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Ammonia Adducts of Chloramphenicol and Chloramphenicol Palmitate and Their Application to Particle Size Reduction[1]

YASUYUKI TSUDA, MOTOKO KANKE,[2a] ISAO MIYACHI,[2b]
KAORU MAENO, and KEIJI SEKIGUCHI[2a]

School of Pharmaceutical Sciences, Kitasato University[2a]
and Seikagaku Kogyo, Co., Ltd.[2b]

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It was confirmed that chloramphenicol and chloramphenicol palmitate form NH_3 adducts with combining ratios of 1:2 (chloramphenicol: NH_3), and 1:2 and 1:1 (chloramphenicol palmitate: NH_3). The thermal and physico-chemical properties of their NH_3 adducts and the recovered chemicals were investigated by differential scanning calorimetry, infrared spectroscopy, X-ray diffractometry, and scanning electron microscopy. Elimination of NH_3 from the adducts of chloramphenicol and chloramphenicol palmitate proceeded readily at normal temperatures and pressure to yield very fine particles of the original chemicals. Further, the effect of repeating the process of sorption and desorption of NH_3 on particle size was investigated in the case of chloramphenicol palmitate by the gas adsorption method. Two or more repetitions of the process produced a significant increase in specific surface area.

Keywords——chloramphenicol; chloramphenicol palmitate; ammonia adduct; particle size reduction; thermal behavior of ammonia adduct; physico-chemical properties of ammonia adduct; ammonia sorption and desorption

The formation of NH_3 adducts of several sulfonamides and barbiturates and the application of these adducts to particle size reduction by desorption of NH_3 have been reported.[3] The solvate formation of chloramphenicol with pyridine and picoline was also confirmed and the thermal properties of the solvates were studied.[4] The particle size of chloramphenicol recovered from its pyridine solvate became much smaller than in the original commercial product pulverized mechanically.[4]

Several preparation procedures for microcrystalline chloramphenicol[5a] as well as chloramphenicol palmitate[5b] have been patented recently. However, some kinds of surfactants or suspending agents, such as hydrophilic high polymers may remain in the final products.

On the other hand, the existence of various polymorphs of chloramphenicol palmitate is well-known, and differences in the absorption of various polymorphic forms following oral administration were reported.[6]

In the present study, chloramphenicol and chloramphenicol palmitate were investigated to determine whether they could form NH_3 adducts. The thermal and physico-chemical

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[1] This forms Part IX of "Studies on Methods of Particle Size Reduction of Medicinal Compounds." Part VIII: E. Suzuki, K. Shirotani, Y. Tsuda, and K. Sekiguchi, *Chem. Pharm. Bull.*, 27, 1214 (1979). A preliminary communication has appeared in *Chem. Pharm. Bull.*, 25, 2470 (1977).

[2] Location: *a) 9-1 Shirokane 5-Chome, Minato-ku, Tokyo, 108, Japan*; *b) 1253 Tatsuno 3-Chome, Higashi-Yamato Shi, Tokyo, 189, Japan.*

[3] K. Sekiguchi, Y. Tsuda, and M. Kanke, *Chem. Pharm. Bull.*, 22, 2972 (1974); K. Sekiguchi, Y. Tsuda, M. Kanke, E. Suzuki, and M. Iwatsuru, *ibid.*, 26, 1279 (1978).

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properties of the NH_3 adducts and recovered chemicals were investigated by differential scanning calorimetry, infrared spectroscopy, X-ray powder diffractometry, and scanning electron microscopy. The specific surface areas of the powders recovered were also measured by a gas adsorption method.

Experimental

Materials——Chloramphenicol (reagent grade from Boehringer Mannheim GmbH) and chloramphenicol palmitate polymorphs A and B (supplied by Sankyo Co., Ltd.) were used. Compressed liquid NH_3 was employed as a source of NH_3.

Preparation of NH_3 Adducts——The NH_3 adduct of chloramphenicol was prepared as described in the previous paper,[3] that is, it was crystallized from an NH_3 solution of chloramphenicol. Chloramphenicol palmitate adducts were prepared not by dissolution but by suspending chloramphenicol palmitate in liquid NH_3 for a few hours.

Differential Scanning Calorimetry (DSC)——A Perkin-Elmer DSC-1B differential scanning calorimeter was used. In the case of the palmitate adducts, measurements were done with the cryogenic cover filled with acetone-dry ice. Evolved gas detection (EGD) was carried out simultaneously and dissociated NH_3 gas was identified with Nessler's reagent solution, if necessary.

Measurement of Weight Decrease——A chemical balance (type 2462, Sartorius) in a cold room maintained at $2 \pm 1°$ was used to obtain weight decrease curves for NH_3 adducts.

Infrared Spectroscopy (IR)——IR spectra (nujol mull method) were determined with a Jasco IRA-1 grating infrared spectrophotometer.

