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Solubilization of Amobarbital by Mechanical Treatment in the Presence of Diluents

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Amobarbital was ball-milled, triturated, mortar-ground or stamp-milled in the absence or in the presence of various kinds of diluents, and the variation of solubility of amobarbital from these samples in the KH_2PO_4–Na_2HPO_4 buffer solution of pH of 6.0 at 30° was investigated.

It was suggested from the results of the elemental analysis, measurement of the mass spectra and the infrared spectra, and the chemical analysis by thin-layer chromatography and gas chromatography that amobarbital in the mechanically treated mixtures of precipitated silica or carbon black was hydrolyzed by the addition of water. It was also suggested that amobarbital decomposed or formed complex by ball-milling in the presence of potato starch. But, it was considered that decomposition of amobarbital by the mechanical treatment in the absence and in the presence of the other diluents was negligible.

Solubility of amobarbital in the buffer solution increased by mechanical treatment in the presence of methyl cellulose or hydroxypropyl cellulose. It was considered that solubilization of amobarbital was related to the decrease in the intensity of the signals of amobarbital in the X-ray diffraction diagrams and dropping of the melting point of amobarbital by mechanical treatment in the presence of the diluent. It was also considered that solubilization of amobarbital was due to the interaction with the diluent in the suspension caused by mechanical treatment and to the kind of the crystal structure of amobarbital formed by recrystalization after dissolution.

Keywords——amobarbital; diluents; methyl cellulose; ball-milling; trituration; stamp-milling; solubilization; the decrease in the intensity of the signals of amobarbital in the X-ray diffraction diagram; dropping of the melting point of amobarbital; interaction of amobarbital with the diluent caused by mechanical treatment

It was reported in the previous paper that the intensity of the signals of amobarbital in the X-ray diffraction diagrams decreased and that the melting point of amobarbital dropped by mechanical treatment in the presence of various kinds of the diluents.[2] In our another previous paper, an increase in solubility in distilled water was reported for the slightly soluble organic powders whose crystallinity changed by ball-milling in the presence of polyvinylpyrrolidone.[3] Nakai *et al.* also reported the same phenomenon by vibro-milling slightly soluble pharmaceutical powders in the presence of microcrystalline cellulose.[4]

In this paper, the variation of solubility of amobarbital in the KH_2PO_4–Na_2HPO_4 buffer solution by mechanical treatment in the presence of various kinds of diluents was investigated, and influence of the kind of the diluents and the mechanical treatment on the variation was discussed.

Experimental

The materials used and the way of the mechanical treatment were referred to the previous paper.[2]

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[1] Location: *Hatanodai, 1–5–8, Shinagawa, Tokyo.*

[2] N. Kaneniwa, A. Ikekawa, and M. Sumi, *Chem. Pharm. Bull.* (Tokyo), **26**, 2734 (1978).

[3] N. Kaneniwa and A. Ikekawa *Chem. Pharm. Bull.* (Tokyo), **23**, 2973 (1975).

[4] K. Yamamoto, M. Nakano, T. Arita, and Y. Nakai, *J. Pharmacokin. Biopharm.*, **2**, 487 (1974); K. Yamamoto, M. Nakano, T. Arita, Y. Takayama, and Y. Nakai, *J. Pharm. Sci.*, **65**, 1484 (1976); K. Yamamoto, S. Matsuda, M. Nakano, T. Arita, and Y. Nakai, *Yakugaku Zasshi*, **97**, 367 (1977); Y. Nakai, E. Fukuoka, S. Nakajima, and K. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **25**, 3340 (1977).

Immediately after the sample containing 60 mg of amobarbital was suspended in 30 ml of the KH_2PO_4–Na_2HPO_4 buffer solution (pH: 6.0, ionic strength: 0.08) at 30° in a flask in capacity of 100 ml, the flask was shaken twice per second in amplitude of 10 cm in a thermobath of 30°. After shaken for 2 hours, the flask was kept standing at 30° for more than a week. Approximately 1.5 ml of the suspension was pipetted at intervals and filtered through a milipore filter of 0.22 μ (GSWPO 1300) and absorbance of the filtrate at 238 nm was measured with a Hitachi Perkin Elmer spectrophotometer (Model 139), in order to obtain the concentration of amobarbital in the filtrate.

The freeze-dried powders of the samples were prepared as follows. The filtrates of the aqueous suspensions of original amobarbital or amobarbital mechanically treated in the absence of the diluent were freeze-dried. The mechanically treated mixtures were dialysed through the cellulose tubing for a week, and the solution out of the tubing was also freeze-dried.

The powders were obtained by drying in vacuum the filtrates of the ethanol suspensions of the mechanically treated mixtures of precipitated silica or carbon black. These powders are called PEE in this paper.

Thin-layer chromatography (TLC) was carried out on precoated TLC plates of silica gel 60F-254 with a solvent system of isopropanol–chloroform–25% ammonium hydroxide (45: 45: 10).[5] The spot was visualized under the short wave length UV by a Toshiba PAN UV lamp. Gas chromatography of the acetone solutions of the samples was carried out with a Shimadzu gas chromatograph (Model GC-4CM PF). Mass spectra of the samples were measured by a Hitachi mass spectrometer (Model RMS-4). Infrared (IR) spectra of the KBr tablets of the samples were measured by a JASCO diffraction granting infrared spectrometer (Model (IRA-2).

Influence of the amount of the diluent in the buffer solution on dissolution of amobarbital from the mixture was investigated as follows. The suspensions of various amounts of the mixtures in 5 ml of the buffer solution were dialysed through the cellulose tubing for more than a week until the equilibrated state was obtained, and the absorbance of the solution out of the tubing at 238 nm was measured. The total volume of the buffer solution in and out of the tubing was 15 ml.

Results and Discussion

1. Dissolution of Amobarbital in a KH_2PO_4–Na_2HPO_4 Buffer Solution from Various Kinds of the Samples

In Fig. 1, 2 and 3, ΔOD is the difference of the absorbance of the filtrate of the suspension of the mixture in the buffer solution at 238 nm to the absorbance for the diluent. It is considered from Fig. 1, 2 and 3 that dissolution of amobarbital is characterized by the values of ΔOD after shaken for 5 minutes, 2 hours and after kept at 30° for a week, $a_{5\text{min}}$, $a_{2\text{hr}}$ and a_e, respectively.

Influence of the mechanical treatment on dissolution of amobarbital is shown by comparing the values of ΔOD for the suspension of original amobarbital or the physical mixtures with the values for the mechanically treated samples. The results are summarized in Table I. The suspension of the ball-milled mixture of potato starch containing 10 w/w % of amobarbital was too viscous to be filtered. The absorbance of the solution of polyvinylpyrrolidone K90 (PVP K90) at 238 nm was too large for the value of ΔOD for the suspension of the mixture to be obtained. The values of a_e for these mixtures in Table I are the values for the solutions obtained by dialysis of the suspensions of these mixtures in the buffer solution through the cellulose tubing for a week.

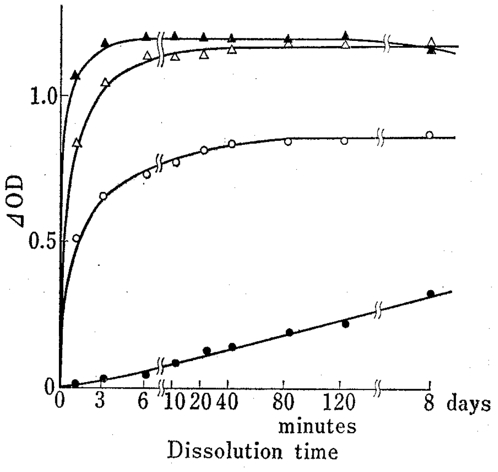

Fig. 1. Dissolution of Amobarbital in the KH_2PO_4–Na_2HPO_4 Buffer Solution (pH; 6.0) from Various Samples

Dissolution temperature; 30°
△, original amobarbital.
▲, amobarbital ball-milled for 100 hours(W_s=10 g).
○, physical mixture of amobarbital and ethyl cellulose in the ratio of 1: 9 by weight.
●, the mixture of amobarbital and ethyl cellulose in the ratio of 1: 9 by weight ball-milled for 60 hours (W_s=10 g).
W_s; the weight amount of the mechanically treated sample.

