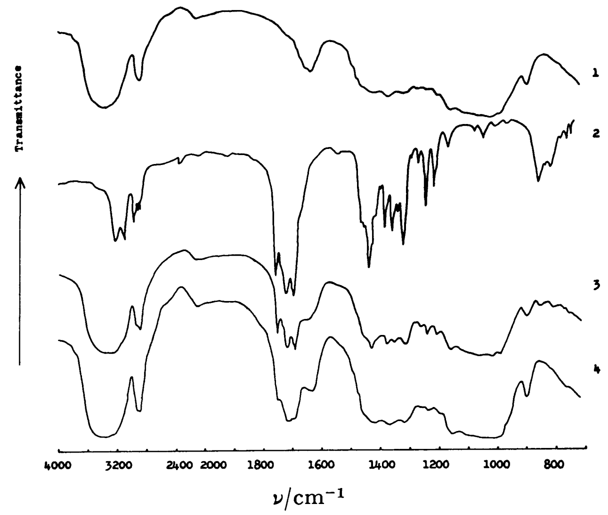

Fig. 1. Effect of ball-milling on IR spectra of the mixture of amobarbital and MCC.
1: MCC ball-milled for 200 h, 2: AB, 3: the mixture of "1" and "2" in the ratio of 9 : 1 by weight, 4: the mixture of 1 g of AC and 9 g of MCC ball-milled for 40 h. The spectra for the mixture ball-milled for 60 h were the same as those for the sample "4".

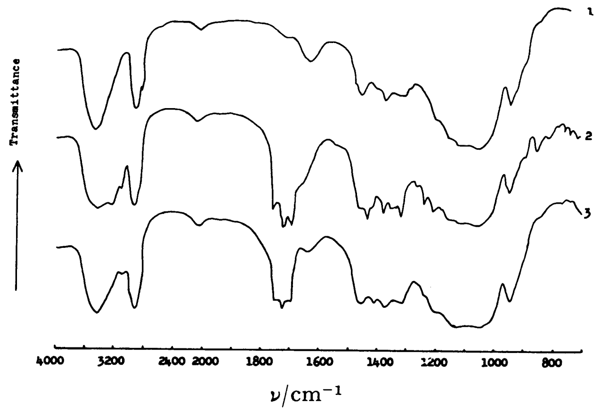

Fig. 2. Effect of ball-milling on IR spectra of the mixture of amobarbital and MC.
1: MC ball-milled for 100 h, 2: the mixture of AB and "1" in the ratio of 1 : 9 by weight, 3: the mixture of 1 g of AC and 9 g of MC ball-milled for 60 h.

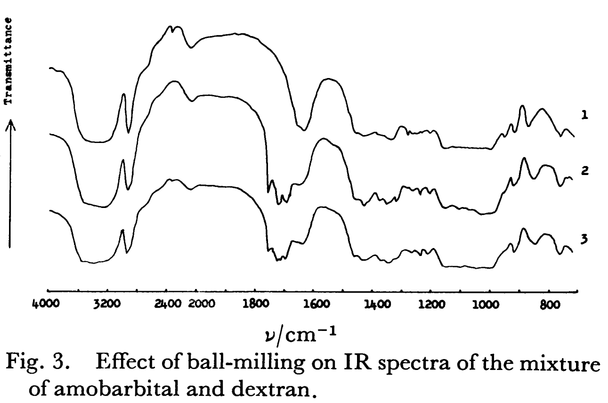

Fig. 3. Effect of ball-milling on IR spectra of the mixture of amobarbital and dextran.
1: Dextran ball-milled for 200 h, 2: the mixture of AB and "1" in the ratio of 1 : 9 by weight, 3: the mixture of 1 g of AC and 9 g of dextran ball-milled for 60 h.

cellulosic OH groups and the fluorine atoms of PTFE.[7] Marchessault *et al.* pointed out that the difference in a hydrogen-bonding system in various crystal-lattice types affected the intensities and frequencies of the bands in the OH stretching region.[8] The band at 1429 cm^{-1} was assigned to the CH_2 symmetric bending mode. The bands at 1315, 1335, and 1370 cm^{-1} were assigned to the CH_2 wagging mode, the C–O–H in-plane bending mode, and the CH bending mode respectively. The band at 1110 cm^{-1} was assigned to the C–O stretching mode, perhaps coupled to the C–C frequency. These bands were also reported to change with the variation in the crystal modifications or crystallinity; this was probably due to the change in the hydrogen-bonding system.[5,9] The above facts indicate that hydrogen bonds are formed between the C=O groups of amobarbital and the OH groups of the diluents.

