[Chem. Pharm. Bull. 31(10)3649—3655(1983)]

Formation of Ammonia Adducts of Several Steroids and Their Application to Particle Size Reduction[1)]

SAWSAN ABD EL FATTAH,[a] KIKUKO OKAMOTO,[b] KAORU CHIHARA,[b] KEN-ICHI SHIROTANI,[b] YASUYUKI TSUDA,[b] ETSUKO SUZUKI,[b] and KEIJI SEKIGUCHI *,[b]

Faculty of Pharmacy, University of Alexandria,[a] Egypt and School of Pharmaceutical Sciences, Kitasato University,[b] 9–1, Shirokane 5-chome, Minato-ku, Tokyo 108, Japan

(Received February 19, 1983)

Adduct formation with ammonia (NH_3) was confirmed to occur with four steroids (cortisone acetate, hydrocortisone acetate, prednisolone, and prednisone). The thermal, physico-chemical, and micromeritic properties of these adducts were investigated by differential scanning calorimetry, thermogravimetry, thermomicroscopy, infrared spectroscopy, X-ray powder diffractometry, electron microscopy, and BET gas adsorption analysis. From the results of thermogravimetry, the combining ratios of the adducts (steroid : NH_3) were determined to be 1 : 1.13 and 1 : 0.95 for cortisone acetate and hydrocortisone acetate, respectively; however, in the cases of prednisolone and prednisone, one molecule of the steroid combined with 0.5—1 or 1—2 molecules of NH_3, respectively. The formation of adducts having nonstoichiometric ratios may be due to the fact that the architecture of molecules rather than their chemical affinity may play an important role in adduct formation. Elimination of NH_3 from adducts proceeded completely at room temperature and under the reduced pressure to yield ultrafine particles of the original steroids.

Keywords——cortisone acetate; hydrocortisone acetate; prednisolone; prednisone; ammonia adduct of steroid; thermal behavior of ammonia adduct; particle size reduction

It has been recognized that the rate of absorption of many slightly soluble drugs from the gastrointestinal tract and other sites is limited by the rate of dissolution of drugs. The particle size of a drug is therefore of importance if the substance in question has a low solubility. Since steroidal substances commonly have very low solubility, the effect of particle size on the availability from various dosage forms has been investigated. For instance, Smith *et al.* studied the gastrointestinal absorption of medroxyprogesterone acetate from tablets containing either micronized or nonmicronized drug and demonstrated that micronization led to a twofold increase in the extent of absorption of the steroid.[2)] In an ophthalmic suspension of dexamethasone, a rank-order correlation was observed between increasing drug level in the aqueous humor or cornea and decreasing particle size.[3)]

In a series of studies, Sekiguchi and his coworkers developed a method of particle size reduction by solvation and subsequent desolvation of certain drug compounds that can form solvates with suitable solvents,[4)] and by desorption of ammonia from drug-NH_3 adducts.[5)]

In this study, we investigated the formation of NH_3 adducts of steroidal substances in an attempt to reduce their particle size and to increase their surface area *via* desorption of NH_3 from their NH_3 adducts.

Experimental

Materials——Four kinds of steroids, namely cortisone acetate and hydrocortisone acetate (Wako Pure Chemical Industries, Ltd.), prednisolone (E. Merck, Darmstadt), and prednisone (Sigma Chemical Company) were of

commercial quality. Compressed liquid NH_3 was used as the source of NH_3.

Preparation of NH_3 Adducts——1) Soaking in Liquid NH_3: This method was used for steroids which are slightly soluble or insoluble in liquid NH_3. The apparatus was assembled from several round-bottomed glass flasks, two gas reservoirs (each with a volume of 1000 ml), a mercury manometer, and stopcocks for the inlet and outlet of gas. Each steroid was placed in a flask and the apparatus was completely evacuated. Thereafter, the stopcock leading to the vacuum pump was closed and NH_3 gas was gradually introduced into the apparatus until the manometer reading was 700 mmHg. As the flask was cooled with acetone–dry ice mixture to about -70 °C, NH_3 gas gradually liquefied in the flask and wetted the sample powder. After a certain period of time, the refrigerant was removed and excess NH_3 was allowed to evaporate. When the manometer reading showed a constant pressure, the flask was detached from the apparatus for the subsequent physico-chemical measurements. Because the NH_3 adduct thus formed lost its combined NH_3 gradually, all measurements were done within a short period after preparation.