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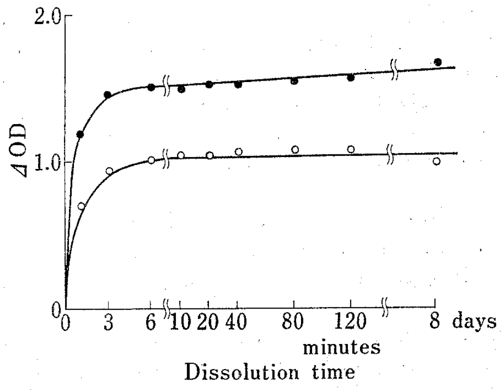

Fig. 2. Dissolution of Amobarbital in the KH_2PO_4–Na_2HPO_4 Buffer Solution (pH; 6.0) from the Mixture of Precipitated Silica

Dissolution temperature; 30°
Content of amobarbital; 10 w/w %
○, physical mixture.
●, the mixture ball-milled for 60 hours (W_s=10 g).

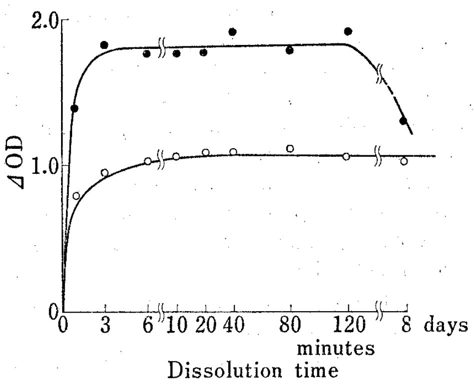

Fig. 3. Dissolution of Amobarbital in the KH_2PO_4–Na_2HPO_4 Buffer Solution (pH; 6.0) from the Mixture of PVA 2000

Dissolution temperature; 30°
Content of amobarbital; 10 w/w %
○, physical mixture.
●, the mixture ball-milled for 60 hours (W_s=10 g).

The following four types were observed between the mechanically treated samples in Table I, concerning the variation of ⊿OD with the time, where a_m was the value of a_e for the physical mixture.

(1) a_{5min}, a_{2hr}, $a_e > a_m$

(2) The values of a_{5min} and a_{2hr} are larger than a_m, but ⊿OD decreases with the lapse of the time.

(3) Apparent difference was not observed between the values of a_{5min}, a_{2hr} and a_e for the mechanically treated samples and those for original amobarbital or the physical mixtures.

(4) a_{5min}, a_{2hr}, $a_e < a_m$

Amobarbital stamp-milled in the absence of the diluent belonged to type (1), while amobarbital ball-milled, triturated or mortar-ground in the absence of the diluent belonged to type (3). The mechanically treated mixtures of carbon black, methyl cellulose (MC) or hydroxypropyl cellulose (HPC) belonged to type (1), and those of the granules of activated charcoal or ethyl cellulose belonged to type (4), independent of the kind of the mechanical treatment. On the contrary, the triturated mixtures of microcrystalline cellulose (MCC), polyvinyl alcohol 2000 (PVA 2000), acacia powders, dextran or dextrin belonged to type (2), but their stamp-milled mixtures belonged to type (1).

The values of a_{5min}, a_{2hr} and a_e for the six kinds of the ball-milled mixtures in Table I were compared with the values for the physical mixture of original or the ball-milled amobarbital and original or the ball-milled diluents. As shown in Table II, the values for the ball-milled mixture of agar powders and those for the physical mixture of the ball-milled amobarbital and the ball-milled agar were both large, though the former belonged to type (2) and the latter belonged to type (1). This fact may be due to the chemical structure of agar partially changed by ball-milling.[6] But the values for the other ball-milled mixtures were different from the values for their physical mixtures, which were identical with one another. It was considered from the results in Table II that the difference of the values of a_{5min}, a_{2hr} and a_e for the ball-milled mixtures from those for the physical mixtures in Table I was caused by the mechanical treatment of amobarbital in the presence of the diluent.

Table III shows the influence of the content of amobarbital in the ball-milled mixture on its dissolution in the buffer solution. The values of ⊿OD for the mixture of precipitated silica containing 80 w/w % of amobarbital were smaller than the values for the physical

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6) N. Kaneniwa and A. Ikekawa, *Chem. Pharm. Bull.* (Tokyo), **22**, 2990 (1974).

TABLE I. Numerical Values of $a_{5\mathrm{min}}$, $a_{2\mathrm{hr}}$ and a_{e} for Amobarbital (A) mechanically Treated in the Absence and in the Presence of Various Kinds of Diluents

Diluent	Content of A (w/w %)	Ball-milling (a_{m}, $a_{5\mathrm{min}}$, $a_{2\mathrm{hr}}$, a_{e})	Trituration ($a_{5\mathrm{min}}$, $a_{2\mathrm{hr}}$, a_{e})	Mortar-grinding ($a_{5\mathrm{min}}$, $a_{2\mathrm{hr}}$, a_{e})	Stamp-milling ($a_{5\mathrm{min}}$, $a_{2\mathrm{hr}}$, a_{e})
— (Absent)	100	(1.18, 1.20, 1.21, 1.16)	(1.06, 1.16, 1.16)	(1.08, 1.17, 1.10)	(1.02, 1.36, 1.38)
Precipitated silica	10	(0.99, 1.50, 1.57, 1.68)	(2.28, 2.44, 2.24)	(1.13, 1.18, 1.11)	(1.47, 1.57, 1.15)
Carbon black	10	(1.05, 1.31, 1.95, 2.44)	(1.37, 1.87, 2.20)	(1.49, 1.58, 1.47)	(0.79, 1.04, 1.38)
Sodium chloride	10	(0.67, 0.83, 0.79, 0.86)	(0.91, 1.07, 0.83)	(1.01, 0.87, 0.97)	(0.69, 0.71, 0.78)
Methyl cellulose	30	(1.21, 1.96, 1.85, 1.75)	(1.56, 1.55, 2.25)	(1.56, 1.63, 1.82)	(1.09, 1.61, 1.40)
HPC, 3—6 cps	30	(1.10, 1.33, 1.41, 1.37)		(0.59, 2.35, 2.15)	(1.16, 1.37, 1.21)
HPC, 1000—4000 cps	30		(0.99, 1.76, 1.63)		
MCC	10	(1.03, 1.28, 1.24, 1.23)	(1.39, 1.19, 1.13)	(1.15, 1.10, 1.04)	(1.56, 1.73, 1.77)
PVA 2000	10	(1.01, 1.76, 1.92, 1.30)	(1.71, 1.45, 1.36)	(1.19, 1.34, 1.30)	(1.13, 1.30, 1.22)
Potato starch	10	(1.09 4.22)	(1.17)	(0.21, 1.34, 1.18)	(1.04)
Potato starch	30	(1.09, 1.37, 1.20, 1.01)	(1.02, 1.07, 0.97)		
Acacia powders	10	(0.57, 2.03, 2.33, 0.94)		(0.75, 0.79, 0.70)	(0.54, 1.06, 1.60)
Acacia powders	30	(0.53, 0.74, 0.39, 0.34)	(1.10, 1.39, 1.13)		
Sodium alginate	30	(1.04, 1.24, 0.69, 0.87)	(1.07, 1.10, 0.99)	(0.33, 0.50, 0.43)	(0.50, 1.14, 1.36)
Agar powders	10	(1.10, 1.98, 1.75, 1.50)	(0.93, 0.96, 0.75)	(0 0.19, 0.92)	(1.20, 1.18, 1.02)
Dextran 2000	10	(1.07, 1.16, 1.08, 1.19)	(1.32, 1.23, 1.14)		
Dextran 10	10			(0.96, 1.07, 1.02)	(1.55, 2.05, 2.56)
Dextrin	10	(1.11, 1.18, 1.19, 0.89)	(1.36, 1.07, 1.13)	(1.24, 1.24, 1.04)	(1.29, 1.55, 1.73)
PEG 6000	10	(1.11, 1.16, 1.20, 1.14)	(1.23, 1.18, 0.98)	(1.16, 1.29, 1.27)	(1.01, 1.22, 0.50)
Lactose	10	(1.08, 0.99, 1.04, 1.03)	(1.22, 1.25, 1.08)	(0.98, 1.01, 0.87)	(1.00, 1.02, 0.80)
Anhydrous dextrose	10	(1.00, 1.05, 1.11, 0.97)	(1.24, 1.12, 0.90)	(1.01, 0.96, 0.83)	(0.74, 1.04, 0.46)
Granules of activated charcoal	10	(0.49, 0 0 0)	(0.02, 0.02, 0.03)	(0 0 0)	(0 0.02, 0.16)
Ethyl cellulose	10	(0.87, 0.04, 0.22, 0.33)	(0.82, 0.88, 0.55)	(0.65, 0.80, 0.51)	(0.44, 0.91, 0.70)
Stylene polymer	10	(1.05, 0.03, 0.22, 0.40)	(1.37, 1.39, 1.25)	(0.17, 0.01, 0.17)	(1.16, 1.37, 1.21)
PVP K90	10	(1.05 0.12)	(0.53)		(0.95)