X-Ray Powder Diffractometry——A JDX-7F X-ray diffraction analyzer made by Japan Electron Optics Laboratory Co., Ltd. was used (Ni filter, Cu-Kα ray $\lambda = 1.542$ Å). Sample powders were held at low temperature, if necessary, by cooling the sample chamber with dry ice.

Scanning Electron Microscopy (SEM)——Particle appearances were observed with a scanning electron microscope (Hitachi-Akashi model MSM-4).

Measurement of Specific Surface Area——A BET gas adsorption apparatus (model P-600, Shibata Chemical Apparatus Mfg. Co., Ltd.) was used. A sample weight of 1—2 g was taken with N_2 gas as the adsorbate.

Results and Discussion

NH_3 Adduct of Chloramphenicol

Using the crystals obtained from liquid NH_3, NH_3 adduct formation of chloramphenicol was confirmed by means of X-ray powder diffractometry, DSC, and isothermal weight decrease measurement.

1) **X-Ray Powder Diffractometry**——X-Ray powder diffraction patterns of chloramphenicol and its supposed NH_3 adduct are shown in Fig. 1(a) and (b), respectively. The pattern (b), obtained while holding the freshly made adduct crystals at a nearly constant temperature of 0°, is different from pattern (a), obtained with the commercial product. However, when the measurement was repeated once or twice with the same samples, the pattern became identical with that for the commercial chloramphenicol. Therefore, the NH_3-treated product is either a different molecular species or possibly another polymorphic form, but is so unstable that it readily changes to ordinary chloramphenicol even at a temperature of about 0°.

2) **Determination of the Combining Ratio**——As the NH_3 adduct is so unstable, its combining ratio could not be determined by conventional thermal gravimetry. Instead, it was determined from a weight decrease curve obtained by placing freshly prepared crystals wetted with excess NH_3 on a chemical balance at $2 \pm 1°$, as shown in Fig. 2. Since free NH_3 would be eliminated rapidly, the break at A in the curve should represent the net weight of the supposed adduct. The subsequent gradual decrease and the final weight equilibrium can be reasonably ascribed to desorption of combined NH_3 and the weight of recovered chloramphenicol, respectively. Thus it is clear that not a polymorph but an NH_3 adduct having a combining ratio of 1:2 (chloramphenicol: NH_3=1:1.98) is formed by recrystallizing chloramphenicol from NH_3 solution.

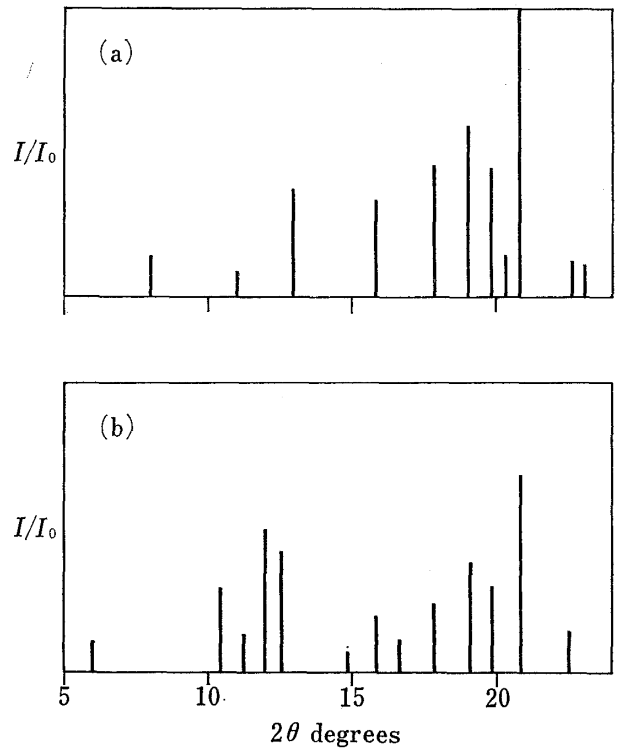

Fig. 1. X-ray Diffraction Patterns for Chloramphenicol and Its NH_3 Adduct (2θ scan speed: 4°/min)

(a) Commercial chloramphenicol.
(b) Chloramphenicol NH_3 (1: 2) adduct.

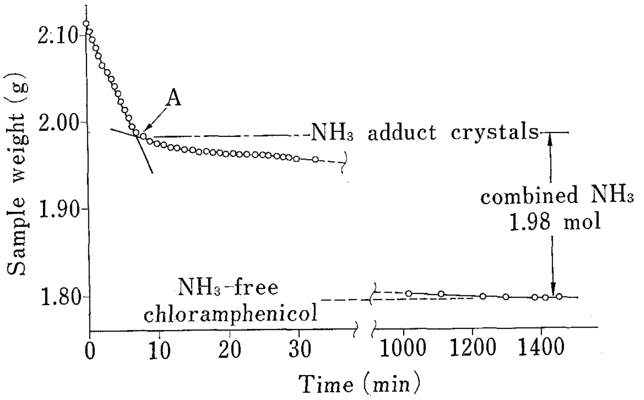

Fig. 2. Weight Decrease Curve of Chloramphenicol NH_3 (1: 2) Adduct obtained on a Chemical Balance at 2±1°