The absorbance at each of the wave numbers, ν, was determined, assuming that the transmittance on the straight line passing through 3800 and 2000 cm^{-1}, or 2000 and 900 cm^{-1}, was 100%. Figure 4 represents the variations in $R_{\nu,\nu'}$ with ball-milling for the mixture with MCC containing 10 w/w% of amobarbital, where $R_{\nu,\nu'}$ is the ratio of the absorbance at the wave number of ν to the absorbance at ν'. $R_{1755,1690}$ was little influenced by ball-milling. $R_{1720,1690}$ increased in the first stage, while it stayed nearly constant after ball-milling for 15 h. $R_{1735,1690}$, $R_{1710,1690}$, and $R_{1700,1690}$ increased after ball-milling for 10 h. In the carbonyl absorptions of AB in Fig. 1, the absorption at 1755 cm^{-1} was considered to be due to the mode associated mainly with the C=O group in the 2-position, and the absorptions at 1720 and 1690 cm^{-1} bands, to be the antisymmetric and symmetric vibrations respectively, involving mainly the groups in the 4- and 6-positions.[4] It was previously reported that the 4- and 6-carbonyl groups formed hydrogen bonds with the NH groups, leaving the 2-carbonyl group unaffected in the solid state.[10] It was also noted that these bands showed a drop in frequency upon hydrogen bonding. The results in Fig. 4 support the following interpretations: (1) some part of the band at 1755 cm^{-1} shifts to a lower frequency upon the hydrogen bonding of the free C=O group in the 2-position, probably with the OH groups of MCC; (2) some part of the band at 1690 cm^{-1} shifts to a higher frequency upon the conversion of the bonds of the C=O group in the 4- or 6-position with the NH group of amobarbital to the bonds with the OH groups.

Figure 5 shows the variation in $R_{\nu,1690}$ (ν/cm^{-1} is between 1700 and 1755) with ball-milling for the mixture with MC. All of the $R_{\nu,1690}$ values in Fig. 5 increased in the first stage, while they stayed nearly constant after ball-milling for 10 h. The increase in $R_{1755,1690}$ indicates that the formation of hydrogen bonds of the C=O group in the 2-position with the OH groups of MC is not so predominant as the formation with those of MCC.

Table 1 presents the numerical values of $R_{\nu,1690}$ obtained by the use of a Nujol method. The band at 1640 cm^{-1} is the OH in-plane bending absorption of water adsorbed on diluents.[5,6] $R_{1640,1690}$ decreased upon ball-milling; this was probably a result of the squeezing

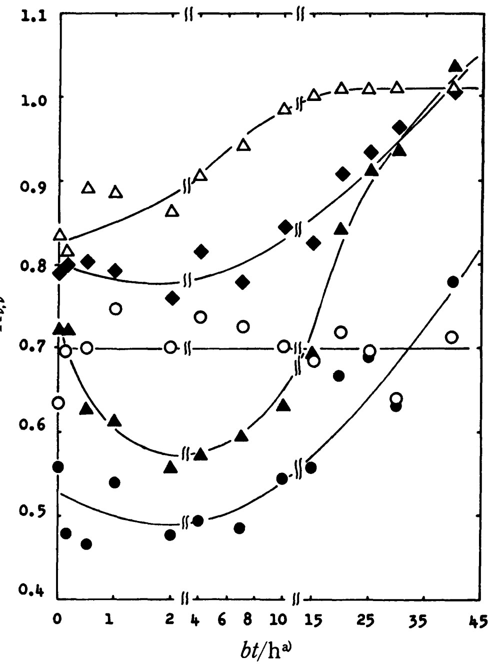

Fig. 4. Variation in $R_{\nu,\nu'}$ with ball-milling for the mixture with MCC containing 10 w/w % of amobarbital by a Nujol method.
ν'/cm^{-1}=1690, ν/cm^{-1}; ○: 1755, ●: 1735, △: 1720, ▲: 1710, ◆: 1700.
a) bt is the ball-milling time.