Table II. Influence of Ball-Milling in the Presence of the Diluents on Solubilization of Amobarbital (A)

Diluent	Kind of A	Content of A (w/w %)	Type of the mixture	($a_{5\text{min}}$, $a_{2\text{hr}}$, a_e)
Precipitated silica		10	Ball-milled	(1.50, 1.57, 1.68)
Precipitated silica	Original	10	Physical	(1.02, 1.08, 0.99)
b-Precipitated silica[a]	Original	10	Physical	(0.84, 0.92, 0.82)
b-Precipitated silica	Ball-milled	10	Physical	(0.99, 0.94, 0.85)
Carbon black		10	Ball-milled	(1.31, 1.95, 2.44)
Carbon black	Original	10	Physical	(0.77, 1.01, 1.05)
b-Carbon black	Original	10	Physical	(0.75, 0.92, 1.12)
b-Carbon black	Ball-milled	10	Physical	(0.84, 0.97, 1.10)
Methyl cellulose		30	Ball-milled	(1.99, 1.86, 1.79)
Methyl cellulose	Original	30	Physical	(0.96, 1.16, 1.16)
b-Methyl cellulose	Original	30	Physical	(0.83, 0.97, 1.10)
b-Methyl cellulose	Ball-milled	30	Physical	(1.09, 1.12, 1.23)
Agar powders		10	Ball-milled	(2.00, 1.79, 1.50)
Agar powders	Original	10	Physical	(0.90, 1.07, 1.11)
b-Agar powders	Original	10	Physical	(1.16, 1.42, 1.20)
b-Agar powders	Ball-milled	10	Physical	(1.72, 1.79, 1.72)
Ethyl cellulose		10	Ball-milled	(0.04, 0.22, 0.33)
Ethyl cellulose	Original	10	Physical	(0.73, 0.84, 0.87)
b-Ethyl cellulose	Original	10	Physical	(0.96, 0.93, 0.85)
b-Ethyl cellulose	Ball-milled	10	Physical	(1.06, 1.06, 0.88)
Stylene polymer		10	Ball-milled	(0.03, 0.22, 0.40)
Stylene polymer	Original	10	Physical	(1.10, 1.13, 1.16)
b-Stylene polymer	Original	10	Physical	(0.90, 1.00, 0.93)
b-Stylene polymer	Ball-milled	10	Physical	(1.08, 1.08, 0.96)

a) For example, b-precipitated silica means ball-milled precipitated silica. Carbon black was ball-milled for 100 hours, and the other diluents were ball-milled for 60 hours. (W_s=10 g).

Table III. Influence of the Content of Amobarbital (A) in the Ball-Milled Mixture of the Diluent (D) on Dissolution in KH_2PO_4–Na_2HPO_4 Buffer Solution (pH; 6.0)

Diluent	Amount of D (g)	Amount of A (g)	Ball-milling time (hr)	($a_{5\text{min}}$, $a_{2\text{hr}}$, a_e)
Precipitated silica	9	1	65	(1.50, 1.57, 1.68)
	8	2	61	(1.31, 1.23, 1.13)
	3	2	32	(1.30, 1.10, 1.21)
	2	3	32	(1.38, 1.38, 1.23)
	1	4	32	(0.57, 0.60, 0.72)
Methyl cellulose	7	3	63	(1.99, 1.86, 1.79)
	3	2	32	(1.72, 1.92, 2.22)
	2	3	32	(1.69, 1.69, 2.13)
	1	4	32	(1.60, 1.62, 1.72)
	1	4	0	(0.80, 0.97, 1.18)
Microcrystalline cellulose	9	1	61	(1.28, 1.24, 1.23)
	4	1	30	(1.40, 1.31, 1.25)
	3	2	33	(1.52, 1.50, 1.22)
	2	3	33	(1.48, 1.34, 1.36)
	1	4	30	(1.33, 1.38, 1.25)
Agar powders	9	1	61	(2.00, 1.79, 1.50)
	4	1	31	(1.61, 1.60, 1.42)
	3	2	31	(1.35, 1.22, 1.18)
	2	3	31	(1.28, 1.28, 1.31)
	1	4	31	(1.15, 1.24, 1.25)
Ethyl cellulose	9	1	62	(0.04, 0.22, 0.33)
	8	2	64	(0.16, 0.50, 1.21)
	6	4	60	(0.39, 0.75, 1.06)
	4	6	61	(0.52, 1.03, 1.21)
	2	8	61	(0.53, 1.15, 1.29)

mixture. But the values for the mixtures containing less than 60 w/w % of amobarbital were larger than the values for the physical mixture. The difference between the values for the ball-milled mixture and those for the physical mixture was remarkable when the weight content of amobarbital was small, and this tendency was also observed for the mixtures of methyl cellulose or ethyl cellulose. The ball-milled mixture of microcrystalline cellulose containing small amount of amobarbital belonged to type (1), and the mixture containing large amount of amobarbital belonged to type (2). The values of ΔOD for the physical mixtures were influenced little by the content of amobarbital.

2. Chemical Analysis of Amobarbital in the Mechanically Treated Mixtures

Elemental analysis, measurement of mass spectra and IR spectra were made for the freeze-dried powders of the mechanically treated mixtures of polymers or the inorganic diluents whose values of ΔOD were remarkably different from the values for the physical mixtures, and these powders were analysed by thin-layer chromatography and gas chromatography.

2.1. Elemental Analysis——The diluent seemed to be mixed in the freeze-dried powders for the mechanically treated mixtures of organic polymers. Then, elemental analysis was made for original amobarbital, amobarbital mechanically treated in the absence of the diluent or in the presence of carbon black or precipitated silica. As shown in Table IV, the results for the elemental analysis were influenced little by mechanical treatment in the absence of the diluent or by freeze-drying. The content of N, C and H for amobarbital stamp-milled in the absence of the diluent was a little smaller than the content for the original amobarbital, but the content for the freeze-dried powders was identical with the content for the original amobarbital. This fact is probably due to the contamination by very small amount of iron from the mill. The values of the content of N, C and H for the freeze-dried powders of the mechanically treated mixtures of precipitated silica or carbon black were much smaller than the values for the original amobarbital, but the values for the powders obtained by drying in vacuum the filtrates of the ethanol suspensions of their mechanically treated mixtures (PEE) were identical with the values for the original amobarbital.

TABLE IV. Elemental Analysis of Amobarbital (A) prepared by Various Kinds of Treatment

Diluent	Content of A (w/w %)	Treatment	Type[a]	N (w/w %)	C (w/w %)	H (w/w %)
—	100	(Original)	Powder	12.45	58.38	8.23
—	100	(Original)	H_2O	12.40	58.48	8.06
—	100	Ball-milling	Powder	12.52	58.70	8.49
—	100	Ball-milling	H_2O	12.00	56.75	7.85
—	100	Trituration	Powder	12.33	57.91	8.38
—	100	Trituration	H_2O	12.26	57.76	7.98
—	100	Mortar-grinding	Powder	12.51	58.63	8.42
—	100	Stamp-milling	Powder	11.52	53.43	7.68
—	100	Stamp-milling	H_2O	12.17	58.30	7.86
Carbon black	10	Ball-milling	H_2O	10.47	41.73	6.07
Carbon black	10	Ball-milling	EtOH	12.03	57.25	7.83
Precipitated silica	10	Ball-milling	EtOH	12.12	58.07	8.06
Precipitated silica	20	Ball-milling	H_2O	10.67	50.43	7.33
Precipitated silica	10	Trituration	H_2O	9.33	46.11	6.40

a) powder; original or mechanically treated powders.
H_2O; the freeze-dried powders of the samples.
EtOH; the powders obtained by drying in vacuum the filtrate of the suspension of the sample in ethanol (PEE).