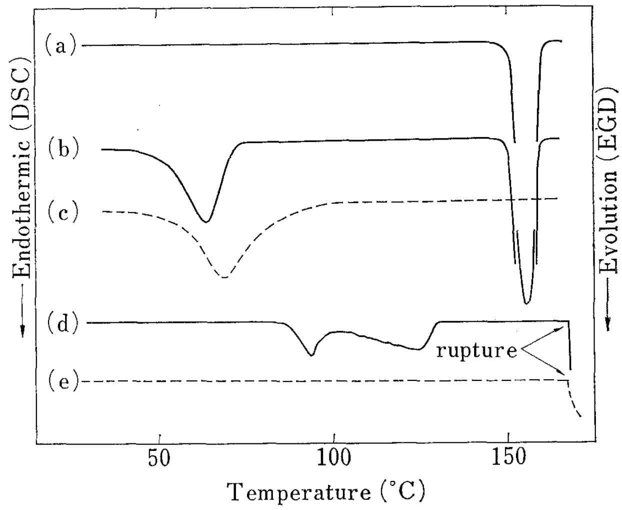

Fig. 3. Thermograms of Chloramphenicol and Its 1: 2 NH_3 Adduct

(a) DSC curve of chloramphenicol under semi-closed conditions: sample weight, 16.85 mg; heating rate, 16°/min.
(b) and (c) Simultaneous DSC and EGD curves of the 1: 2 NH_3 adduct under semi-closed conditions: sample weight, 18.81 mg; heating rate, 16°/min.
(d) and (e) Simultaneous DSC and EGD curves of the 1: 2 NH_3 adduct under closed conditions: sample weight, 6.56 mg; heating rate, 8°/min.

3) **Thermal Behavior**——The thermograms of chloramphenicol and its NH_3 adduct are shown in Fig. 3. In the DSC curve of the adduct under semi-closed conditions, endothermic peaks at 50—70° and at around 150° always appeared (Fig. 3(b)). The latter peak, due to the melting of NH_3-free chloramphenicol, indicates that chloramphenicol was recovered by thermal decomposition of the adduct on heating, and the former one is attributable to the heat effect of NH_3 desorption from the adduct on the basis of simultaneous EGD measurement.

On the other hand, two successive endothermic peaks appeared between 85° and 130° under closed conditions, as shown by curve (d). In the closed heating system, evaporation of NH_3 was restricted inside a container, unless the container was ruptured by the pressure increase; therefore, the change in the sample composition would be small. The endothermic peak at 85—100° is probably attributable to liquefaction caused by a peritectic or eutectic reaction, while a broad endothermic peak at about 100—130° should represent melting either of NH_3-free chloramphenicol or of the adduct; this was lowered by the coexistence of the liquid derived from the adduct. Thus, it can be reasonably estimated that NH_3 desorption from the adduct on heating under semi-closed conditions occurred in a solid-vapor phase reaction. It was also confirmed that no adduct with a ratio other than 1: 2 nor any intermediate adduct in the process of desorption from the 1: 2 adduct was formed.

NH_3 Adducts of Chloramphenicol Palmitate

Although chloramphenicol palmitate did not dissolve in liquid NH_3, two kinds of NH_3 adducts were successfully formed by suspending chloramphenicol palmitate crystals in liquid NH_3. These adducts were identified from the following experimental results.

1) **X-Ray Diffractometry and Determination of the Combining Ratio**——Irrespective of the polymorphic form of chloramphenicol palmitate, the same product containing NH_3 was almost always obtained by treating the palmitate with liquid NH_3. As shown in Fig. 4 (c), its X-ray pattern was quite different from those of the known polymorphs of the palmitate (Fig. 4 (a) and (b)); however, after the product had been allowed to stand at room temperature, the pattern changed to that of form B of the palmitate.

When the freshly prepared product was maintained at 2±1°, and then at 25±1°, a weight decrease curve was obtained in the same way as for the NH_3 adduct of chloramphenicol. Judging from the difference between the weight at the break point and the final weight, the palmitate was converted to a 1: 2 NH_3 adduct (chloramphenicol palmitate: NH_3=1: 1.98) by soaking in