2) Crystallization from Liquid NH_3: Sample powder was placed in a flask as above and cooled with acetone–dry ice mixture. NH_3 gas was gradually introduced into a flask until the powder dissolved completely. The flask was then immersed in an ice bath, and excess NH_3 was allowed to evaporate.

Differential Scanning Calorimetry (DSC)——A Perkin–Elmer DSC-1B differential scanning calorimeter was used. Evolved gas detection (EGD) was carried out simultaneously. The scanning speed was 16 °C/min and a sample weight of 4—12 mg was used after precise weighing with a semi-micro balance. All measurements were carried out with cooling of the furnace with a cryogenic cover filled with acetone–dry ice mixture.

Thermogravimetry (TG)——A Perkin–Elmer TGS-1 thermobalance was used. A sample weight was 2—9 mg and the scanning speed was 16 °C/min.

Infrared (IR) Spectroscopy——IR spectra were measured by the Nujol mull method with a Jasco IRA-1 grating infrared spectrometer.

X-Ray Powder Diffractometry——A JDX-7F X-ray diffraction analyzer from Japan Electron Optics Laboratory Co., Ltd. was used (Ni filter, Cu-$K\alpha$ radiation, $\lambda = 1.542$ Å).

Thermomicroscopy——The thermal behavior of the NH_3 adducts of steroids was observed microscopically with Mettler FP 5 and FP 52 instruments (Mettler Instrument Co., Ltd.).

Scanning Electron Microscopy——The surface appearance of steroids was observed using a scanning electron microscope (MINI SEM model MSM-4, Hitachi-Akashi Co., Ltd).

Measurements of Specific Surface Area——A BET gas adsorption apparatus (model P-600, Shibata Chemical Apparatus Mfg. Co., Ltd.) was used. A sample weight of 0.7—1 g was used and the gas employed was N_2.

Measurements of Density——The densities of prednisolone (form A) and prednisone at 25 °C were determined by the flotation method using crystals obtained by gradual recrystallization. The floating solvents were CCl_4–cyclohexane mixtures in suitable proportions.

Results and Discussion

Thermal Behavior of NH_3 Adducts of Steroids

1. NH_3 Adducts of Cortisone Acetate——The DSC, EGD, and TG curves of the NH_3 adduct obtained by soaking cortisone acetate powder in liquid NH_3 are shown in Fig. 1 (b)—(d). The DSC pattern of pure cortisone acetate is shown by curve (a) in the same figure. It was considered that the first endothermic peak on the DSC curve (b) which appeared between 60 °C and 85 °C was due to decomposition of the adduct and release of gas, because the simultaneously obtained EGD curve showed gas evolution corresponding to the DSC peak. The evolved gas from the DSC furnace was passed into 0.1 N H_2SO_4 solution and an aliquot of this solution was tested by means of the phenol-hypochlorite reaction.[6] The development of intense blue color confirmed that the gas is NH_3. The second DSC peak at aobut 250 °C is due to melting of free cortisone acetate, since curve (a) has a peak in the same range of temperature.

The combining ratios of the adducts were determined to be 1 : 1.13 (cortisone acetate: $NH_3 = 1 : 1.13 \pm 0.14$) from the weight decrease by TG measurements. As the adduct decomposed easily on standing at room temperature, elemental analysis could not be performed.

2. NH_3 Adducts of Hydrocortisone Acetate——The thermograms of free hydrocortisone acetate and its NH_3 adduct prepared by soaking the steroid in liquid NH_3 are depicted in Fig. 2. Curve (b) shows two successive endothermic peaks between 30 °C and 90 °C (the lower one