2.2. Analysis by Thin-Layer Chromatography and Gas Chromatography——In Table V are shown the results of the thin-layer chromatography. One spot of the same *Rf* value as that of original amobarbital was obtained for the filtrates of the ethanol suspensions of the mechanically treated mixtures of precipitated silica or carbon black and for the ethanol solutions of their PEE. But, in addition to this spot, another spot of the *Rf* value of zero was obtained for the aqueous ethanol solution of their freeze-dried powders, or the filtrates of the aqueous ethanol suspensions of these mixtures.

The *Rf* value of the spot for the freeze-dried powders of the ball-milled mixture of potato starch containing 10 w/w % of amobarbital was smaller than the value for original amobarbital, though the values for the freeze-dried powders of their triturated and stamp-milled mixtures were identical with the value for original amobarbital.

The *Rf* value of the spot for the freeze-dried powders of the ball-milled mixture of HPC was identical with the value for original amobarbital. But, in case of their triturated and stamp-milled mixtures, more than two spots were observed, and the *Rf* value of one spot was smaller and the values for the other spots were larger than the value for original amobarbital. The *Rf* value of the former spot was low in the presence of water and large amount of HPC. The spots of high *Rf* value were large and strong for the filtrates of the suspensions of the mechanically treated mixtures, and these spots were not observed for the aqueous ethanol suspension of the freeze-dried powders of the triturated mixture. Probably, the spots of a large *Rf* value are contributed to HPC, and the spot of a low *Rf* value is due to the interaction between HPC and amobarbital. The similar phenomena were also observed for the mixtures of methyl cellulose.

TABLE V. Thin-Layer Chromatography of Amobarbital (A) prepared by Various Kinds of Treatment

Diluent	Content of A (w/w %)	Treatment	Type[a]	Solvent[b]	*Rf* value
—	100	(Original)	Powder	EtOH–H_2O	0.68—0.73
Precipitated silica	10	Ball-milling	H_2O	EtOH–H_2O	0.67, 0
Precipitated silica	10	Ball-milling	EtOH	EtOH	0.68
Precipitated silica	10	Trituration	H_2O	EtOH	0
Precipitated silica	10	Trituration	Powder	EtOH	0.67
Carbon black	10	Ball-milling	H_2O	EtOH–H_2O	0.70, 0
Carbon black	10	Ball-milling	EtOH	EtOH	0.70
Carbon black	10	Trituration	Powder	H_2O	0
Carbon black	10	Trituration	Powder	EtOH	0.73
Carbon black	10	Stamp-milling	Powder	EtOH–H_2O	0.67, (0)[c]
Potato starch	10	Ball-milling	H_2O	EtOH–H_2O	0.46
Potato starch	10	Ball-milling	Powder	EtOH	0.50
Methyl cellulose	30	Ball-milling	H_2O	EtOH–H_2O	0.70
Methyl cellulose	30	Trituration	H_2O	EtOH–H_2O	0.64
Methyl cellulose	30	Trituration	H_2O	EtOH	0.73
Methyl cellulose	30	Trituration	Powder	EtOH	0.86 (large spot), 0.70 (small spot)
Methyl cellulose	30	Stamp-milling	H_2O	EtOH–H_2O	0.65—0.54 (tailing)
Methyl cellulose	30	Stamp-milling	H_2O	EtOH	0.71
HPC, 3—6 cps	30	Ball-milling	H_2O	EtOH–H_2O	0.70
HPC, 3—6 cps	30	Trituration	H_2O	EtOH–H_2O	0.52, 0.48
HPC, 3—6 cps	30	Trituration	H_2O	EtOH	0.92, 0.68
HPC, 3—6 cps	30	Trituration	Powder	EtOH	0.88, 0.85, 0.81, 0.49
HPC, 3—6 cps	30	Stamp-milling	H_2O	EtOH	0.93, 0.60
HPC, 3—6 cps	30	Stamp-milling	Powder	EtOH	0.92, 0.47

a) Powder; original or mechanically treated powders.
H_2O; the freeze-dried powders of the samples.
EtOH; the powders obtained by drying in vacuum the filtrate of the suspension of the sample in ethanol (PEE).
b) The filtrate of the suspension of the sample in ethanol or the aqueous ethanol solution was separated.
c) A faint spot of *Rf* value of zero seemed to be observed.
Solvent system; The mixture of isopropyl alcohol, chloroform and the 25% aqueous solution of ammonium hydroxide in the ratio of 9: 9: 2 by volume.

An indistinct spot of *Rf* value of zero seemed to be observed for the aqueous ethanol solutions of the freeze-dried powders of the stamp-milled mixture of dextran 10 or dextrin, which may be due to the diluents mixing in the freeze-dried powders.

Figure 4 shows the results of the analysis of amobarbital in the ball-milled mixtures of carbon black by gas chromatography. The filtrates of the acetone solutions of their freeze-

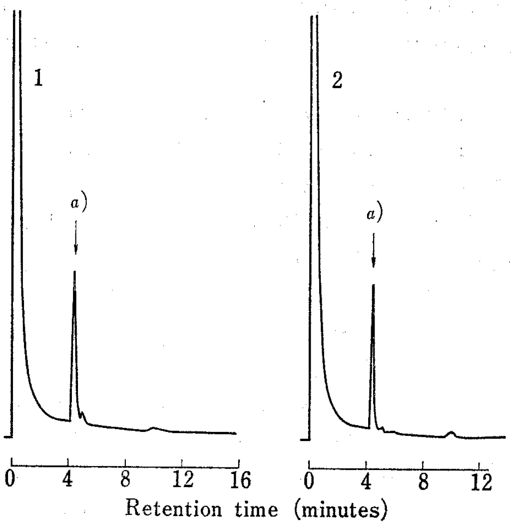

Fig. 4. Gas Chromatography of Amobarbital Ball-Milled in the Presence of Carbon Black

Ball-milling condition; $W_s=10$g, Content of amobarbital: 10 w/w %, Ball-milling time: 100 hours.

1, acetone solution of the powders obtained by drying in vacuum the filtrate of the suspension of the ball-milled mixture in ethanol (PEE).

2, the filtrate of the acetone suspension of the freeze-dried powders (A large amount of the powder was not dissolved in acetone).

a) The retention time for the signal with an arrow is identical with the time for original amobarbital.

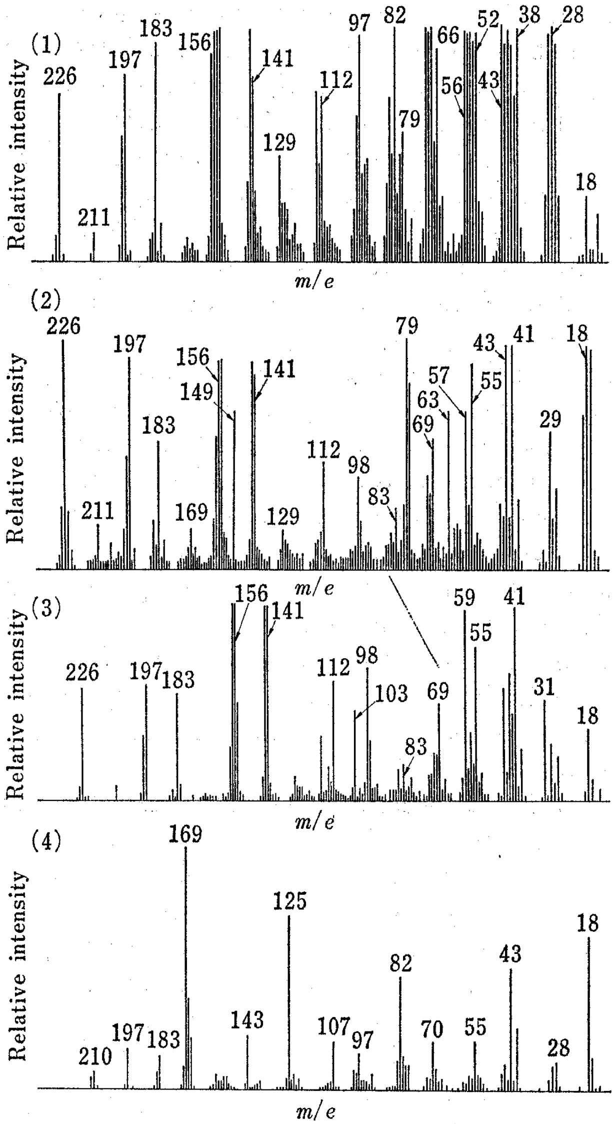

Fig. 5. Influence of Mechanical Treatment in the Presence of Diluents on Mass Spectra of Amobarbital (A)