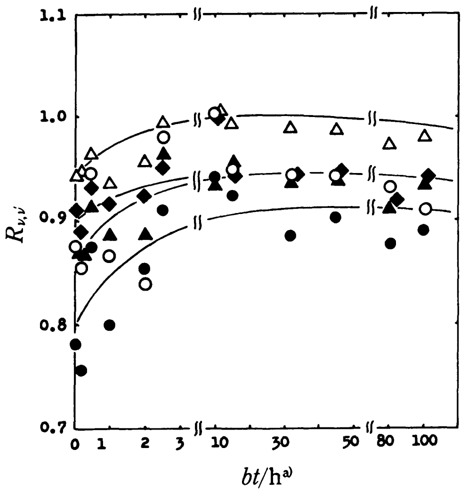

Fig. 5. Variation in $R_{\nu,\nu'}$ with ball-milling for the mixture with MC by a Nujol method.
ν'/cm^{-1}=1690, ν/cm^{-1}; ○: 1755, ●: 1735, △: 1720, ▲: 1710, ◆: 1700.
a) bt is the ball-milling time.

out of the adsorbed water caused by the formation of hydrogen bonds between the amobarbital and the diluents. It seemed probable from the change in $R_{1755,1690}$ that the absorbance at 1690 cm^{-1} after ball-milling was approximately 0.9 times as large as that before ball-milling for the mixture containing 30—40 w/w% of amobarbital. In this case, the $R_{3420,1690}$ value after ball-milling was 1.7—2.4 times as large as the value before ball-milling, though $R_{1640,1690}$ decreased upon ball-milling. The $R_{1110,1690}$ and $R_{1050,1690}$ values also doubled upon ball-milling. It is clear from these facts that the absorbances at 3420, 1110, and 1050 cm^{-1} increase upon ball-milling, though the variation in $R_{\nu,1690}$ for ν/cm^{-1} from 1450 to 1300 could not be discussed on the basis of a Nujol method. The band in the 1020—1060 cm^{-1} region has been suggested to be a skeletal vibration characteristic of the barbituric acid ring.[4] The skeletal vibration of amobarbital is probably affected by the change in the hydrogen bonds of the C=O groups. As is shown in Figs. 1, 2, 3, and Table 1, the absorbances of the NH stretching modes at 3200 and 3080 cm^{-1} and those of the NH out-of-plane deformation modes at 855 and 815 cm^{-1} decreased upon ball-milling, though the locations of these bands were not affected. The band of the stretching vibration of free NH has been reported to be observed in the 3300—3400 cm^{-1} region.[4] Probably, the increase in the absorbance at 3400 cm^{-1} is partly attributable to the free NH formed by the hydrogen bonding of the C=O groups with the OH groups of the diluents.

Effect of Dissolution on the Solid State of Amobarbital. A mixture with a water-soluble diluent containing approximately twice as much as the solubility of amobarbital was added to distilled water. After the suspension

Table 1. Numerical values of $R_{\nu,1690}$ for various kinds of mixtures (by a Nujol method)

Diluent	$CA^{a)}$ w/w%	$bt^{b)}$ h	ν/cm^{-1} 3420	1755	1735	1720	1710	1700	1640	1110	1050	850	815
Dextran	10	0$^{c)}$	1.04	0.67	0.56	0.83	0.71	0.85	0.78	0.64	0.61		
Dextran	10	60	1.40	0.81	0.75	1.04	0.89	0.93	0.57	0.91	0.92		
MCC	10	0$^{c)}$	1.16	0.67	0.56	0.85	0.72	0.81	0.81	0.74	0.73		
MCC	10	40	1.37	0.71	0.80	1.06	1.03	1.01	0.67	0.93	0.91		
MC	10	0$^{c)}$	1.00	0.79	0.70	0.93	0.74	0.84	0.78	0.81	0.70		
MC	10	60	1.19	1.00	1.01	1.10	0.98	1.02	0.54	1.04	1.01		
Dextran	30	0$^{c)}$	0.55	0.82	0.73	0.91	0.82	0.89	0.63	0.35	0.33	0.28	0.16
Dextran	30	203	0.95	0.92	0.82	0.98	0.86	0.91	0.45	0.61	0.65	0.27	0.13
MCC	30	0$^{c)}$	0.40	0.80	0.65	0.91	0.77	0.87	0.50	0.30	0.31	0.26	0.16
MCC	30	203	0.94	0.88	0.74	0.99	0.84	0.92	0.39	0.69	0.83	0.16	0.08
MC	40	0$^{c)}$	0.29	0.88	0.78	0.95	0.87	0.91	0.54	0.37	0.35	0.44	0.32
MC	40	101	0.70	0.91	0.89	0.98	0.93	0.94	0.33	0.73	0.75	0.33	0.23