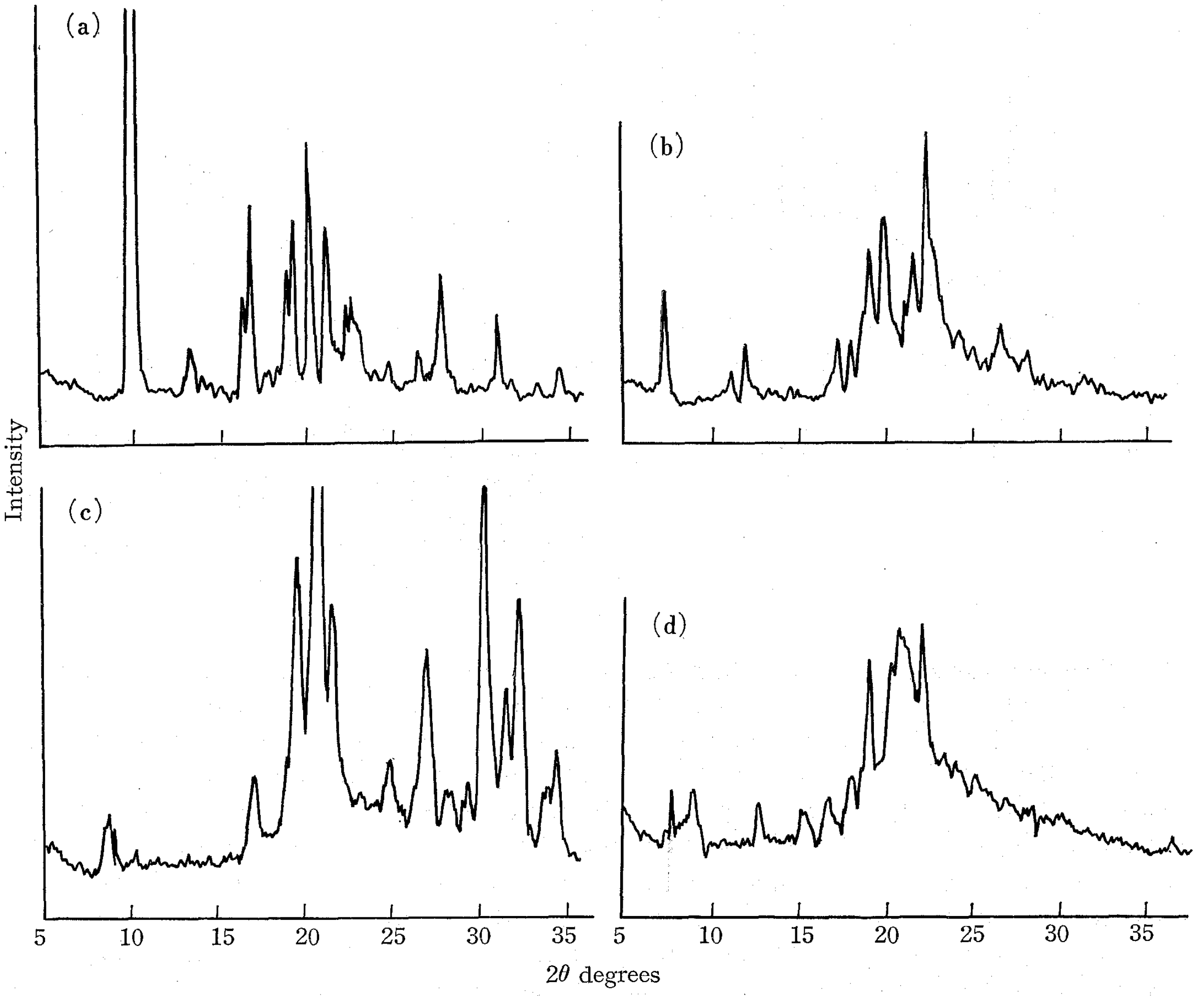

Fig. 4. X-Ray Diffraction Patterns for Polymorphs of Chloramphenicol Palmitate and Their NH_3 Adducts (2θ scan speed: 4°/min)

(a) polymorphic form A, (b) polymorphic form B,
(c) 1: 2 NH_3 adduct, (d) 1: 1 NH_3 adduct.