Samples; freeze-dried powders of the samples

	Diluent	Content of A (w/w %)	Treatment
(1),	—	100	— (original amobarbital)
(2),	carbon black	10	ball-milling for 100 hours ($W_s=10$ g)
(3),	HPC, 3—6 cps	30	trituration ($W_s=3$ g)
(4),	potato starch	10	ball-milling for 60 hours ($W_s=10$ g)

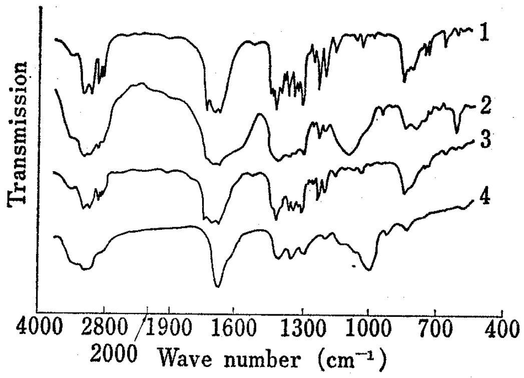

Fig. 6. IR Spectra of Amobarbital extracted from the Ball-Milled Mixtures

1, original amobarbital.

2, the freeze-dried powders of the mixture of carbon black ball-milled for 100 hours.

3, powders obtained by drying in vacuum the filtrate of the suspension of the mixture of carbon black ball-milled for 100 hours in ethanol (PEE).

4, the freeze-dried powders of the mixture of potato starch ball-milled for 60 hours (content of amobarbital; 10 w/w %).

dried powders were analysed, as a large part of the powders was not soluble in acetone. In addition to the large signal at the same retention time as for the original amobarbital, small signals were observed at the retention times different from the time for original amobarbital. The similar phenomenon was also observed for the mixtures of precipitated silica. The ratio of the retention time for the signal of the freeze-dried powders of the ball-milled mixture of potato starch containing 10 w/w % of amobarbital to the time for original amobarbital was 0.78. The retention time for the signals of the freeze-dried powders of the other mixtures was identical with the time for original amobarbital.

2.3. Mass Spectra——In the mass spectrum of the freeze-dried powders of the ball-milled mixture of carbon black, new signals were observed at *m/e* of 149 and at *m/e* larger than the molecular weight of amobarbital of 226, and the intensity of the signals at *m/e* of 57, 63 and 79 was much larger than the intensity for original amobarbital, as shown in Fig. 5. But the spectrum of PEE was identical with the spectrum for original amobarbital. The similar phenomenon was also observed for the mixtures of precipitated silica.

New signals were observed at *m/e* of 107, 125, 143 and 169, and the signals of amobarbital at *m/e* of 112, 129, 141, 156 and 226 were not observed in the spectrum of the freeze-dried powders of the ball-milled mixture of potato starch. In the spectrum of the freeze-dried powders of the triturated mixture of HPC, new signals were observed at *m/e* of 31, 59, 98, 103 and at *m/e* larger than 226, and the intensity of the signal at *m/e* of 82 was smaller than the intensity for original amobarbital. Signals were also observed at *m/e* larger than 226 for the spectra of the powders and the freeze-dried powders of amobarbital triturated in the absence of the diluent, the freeze-dried powders of the ball-milled mixture of PVA, the stamp-milled mixture of methyl cellulose and the triturated mixtures of the diluents other than PVA. But signals were not observed at *m/e* larger than 226 for the spectra of the diluents. Signals were observed in the ESR spectra of most of the triturated powders, though signals were not observed for the spectra of most of the stamp-milled and the ball-milled powders.[2] This fact may be related to the fact that signals were observed at *m/e* larger than 226 in the mass spectra of most of the triturated mixtures, but not in the spectra of most of the stamp-milled and the ball-milled mixtures.

The mass spectra of the other samples were identical with the spectrum of the original amobarbital.

2.4. IR Spectra of the Freeze-Dried Powders of Various Samples——As shown in Fig. 6, the IR spectrum of the freeze-dried powders of the ball-milled mixture of carbon black was different from the spectrum of the original amobarbital, but the spectrum of the PEE was nearly identical with the spectrum of the original amobarbital. The similar phenomenon was also observed for the mixtures of precipitated silica. It is considered from the findings mentioned above that amobarbital in the mechanically treated mixtures of carbon black or precipitated silica is hydrolyzed by the addition of water, though details are not investigated in this paper. Many studies were made on the hydrolysis of barbituric acid derivatives in the aqueous solutions.[7]

In the spectrum of the freeze-dried powders of the ball-milled mixture of potato starch, the carbonyl absorption at 1750 cm^{-1} was not observed, a new strong signal was observed at 1000 cm^{-1} region and the absorption band around 1300—1500 cm^{-1} region was different from the band for the original amobarbital. Mesley found that the carbonyl absorption around 1750 cm^{-1} was not observed and that two strong signals were observed at the region of 1700—1600 cm^{-1} in the spectra of the sodium salts of phenobarbital and considered this fact to be due to the enolization of the 2-carbonyl groups.[8] It was considered from the results of the elemental analysis that a relatively large amount of potato starch was mixed

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[7] E.R. Garrett, J.T. Bojarski, and G.J. Yakatan, *J. Pharm. Sci.*, **60**, 1145 (1971); L.A. Gardner and J.E. Goyan, *J. Pharm. Sci.*, **62**, 1027 (1973).

[8] R.J. Mesley, *Spectrochimica Acta.*, **26A**, 1427 (1970).

in the freeze-dried powders. In case of ball-milling the mixture of potato starch containing 10 w/w % of amobarbital, the following two possibilities were suggested from the above facts; amobarbital decomposed, or amobarbital formed complex with potato starch by ball-milling.

In the spectra of the freeze-dried powders of original amobarbital, the signal at 3200 cm^{-1} seemed to shift slightly to the low frequency, the carbonyl absorption at 1750 cm^{-1} disappeared, and broad absorption bands were observed at the region of 1690—1720 cm^{-1} and around 830 cm^{-1}. The similar phenomenon was also observed for the freeze-dried powders of the ball-milled mixtures of the diluents other than agar powders, and amobarbital stamp-milled in the absence of the diluents or in the presence of methyl cellulose or dextrin. In the spectra of the freeze-dried powders of amobarbital ball-milled in the absence of the diluents or in the presence of agar, stamp-milled in the presence of MCC or stylene polymer, or triturated in the absence or in the presence of the diluents, the intensity of the signal at 3200 cm^{-1} was a little larger than the intensity at 3080 cm^{-1}, and the signal at 850 cm^{-1} shifted slightly to the low frequency, but the carbonyl absorption around 1600—1800 cm^{-1} was identical with the absorption for the original amobarbital (Fig. 7).