a) CA is the content of amobarbital in the mixture. b) bt is the ball-milling time. c) A physical mixture of AB and the ball-milled diluent (Ten g of dextran or MCC was ball-milled for 200 h, and 5 g of MC was ball-milled for 100 h.)

had been allowed to stand for a week, the solid state of the amobarbital remaining undissolved was compared with the state before dissolution. As has been described previously, there are three polymorphs for amobarbital: Crystals I, II, and III. Crystal I was given by heating Crystal II (at 150 °C), which had been recrystallized from a 25 v/v% aqueous ethanol solution. Crystal III was given by freeze-drying an aqueous solution. It was also reported previously that AC and AB particles were composed of Crystal I, Crystal III and an amorphous part. The X-ray diffraction intensity at 12.7° of 2θ was lower than the intensity at 16.0° of 2θ in the diagram of Crystal I, while the opposite was observed in the diagram of Crystal III.[2] Figure 6 shows the X-ray-diffraction patterns of various kinds of amobarbital. Diffraction lines were not observed in the digram of dextran or MC. The crystallinity of amobarbital was determined by Ruland's method.[11] The ratio of the weight content of Crystal III to the content of Crystal I was determined from the ratio of the intensity of the diffraction line at 12.7° of 2θ to the intensity at 16.0° of 2θ.[2] The results of these determinations are shown in Table 2, where the MC content in MCABS was assumed to be 7.1 w/w% and where the contribution of MC to the scattering intensity was assumed to be identical with the contribution of the amorphous part of amobarbital. The contents of Crystal III in DexABS and MCABS were larger than those in ACS and ABS. It was noted previously that the $R_{3085,3020}$ and $R_{850,815}$ values for Crystal III were larger than those for Crystal I.[2] Table 3 presents the numerical values of $R_{\nu,\nu'}$ for various samples. The $R_{3085,3020}$ and $R_{850,815}$ values for DexABS and MCABS were larger than those for the other samples. This fact also shows that the content of Crystal III in DexABS and MCABS is larger than the content in the other samples. By the use of the solubility of amobarbital presented in Table 4, the solid state of the amobarbital remaining undissolved in the suspension was obtained on the basis of the following assumptions; (1) only a part of Crystal III or the amorphous part dissolved, and (2) no transformation occurred between Crystal I, Crystal III, and the amorphous state. A comparison of the results in Tables 2 and 4 demonstrates that Crystal III and the amorphous part are transformed into Crystal I by the dissolution of AB. The presence of dextran ball-milled with amobarbital is shown to prevent Crystal III from being transformed into Crystal I. The presence of MC ball-

TABLE 2. SOLID STATE OF AMOBARBITAL OBTAINED UPON VARIOUS KINDS OF PREPARATIONS

Sample	I[a)] w/w%	III[b)] w/w%	A_m[c)] w/w%
AC	76±4[d)]	11±1	13±3
ACS	74±3	14±1	12±4
AB	8±1	71±3	21±3
ABS	68±5	16±2	15±3
DexAB	6±1	34±6	60±5
DexABS	14±2	66±6	20±3
MCAB	3±1	25±5	73±12
MCABS	8±1	59±2	33±2

a) I: Crystal I. b) III: Crystal III .c) A_m: Amorphous part. d) The figure succeeding to ± is a standard deviation.