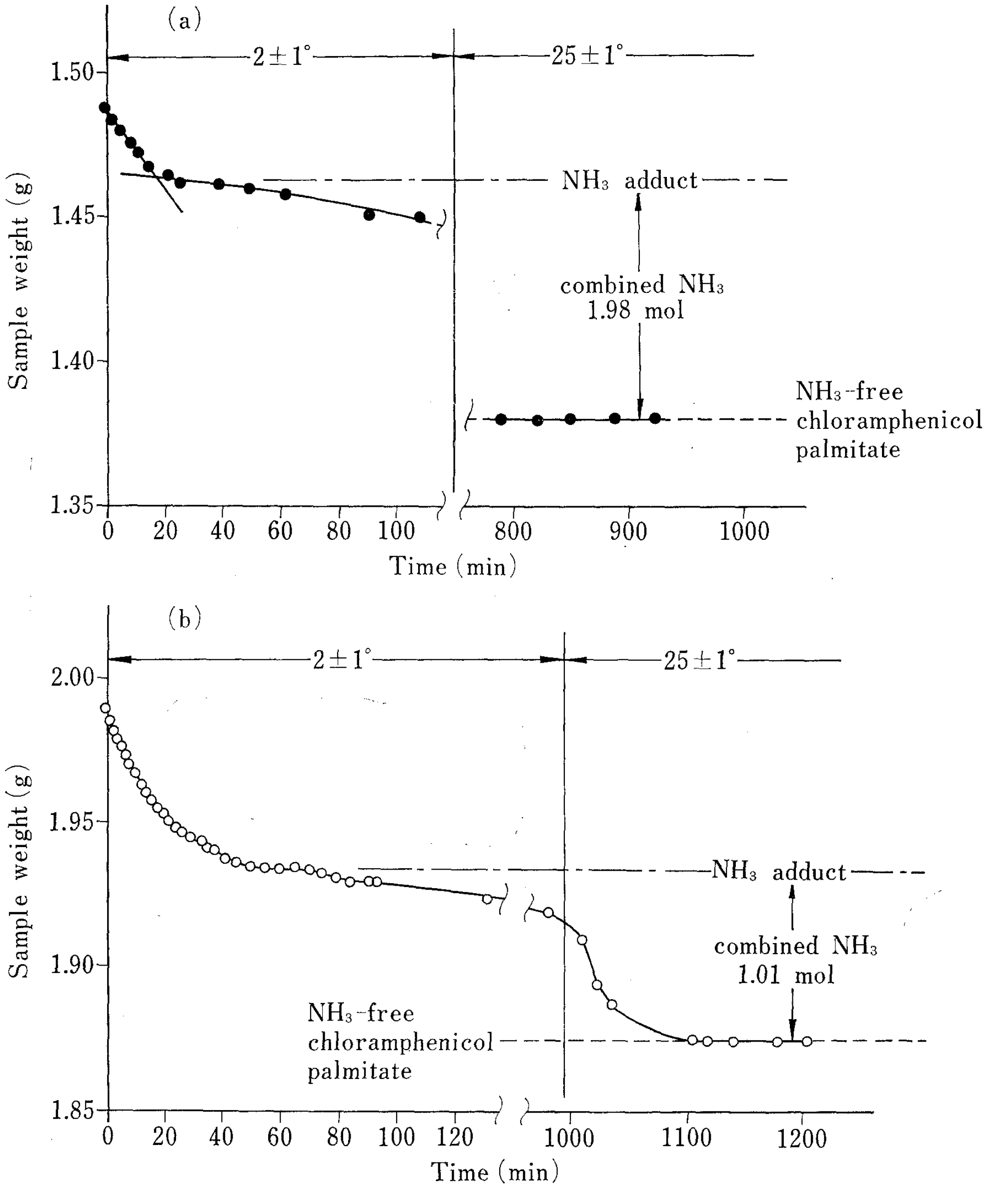

Fig. 5. Weight Decrease Curves of Chloramphenicol Palmitate NH_3 Adducts obtained on a Chemical Balance

(a) 1: 2 NH_3 adduct. (b) 1:1 NH_3 adduct.

liquid NH_3 (Fig. 5 (a)). This 1: 2 adduct was almost invariably formed, except that the product of a single batch showed a different X-ray pattern, as shown in Fig. 4 (d). The weight decrease curve in Fig. 5 (b) exhibited a plateau between the initial loss and final equilibrium in weight. It is thought from this curve that another kind of NH_3 adduct having a combining ratio of 1: 1 (chloramphenicol palmitate: NH_3=1: 1.01) was formed exceptionally.

In addition, when the ordinary 1: 2 NH_3 adduct was kept in an acetone-dry ice bath (—20 ±10°) for 12 hours or more, a mixed X-ray pattern of the 1: 2 and 1: 1 adducts was obtained. Therefore, it appears that the 1: 1 adduct was formed as an intermediate upon very gradual deammoniation of the 1: 2 adduct.

2) **Thermal Behavior (DSC)**——The characteristic DSC curves of polymorphic forms A and B of chloramphenicol palmitate, its 1: 2 NH_3 adduct and the recovered palmitate after desorption of NH_3 from the adduct are shown in Fig. 6.

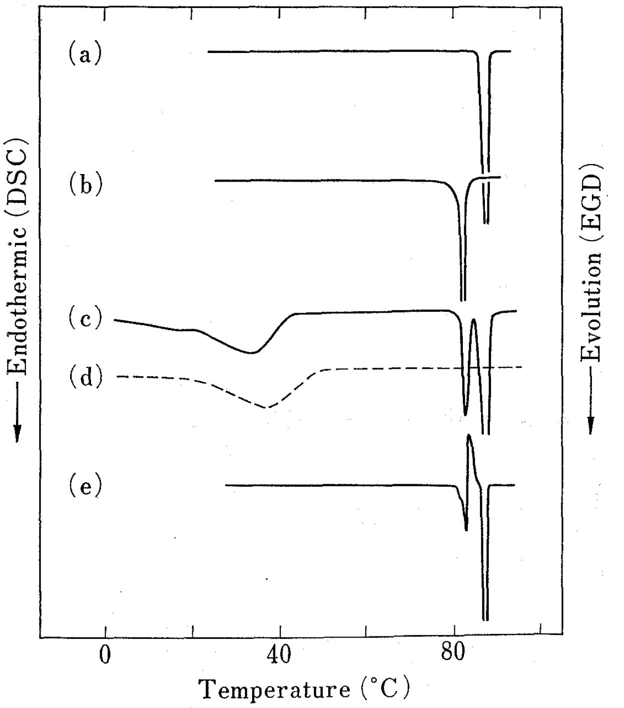

Fig. 6. Thermograms of Various Physical Forms of Chloramphenicol Palmitate and Their 1: 2 NH_3 Adduct