Mesley found two major differences in the spectra of phenobarbitone IV, V and VI and nealbarbitone from the other barbiturates: the peak near 3200 cm^{-1} was significantly stronger than the peak at 3100 cm^{-1}, the latter being shifted from its usual position of 3080 cm^{-1}, though the two bands of the other barbiturates were of approximately equal intensity, and the highest frequency carbonyl absorption band around 1750 cm^{-1} was missing, leaving a single broad band centered around 1705 cm^{-1}. Mesley considered this finding to be due to hydrogen-bonding of the 2-carbonyl group with an NH group, presumably at the expence of either the 4- or 6-carbonyl group. Mesley also observed two signals at 850 cm^{-1} and 815 cm^{-1} in the spectra of

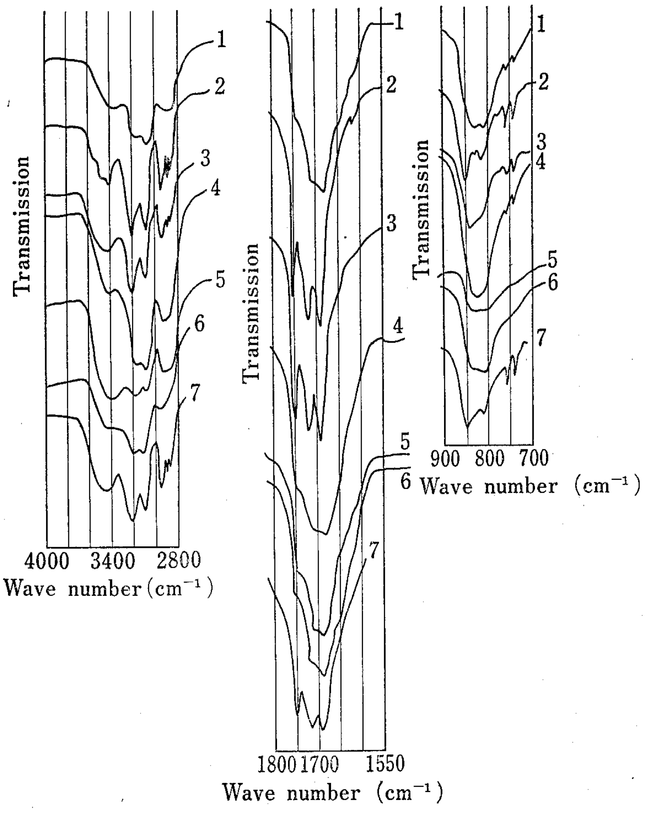

Fig. 7. IR Spectra of the Freeze-Dried Powders of Amobarbital mechanically treated in the Absence and in the Presence of Methyl Cellulose

	Diluent	Mechanical treatment	Weight amount of amobarbital (g)	Weight amount of diluent (g)
1,	absent	—	—	—
2,	absent	100 hours ball-milling	10	0
3,	absent	trituration	3	0
4,	absent	30 minutes stamp-milling	5	0
5,	methyl cellulose	60 hours ball-milling	3	7
6,	methyl cellulose	trituration	0.3	2.7
7,	methyl cellulose	30 minutes stamp-milling	0.5	4.5

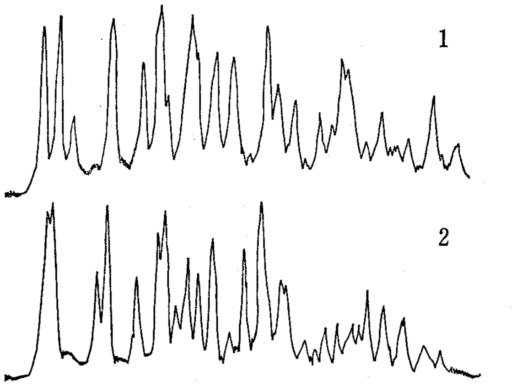

Fig. 8. X-Ray Diffraction Diagrams of Stamp-Milled Amobarbital

1, amobarbital stamp-milled for 30 minutes ($W_s=5$ g).
2, the freeze-dried powders of amobarbital stamp-milled for 30 minutes ($W_s=5$ g).

amobarbital I, and 841 cm^{-1} and 822 cm^{-1} in the spectra of amobarbital II, and found that the band near 1220 cm^{-1} was split in type II, but not in type I.[8)]

The X-ray diffraction diagram of amobarbital stamp-milled in the absence of the diluents was different from the diagram of the freeze-dried powders, as shown in Fig. 8. This finding is probably due to the difference in the crystal structure of amobarbital. Then, amobarbital in the structure of the stamp-milled powders is called type I, and amobarbital in the structure of the freeze-dried powders is called type II in this paper, though these types may be the mixtures of the powders of various kinds of the crystal structures. Original amobarbital and amobarbital mechanically treated in the absence or in the presence of the diluents were type I, when signals were observed in the X-ray diffraction diagram. The freeze-dried powders of the ball-milled mixture of methyl cellulose and all of the triturated mixtures tested were type I, and those of the other samples were type II. It is considered from this fact that the results in Fig. 7 is due to the change in the crystal structures and not to the change in the chemical structure of amobarbital. Type II in this work is different from type II of amobarbital reported by Mesley, from the absorption around 1220 cm^{-1} in the IR spectra.[8)] As shown in Table VI, the melting point of type II of amobarbital by DTA was 152—153°, and the solubility in the buffer solution was larger than the solubility of type I by approximately 20%.

Table VI. Melting Points and Solubility of the Freeze-Dried Powders of Various Kinds of the Samples in the KH_2PO_4–Na_2HPO_4 Buffer Solution

Dialysed sample Diluent	Dialysed sample Content of A[a)] (w/w %)	Treatment	mp by DTA (°)	($a_{5\text{min}}$, $a_{2\text{hr}}$, a_e)
—	100	(Original)	$T_1=152$	(1.30, 1.30, 1.13)
—	100	Ball-milling	$T_1=153$	
—	100	Trituration	$T_1=157$	
—	100	Stamp-milling	$T_1=152$	
Methyl cellulose	30	Ball-milling	$T_1=142$, $T_2=113$	(0.75, 1.57, 1.53)
HPC, 3—6 cps	30	Ball-milling	$T_1=143$, $T_2=141$, $T_3=126$	
MCC	10	Ball-milling	$T_1=144$	
PVA 2000	10	Ball-milling	$T_1=146$, $T_2=145$, $T_3=116$	
Acacia powders	10	Ball-milling	$T_1=152$	
Sodium alginate	30	Ball-milling	$T_1=151$	

a) A; amobarbital.

The mass spectra of the freeze-dried powders of the triturated mixture of HPC were different from the spectrum of original amobarbital. The *Rf* values of the spots for the triturated and the stamp-milled mixtures of HPC or methyl cellulose were identical with the value for the original amobarbital in one condition and different in another condition, in the analysis by the thin-layer chromatography. One signal was observed in the analysis of the freeze-dried powders of these samples by gas chromatography, and the retention time for the signal was identical with the time for the original amobarbital. It is probable from the above facts that interaction causes between amobarbital and HPC or methyl cellulose by mechanical treatment, which may be attributed to the signals at *m/e* larger than 226 in the mass spectra of the freeze-dried powders of their triturated mixtures. Probably, decomposition of amobarbital by mechanical treatment in the presence of the diluents other than precipitated silica, carbon black or potato starch is negligible, and the difference of the value of ΔOD for their mechanically treated mixtures from the values for their physical mixtures is due to the change in the solubility of amobarbital in the buffer solution.

3. Relation between the Decrease in Crystallinity and Variation in Solubility of Amobarbital in the Buffer Solution by Ball-Milling

No signals were observed in the X-ray diffraction diagram and in the DTA thermogram of the mixture of precipitated silica ball-milled for 2 hours, but ΔOD increased gradually by ball-milling (Fig. 9). The similar tendency was also observed for the mixture of carbon black. The signal of amobarbital in the DTA thermogram disappeared, and the intensity of the signal in the X-ray diffraction diagram decreased, but ΔOD was influenced slightly at the first stage, and ΔOD began to decrease after the intensity of the signal in the X-ray diffraction diagram decreased remarkably by ball-milling in the presence of ethyl cellulose or the granules of activated charcoal (Fig. 10). In contrast with this finding, the intensity of the signal of amobarbital in the X-ray diffraction diagram decreased, the melting point of amobarbital by DTA lowered and ΔOD increased gradually by ball-milling in the presence of methyl cellulose, and the similar tendency was also observed for the mixture of PVA 2000 (Fig. 11, 12 and 13). The mixture of methyl cellulose containing 40 w/w % of amobarbital belonged to type (2) at the first stage, and to type (1) at the last stage of ball-milling, concerning the values of ΔOD.