TABLE 3. NUMERICAL VALUES OF $R_{\nu,\nu'}$ FOR VARIOUS KINDS OF SAMPLES

Sample	ν/cm^{-1}	3085	1690	850
	ν'/cm^{-1}	3210	1750	815
ACS		1.07±0.01[a)]	1.16±0.05	0.94±0.07
ABS		1.08±0.01	1.27±0.03	1.07±0.03
DexABS		1.13±0.02	1.26±0.01	1.11±0.02
MCABS		1.12±0.04	1.22±0.09	1.28±0.05

a) The figure succeeding to ± is a standard deviation.

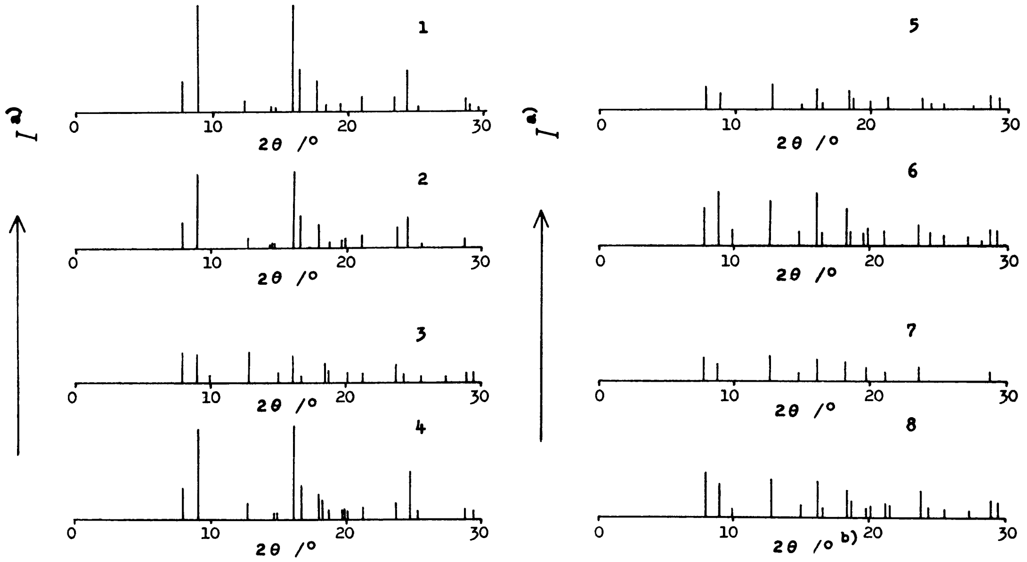

Fig. 6. X-Ray-diffraction patterns of various kinds of amobarbital.
1: AC, 2: ACS, 3: AB, 4: ABS, 5: DexAB, 6: DexABS, 7: MCAB, 8: MCABS.
a) I is the diffraction intensity. b) 2θ is the angle between the incident X-rays and the scattering X-rays.

Table 4. Solid state of amobarbital expected when only the part of crystal III or the amorphous part is assumed to be dissolved

Sample	Assumption		Expected crystal state		
	c a) mg(100 cm^3)$^{-1}$	Dissolved part	I b) w/w%	III c) w/w%	A_m d) w/w%
ABS	66	III	12	57	31
DexABS	66	A_m	9	54	37
MCABS	95	A_m	6	53	41

a) c is the solubility of amobarbital in distilled water. b) I: Crystal I. c) III: Crystal III. d) A_m: Amorphous part.

Table 5. Effect of ball-milling on the dissolution of amobarbital from various samples into a KH_2PO_4–Na_2HPO_4 buffer solution

Diluent	CA a) w/w%	bt b) h	c_{5min} mg(100 cm^3)$^{-1}$	c_{2h} mg(100 cm^3)$^{-1}$	c_e mg(100 cm^3)$^{-1}$
—	100	0		63.4±0.6 c)	61.5±1.2
—	100	60		65.7±1.1	63.0±1.3
Dextran	30	36	71.1±2.5		57.2±1.9
Dextran	30	200	62.8±2.5		58.3±1.9
MCC	10	20	87.4±2.6		59.6±1.7
MCC	10	40	76.1±2.3		62.9±1.7
MCC	30	200	80.0±2.1		59.7±2.2
MC	40	100		93.4±9.1	95.3±12.0

a) CA is the content of amobarbital in the mixture. b) bt is the ball-milling time. c) The figure succeeding to ± is a standard deviation.

milled with amobarbital is also shown to prevent the amorphous part from crystallization, as well as preventing Crystal III from being transformed into Crystal I.