(a) DSC curve of chloramphenicol palmitate polymorphic form A under semi-closed conditions: sample weight, 2.89 mg; heating rate, 8°/min.
(b) DSC curve of chloramphenicol palmitate polymorphic form B under semi-closed conditions: sample weight, 5.03 mg; heating rate, 16°/min.
(c) and (d) Simultaneous DSC and EGD curves of the 1: 2 NH_3 adduct under semi-closed conditions: sample weight, 2.21 mg as chloramphenicol palmitate; heating rate, 8°/min.
(e) DSC curve of chloramphenicol palmitate polymorphic form B obtained *via* the NH_3 adduct under semi-closed conditions: sample weight, 4.66 mg; heating rate, 1°/min.

The stable form A exhibited a melting peak at about 85°, while that of the metastable form B appeared at about 81°. From the areas of these peaks, the heats of fusion of polymorphs A and B were determined to be 15.8±0.2 kcal/mol and 11.2±0.2 kcal/mol, respectively.

In the case of the 1: 2 adduct, there were two separate peaks, one of which is attributable to NH_3 desorption and the other to melting of the recovered palmitate (Fig. 6 (c)). The first endothermic heat effect appeared as either one or two peaks depending on the conditions, such as sample amount and heating rate. This suggests that the combined NH_3 could be desorbed directly to give free palmitate or indirectly by way of the 1: 1 adduct. The second heat effect due to melting consisted of two peaks, as if the recovered palmitate was a mixture of its two polymorphs. When DSC was carried out with the palmitate recovered from the 1: 2 adduct by desorption at 3±2° or 10±5°, the curve showed two melting peaks, as shown in Fig. 6 (e), with or without an exothermic peak between them.

However, in spite of the appearance of the two endothermic peaks, the palmitate recovered at lower temperatures was identified as polymorph B for the following reasons:

(1) All of the recovered palmitate samples were indicated to be not a mixture but pure polymorph B by X-ray measurements and IR.

(2) The sample removed from a DSC furnace at 75° immediately before the melting of form B showed characteristic X-ray as well as IR patterns of form B.

(3) The heat of fusion was determined to be 11.4±0.3 kcal/mol from the area of the melting peaks. This value is practically the same as that for polymorph B.

Therefore, it is considered that, presumably due to size reduction by deammoniation at lower temperatures, the polymorph B of the palmitate thus formed is liable to change to polymorph A during melting.

3) **IR Spectra**——The IR spectra of chloramphenicol palmitate forms A and B, and of the 1: 2 and 1: 1 NH_3 adducts are shown in Fig. 7. The spectrum of the 1: 2 adduct, which shows characteristic absorption bands between 3700 and 3100 cm^{-1}, is different from those of forms A and B. In contrast, the spectrum of the 1: 1 adduct is close to that for form B, except for the absorption peaks at 3340, 1630, and 1150 cm^{-1} in the former. Accordingly, it appears that the intermolecular arrangement of chloramphenicol palmitate in the 1: 1 adduct is similar to that of form B; therefore, the latter is easily obtained by deammoniation from the 1: 1 adduct.