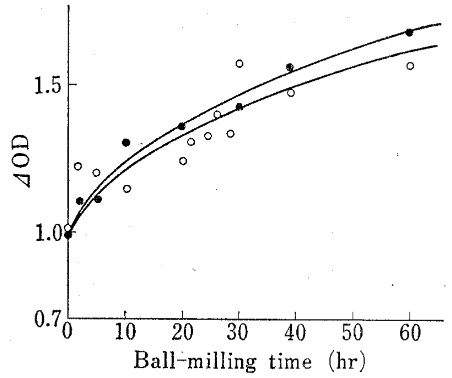

Fig. 9. Variation in Dissolution of Amobarbital in the KH_2PO_4–Na_2HPO_4 Buffer Solution (pH; 6.0) from the Mixture of Precipitated Silica with Ball-Milling Time

Dissolution temperature; 30°.
Dissolution time
○, 2 hours.
●, 1 week.
Content of amobarbital; 10 w/w %, W_s=10 g.

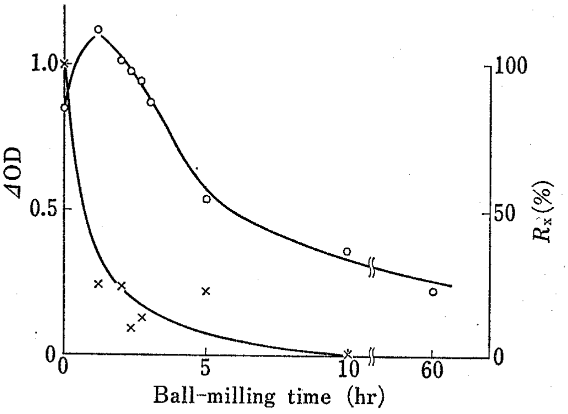

Fig. 10. Variation in Crystallinity and Dissolution of Amobarbital in the KH_2PO_4–Na_2HPO_4 Buffer Solution (pH; 6.0) from the Mixture of Ethyl Cellulose with Ball-Milling Time

Content of amobarbital; 10 w/w %, W_s=10 g.
○, ΔOD for the suspension after shaken for 2 hours at 30°.
×, R_x.

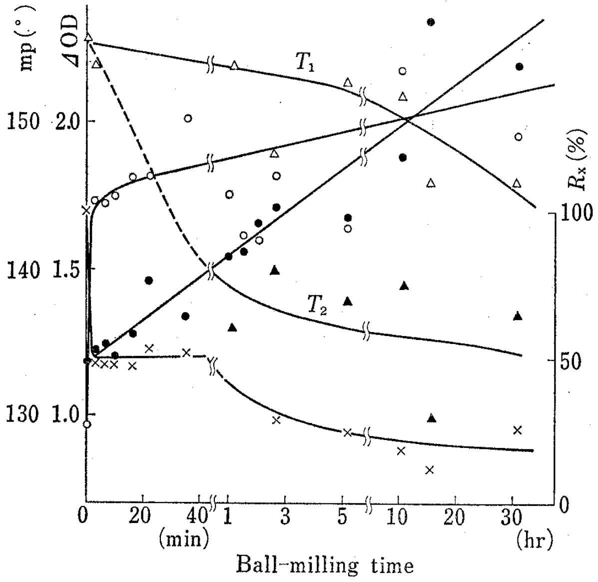

Fig. 11. Variation in Crystallinity and Dissolution of Amobarbital in the KH_2PO_4–Na_2HPO_4 Buffer Solution (pH; 6.0) from the Mixture of Methyl Cellulose with Ball-Milling Time

Content of amobarbital; 40 w/w %, W_s=5 g.
○, ΔOD for the suspension after shaken for 2 hours at 30°.
●, ΔOD for the suspension after kept at 30° for a week.
×, R_x.
△, mp T_1 by DTA.
▲, mp T_2 by DTA.

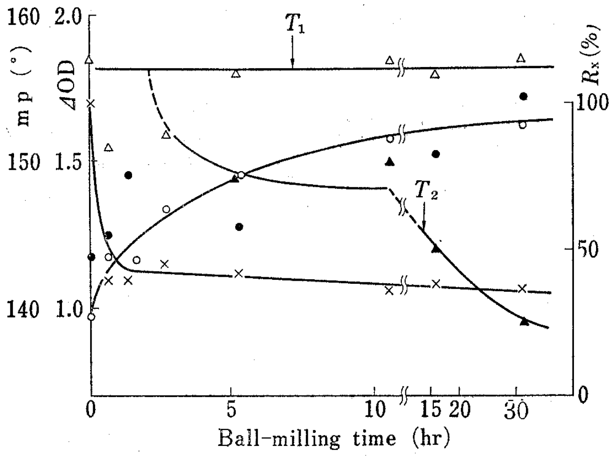

Fig. 12. Variation in Crystallinity and Dissolution of Amobarbital in the KH_2PO_4–Na_2HPO_4 Buffer Solution (pH; 6.0) from the Mixture of Methyl Cellulose with Ball-Milling Time

Content of amobarbital; 80 w/w %, W_s=5 g.
○, ΔOD for the suspension after shaken for 2 hours at 30°.
●, ΔOD for the suspension after kept at 30° for a week.
×, R_x.
△, mp T_1 by DTA.
▲, mp T_2 by DTA.

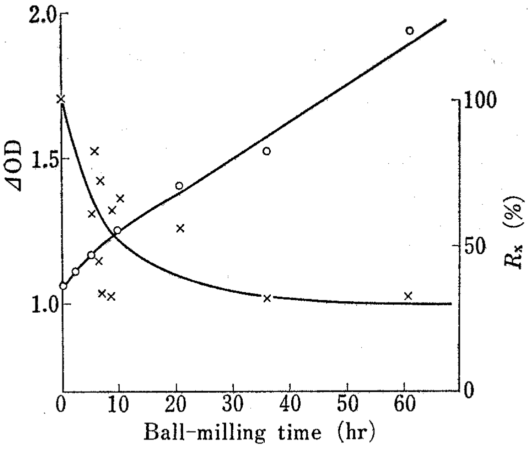

Fig. 13. Variation in Crystallinity and Dissolution of Amobarbital in the KH_2PO_4–Na_2HPO_4 Buffer Solution (pH; 6.0) from the Mixture of PVA 2000 with Ball-Milling Time

Content of amobarbital; 10 w/w %, W_s=10 g.
○, ΔOD for the suspension after shaken for 2 hours at 30°.
×, R_x.

4. Interaction of Amobarbital with the Diluents in the Suspension of the KH_2PO_4-Na_2HPO_4 Buffer Solution

Fig. 14 shows the influence of the amount of the diluent in the buffer solution on dissolution of amobarbital from the mixture. The values of ΔOD were independent of the amount of the diluent for the physical mixtures of MCC, dextrin, dextran 10, agar powders or stylene polymers. The values of ΔOD increased slightly with the increase in the amount of the diluent for the physical mixtures of HPC, PVA 2000 or PVP K90, and the value decreased for the mixture of acacia powders. The mixture of methyl cellulose containing 40 w/w % of amobarbital ball-milled for 10 minutes belongs to type (2), and the mixture ball-milled for 30 hours belongs to type (1), concerning the values of ΔOD. The values of ΔOD for the mixture ball-milled for 10 minutes were independent of the amount of methyl cellulose, but the values for the mixture ball-milled for 30 hours increased with the increase in the amount of the methyl cellulose. The value of ΔOD decreased with the increase in the amount of the diluent for the ball-milled mixtures of acacia powders, PVA 2000 or HPC. The values of ΔOD increased with the increase in the amount of the diluent for the stamp-milled mixtures, and this tendency was more remarkable than the tendency for the physical mixtures. Probably, amobarbital interacts with the diluent in the suspension in these cases. But, ΔOD was independent of the amount of the diluent for the triturated mixtures. This finding may be related to the fact that many kinds of the triturated mixtures belong to type (2) and that many kinds of the stamp-milled mixtures belong to type (1), concerning ΔOD.

Amobarbital stamp-milled in the absence of the diluent belonged to type (1), concerning ΔOD. In the previous paper, it was suggested from the scanning electron microscopic photographs that a disturbed layer was formed in the surface of the particles of amobarbital by stamp-milling in the absence of the diluent.2) It is probable from these facts that solubilization of amobarbital in the buffer solution by stamp-milling in the absence of the diluents is attributed to the disturbed layer formed in the surface of the particles.