Solubility of Amobarbital and the Stability of Its Amorphous State. Previously, a suspension of various kinds of samples containing amobarbital in a KH_2PO_4–Na_2HPO_4 buffer solution with a pH of 6.0 was shaken at 30 °C immediately after preparation, withdrawn from time to time, and filtered. Then the concentration of amobarbital in the filtrate was determined. In the case of amobarbital ball-milled with MC or without any diluent, the concentration increased with shaking and stayed nearly constant after 2 hs' shaking. The concentration of the mixture with dextran or MCC was at a maximum at the shaking time of 5 min. In Table 5 are summarized the results reported previously, where c_{5min} and c_{2h} are the concentrations after shaking for 5 min and for 2 h respectively, and where c_e is the concentration for the suspension allowed to stand at 30 °C for a week after shaking for 2 h. The concentrations increased more remarkably upon ball-milling with diluents than without any diluent. The c_{5min} values for the mixture with dextran were smaller than the values of c_{5min} or c_{2h} for the other mixtures. In the case of the mixture with MC, c_e was nearly identical with c_{2h}, while c_e was smaller than c_{5min} and approximately equal to the solubility of AC for the other mixtures. It was suggested that the increase in surface energy upon ball-milling was not the main reason for the increase in the solubility. Though the amorphous part seemed to control the solubility of amobarbital, the rate and extent of the increase in the amorphous part upon ball-milling were little influenced by the kind of diluent. After all, it was considered that the interaction of amobarbital with diluents resulting from ball-milling was one of the most important reasons for the increase in the solubility.1,2)

In the case of the mixture with MCC containing 10 w/w% of amobarbital, c_{5min} was at its maximum around a ball-milling time of 20—25 h.1) The decrease in c_{5min} after ball-milling for 25 h is probably to be attributed to the adsorption of amobarbital on MCC, as MCC is not soluble in distilled water. In the case of the mixture with MC, the variation in c_e with ball-milling was parallel to the variation in $R_{\nu,1690}$ in Fig. 5.1) This fact shows that the large value of c_e is attributable to hydrogen bonding between amobarbital and MC. In Figs. 2 and 3, the variation in the carbonyl groups of amobarbital upon ball-milling with dextran was not so remarkable as the variation with MC; this fact shows that the interaction of amobarbital with dextran is weaker than the interaction with MC. The above results show that the solubilization of amobarbital upon ball-milling is attributable to the stabilization of the amorphous state caused by the interaction with diluents.

The authors are grateful to the members of the Analytical Center of Showa University for their measurements of the IR spectra and for the elemental analysis.

References

1) A. Ikekawa, and S. Hayakawa, *Bull. Chem. Soc. Jpn.*, **55**, 1261 (1982).

2) A. Ikekawa and S. Hayakawa, *Bull. Chem. Soc. Jpn.*, **54**, 2587 (1981).

3) N. Kaneniwa, A. Ikekawa, and M. Sumi, *Chem. Pharm. Bull.*, **26**, 2734, 2744 (1978).

4) R. J. Mesley, *Spectrochim. Acta, Part A*, **26**, 1427 (1970).

B. L. Chang, N. O. Neussle, and W. G. Honey, Jr., *J. Pharm. Sci.*, **64**, 1787 (1975).

5) H. G. Higgins, G. M. Stewart, and K. J. Harrington, *J. Polym. Sci.*, **51**, 59 (1961).

6) Anton N. J. Heyn, *Biopolymers*, **13**, 475 (1974).

7) J. H. Keighley and S. N. Pandey, *J. Text. Inst.*, **1**, 23 (1976).

8) R. H. Marchessault and C. Y. Liang, *J. Polym. Sci.*, **43**, 71 (1960).

9) M. L. Nelson and R. T. O'Connor, *J. Appl. Polym. Sci.*, **8**, 1311 (1964).

10) B. M. Craven and E. A. Vizzini, *Acta Crystallogr., Part B*, **25**, 1993 (1969); J. Caillet and P. Claverie, *ibid.*, **36**, 2642 (1980).

11) W. Ruland, *Acta Crystallogr.*, **14**, 1180 (1961).