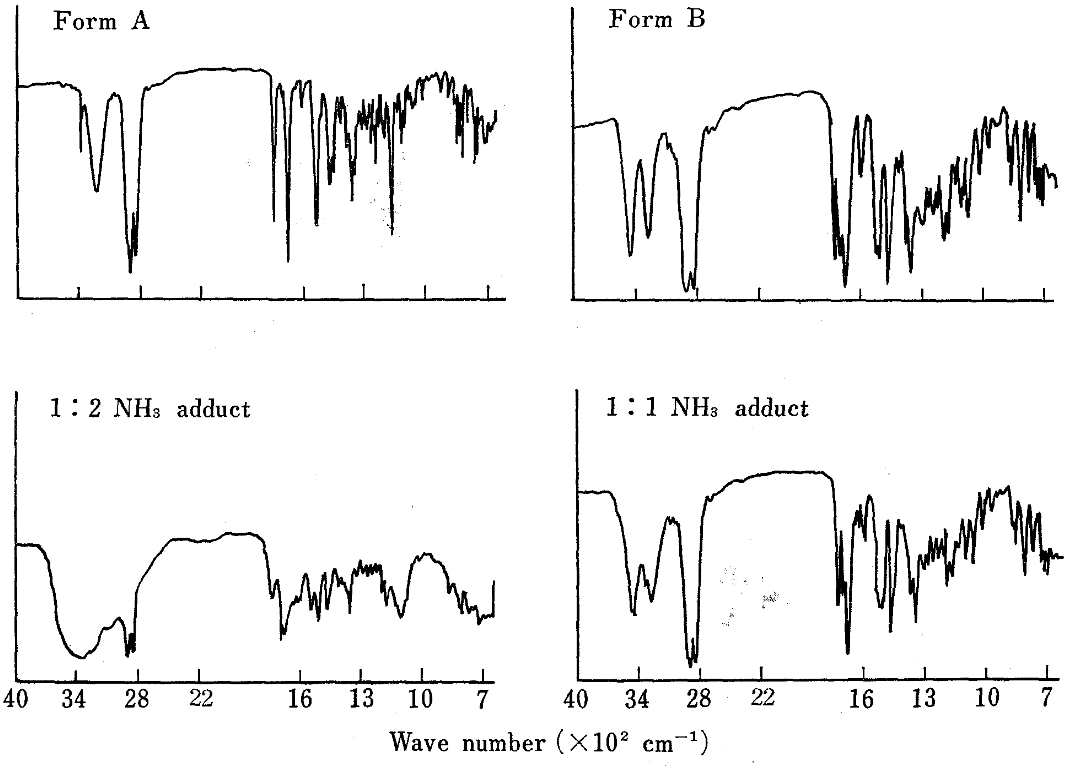

Fig. 7. Infrared Spectra of the Polymorphs of Chloramphenicol Palmitate and Their NH_3 Adducts (Nujol mulls)

Particle Size Reduction of Chloramphenicol and Chloramphenicol Palmitate by Desorption of NH_3 from Their NH_3 Adducts

1) **Chloramphenicol**——The specific surface area of chloramphenicol recovered by desorption of NH_3 from its NH_3 adduct was measured by the BET gas adsorption method. A value of 2.0±0.5 m^2/g (2.2±0.6 μm) was obtained for the particles recovered by deammoniation under atmospheric pressure at 10—25°, while 2.9—3.1 m^2/g (1.3—1.4 μm) was obtained for particles recovered *via* chloramphenicol pyridine solvate, as reported previously.[4] Although thermal stability and elimination conditions are different for the NH_3 adduct and the pyridine solvate of chloramphenicol, the degree of particle size reduction of the two may be affected by the molecular volume difference between two mole of NH_3 and one mole of pyridine included in the NH_3 adduct and the pyridine solvate, respectively. That is, chloramphenicol crystal growth in the solid state by molecular rearrangement following the desolvation of pyridine may be disturbed to a greater degree than following the desorption of NH_3; this might result in the difference in the values of their specific surface areas.

2) **Chloramphenicol Palmitate**——Fig. 8 (a) shows crystals of chloramphenicol palmitate form A, and (b) and (c) show products obtained by desorption of NH_3 from the chloramphenicol palmitate NH_3 adduct, prepared by suspending polymorphic form A in liquid NH_3 for a few hours. The external appearance of the original crystals was retained in the NH_3-free chloramphenicol palmitate crystals recovered by one cycle of the adsorption and desorption process. However, on repeating the process of adsorption and desorption of NH_3 for the same sample without applying mechanical pulverization, the appearance of the original platy crystals gradually changed; the long axis of the primary particles became shorter and finally small spherical particles were formed, as seen in Fig. 8 (d). It is clear visually that the effects of repeating the process of adsorption and desorption of NH_3 on particle size reduction are cumulative. This phenomenon was quantitatively confirmed by measuring the specific surface area of recovered