It is suggested from the above findings that solubilization of amobarbital by mechanical treatment in the presence of the diluent is related to the decrease in the intensity of the signals of amobarbital in the X-ray diffraction diagrams and dropping of the melting point

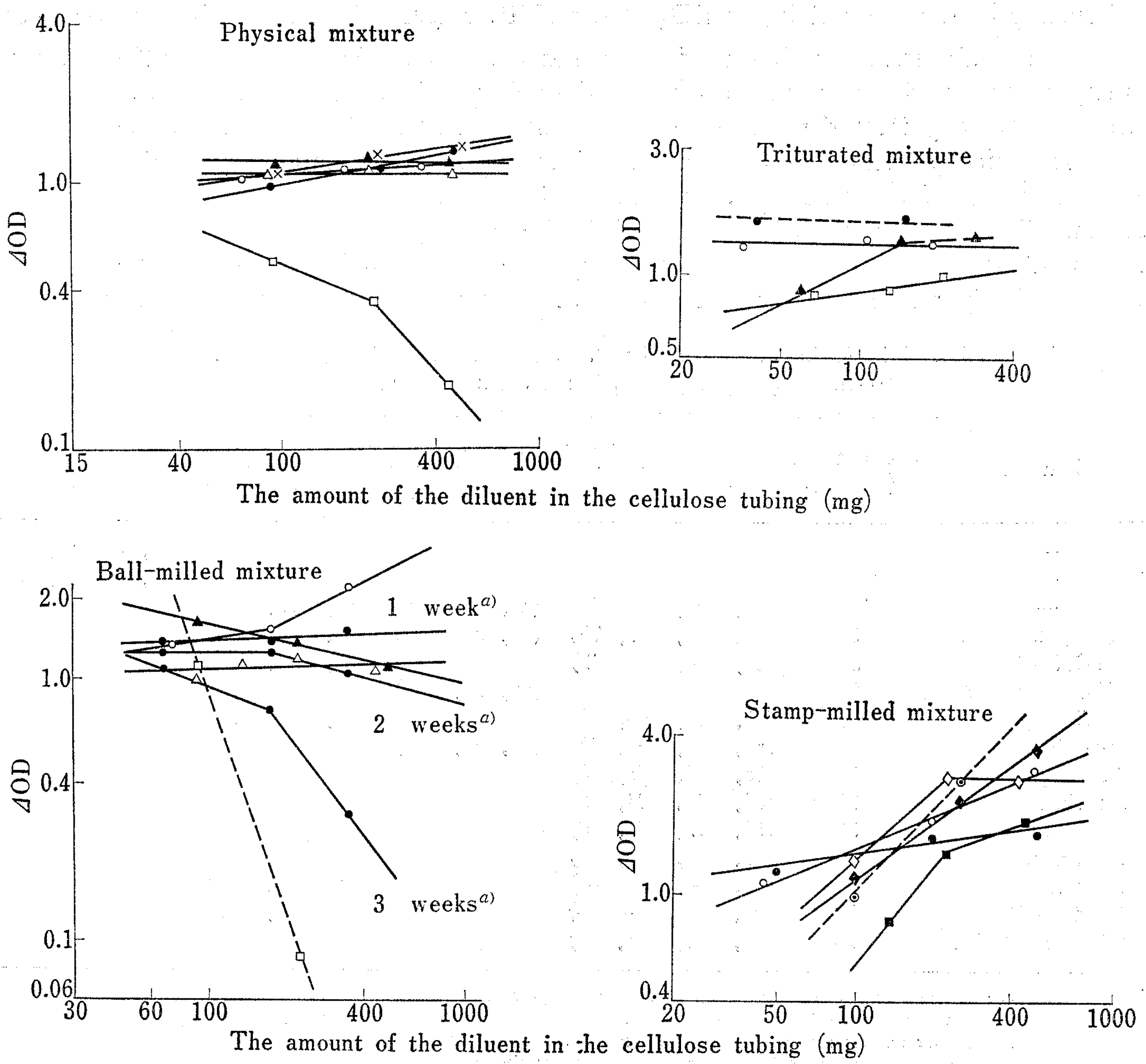

Fig. 14. Influence of the Amount of the Diluent on Dialysis of Amobarbital in the Suspension of Their Mixture in the KH_2PO_4-Na_2HPO_4 Buffer Solution (pH; 6.0) through the Seamless Cellulose Tubing

Total volume of the buffer; 15 ml.
The equilibrated value of ΔOD of the solution out of the tubing was measured after dialysis for more than a week.

Diluent	Content of amobarbital (w/w %)	Diluent	Content of amobarbital (w/w %)
○, methyl cellulose	30	×, PVP K90	10
●, HPC, 3—6 cps	30	⊙, MCC	10
▲, PVA 2000	10	◇, dextrin	10
□, acacia powders	10	◆, dextran 10	10
△, agar powders	10	■, stylene polymer	10

a) The results after 1, 2, and 3 weeks of dialysis, respectively. For the other cases, the results were the same after dialysis of more than 10 days.

of amobarbital, which are probably due to the formation of the particles of amobarbital smaller than 0.1 μ in diameter, the coating films of amobarbital covering the particles of the diluent and thinner than 0.1 μ in thickness, or formation of the disturbed layer in the surface of the particles of amobarbital in diameter larger than 0.1 μ.[2] It is also suggested that the high value of a_e for the mechanically treated mixtures are due to the interaction between amobarbital and the diluent in the suspension or the kind of the crystal structure of amobarbital formed by recrystallization after dissolution.

5. Solubility of Amobarbital in the KH_2PO_4–Na_2HPO_4 Buffer Solution from Various Samples

The molar absorption coefficient of original amobarbital at 238 nm in the buffer solution was expected to be different from the value of amobarbital in the filtrate of the suspension of the mechanically treated mixture because of the interaction between them. Then, each of the mechanically treated mixtures was dissolved in twice the volume of the buffer solution necessary for all of the amobarbital in the mixture to dissolve, and the relation between ΔOD and the concentration of amobarbital in the solution was obtained for each of the samples. And, using these relations, solubility of amobarbital in the buffer solution from the mechanically treated mixture was obtained. The results are shown in Table VII.

TABLE VII. Dissolution of Amobarbital (A) from the mechanically Treated Mixtures into the KH_2PO_4–Na_2HPO_4 Buffer Solution (pH; 6.0) at 30°

Diluent	Content of A (w/w %)	$(c_m, c_{5min}, c_{2hr}, c_e)^{a)}$ or $(c_{5min}, c_{2hr}, c_e)^{a)}$			
		Ball-milling	Trituration	Stamp-milling	No treatment
—	100				(60, 62, 62)
—	100				(74, 74, 64)[b]
—	100	(62, 69, 69, 66)	(61, 66, 66)	(61, 69, 70)	
Methyl cellulose	30	(69, 83, 79, 75)	(89, 89, 131)	(62, 90, 85)	
HPC, 3—6 cps	30	(61, 66, 70, 68)		(76, 85, 78)	
HPC, 1000—4000 cps	30		(50, 95, 85)		
MCC	10	(61, 63, 63, 61)		(87, 95, 97)	
PVA 2000	10	(62, 85, 93, 65)	(99, 99, 95)		
Acacia powders	10	(35, 120, 139, 53)			
Acacia powders	30	(32, 41, 21, 18)			
Sodium alginate	30	(60, 72, 39, 49)			
Agar powders	10	(63, 79, 70, 60)			
Dextrin	10	(63, — — —)		(76, 87, 97)	
Dextran 10	10	(61, — — —)		(90, 120, 152)	
Sodium chloride	10	(40, 47, 45, 49)			

a) c_m; concentration of amobarbital in the filtrate of the suspension of original amobarbital or the physical mixture after keeping at 30° for a week (mg/100 ml).
c_{5min}, c_{2hr}, c_e; concentration of amobarbital in the filtrate of the suspension of the sample after shaking for 5 min, 2 hr, and after keeping at 30° for a week, respectively (mg/100 ml).
b) the value for the freeze-dried powders of the original amobarbital.

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