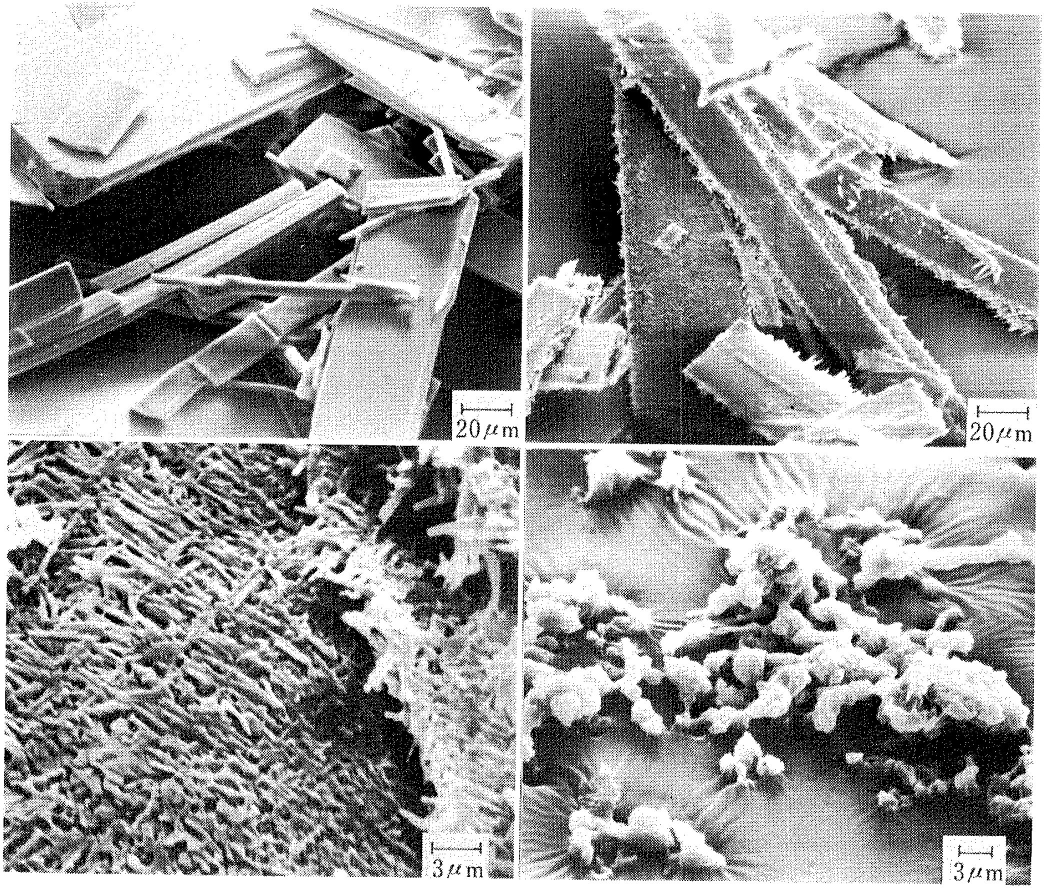

Fig. 8. Scanning Electron Micrographs of Chloramphenicol Palmitate

(a) (b)
(c) (d)

(a) Original crystals of polymorphic form A.
(b) and (c) Deammoniated form (polymorphic form B) after one cycle of sorption-desorption
(d) Deammoniated particles (polymorphic form B) after four cycles of sorption-desorption.
Magnification: (a) 400×, (b) 400×, (c) 3000×, (d) 2000×.

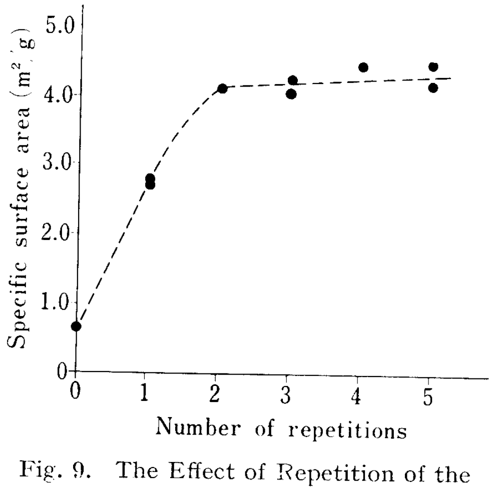

Fig. 9. The Effect of Repetition of the NH_3 Sorption-Desorption Cycle upon the Specific Surface Area of Chloramphenicol Palmitate

chloramphenicol palmitate particles, as shown in Fig. 9. Prior to BET measurements, the deammoniation proceeded at about 10° under atmospheric pressure and samples were not mechanically pulverized throughout the series of experiments. The specific surface area of the original chloramphenicol palmitate is 0.6 m^2/g, while values of 2.7 m^2/g and 4.1 m^2/g (1.2 μm) were obtained for the samples recovered after one and two cycles of adsorption and subsequent desorption of NH_3, respectively. No further marked increase in specific surface area was observed on repeating the process again with the same sample. The polymorphic form of the sample recovered was always determined to be form B by X-ray diffractometry and IR.

Conclusion

1) Chloramphenicol was confirmed to form an NH_3 adduct having a molecular ratio of 1: 2 (chloramphenicol: NH_3), while chloramphenicol palmitate was found to give two different adducts having combining ratios of 1: 2 and 1: 1, although the latter was formed only occasionally.

2) All of these NH_3 adducts were unstable; elimination of combined NH_3 proceeded rapidly even at room temperature and yielded fine particles of the original chemicals.

3) The effect of two or more repetitions of the NH_3 sorption-desorption process was cumulative for chloramphenicol palmitate crystals and caused a significant increase in the specific surface area. The polymorphic form of chloramphenicol palmitate recovered *via* the NH_3 adducts was determined to be form B.

4) Chloramphenicol and chloramphenicol palmitate, both of which are neutral substances, are considered to form adducts with NH_3 by some weak force such as dipole-dipole interaction or hydrogen bonding.

It is suggested that rather more chemicals may have the ability to form NH_3 adducts than might be expected, since modern medicinal compounds contain a variety of functional groups that have affinity for other molecules. It seems clear that the lower the stability of an adduct or a solvate, the more easily particle size reduction by sorption-desorption will occur, although adduct or solvate formation may become difficult to identify, as in the cases of the NH_3 adducts of chloramphenicol and chloramphenicol palmitate.

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