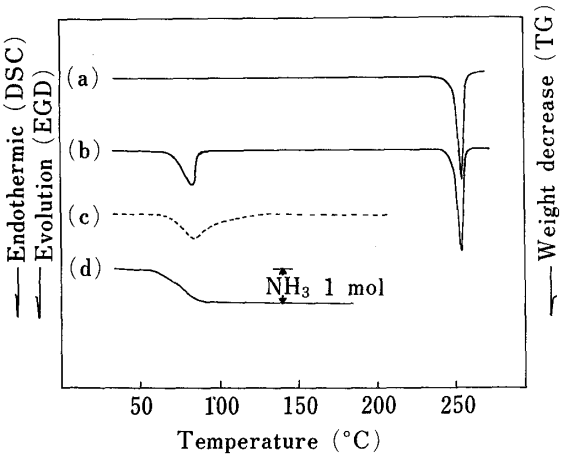


Fig. 1. DSC, EGD, and TG Curves of Cortisone Acetate and Its NH_3 Adduct

(a) DSC curve of cortisone acetate under semi-closed conditions: sample weight, 4.30 mg; heating rate, 16 °C/min.
(b) and (c) Simultaneous DSC and EGD curves of the adduct under semi-closed conditions: sample weight, 7.07 mg; heating rate, 16 °C/min.
(d) TG curve of the adduct corresponding to the DSC curve (b): sample weight, 3.18 mg; heating rate, 16 °C/min.

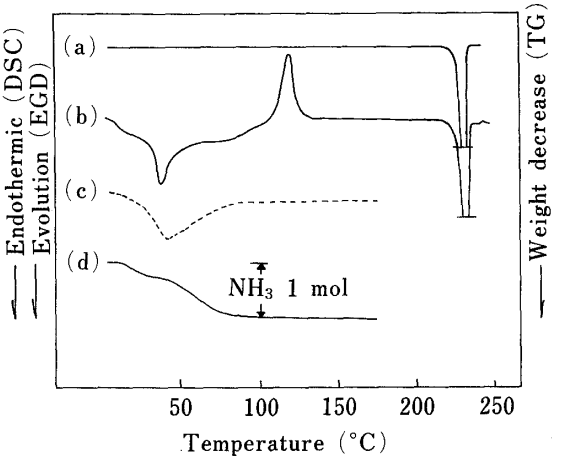


Fig. 2. DSC, EGD, and TG Curves of Hydrocortisone Acetate and Its NH_3 Adduct

(a) DSC curve of hydrocortisone acetate under semi-closed conditions: sample weight, 4.69 mg; heating rate, 16 °C/min.
(b) and (c) Simultaneous DSC and EGD curves of the adduct under semi-closed conditions: sample weight, 13.88 mg; heating rate, 16 °C/min.
(d) TG curve of the adduct corresponding to the DSC curve (b): sample weight, 4.47 mg; heating rate, 16 °C/min.

is sharp and the higher one is shallow) and one exothermic peak between 100 °C and 130 °C. When this adduct was observed through a hot-stage microscope, immediately after the melting at about 80—90 °C, crystal growth in the melt was noticed. The DSC curve can be reasonably explained by the results of microscopic observation, that is, the adduct releases all of its NH_3 at the first two DSC peaks with liquefaction and thereafter delayed crystallization of free hydrocortisone acetate takes place at the temperature around the exothermic peak. The sharp peak at about 230 °C is due to the melting of recovered steroid, because curve (a) has an endothermic peak in the same range of temperature.

The molecular ratios of the adducts obtained by TG measurements were 1 : 0.95 (hydrocortisone acetate : $NH_3 = 1 : 0.95 \pm 0.09$).

3. NH_3 Adducts of Prednisolone——Among the four steroids used in this study, prednisolone is the most soluble in liquid NH_3. Therefore, the NH_3 adducts could be prepared by either of the methods mentioned in Experimental. The adducts obtained by the two methods gave almost identical characteristic patterns of DSC, EGD, TG, IR, and X-ray powder diffraction. These results suggest that the soaking method produces homogeneous NH_3 adducts. The simultaneously obtained DSC and EGD curves are shown in Fig. 3 (b) and (c). The DSC curve (b) consists of two endothermic peaks and one gentle exothermic peak. The first endothermic peak appears around 50 °C, while the second one arises between 240—250 °C. The EGD curve shows one peak in nearly the same range of temperature as the first DSC peak. This result indicates that the first endothermic peak can be attributed to the evolution of NH_3 from the adduct. However, observation with a hot-stage microscope confirmed the thawing of the adduct around the temperature corresponding to the first endothermic peak on the DSC curve. Therefore, it is thought that this endothermic peak is due to a phase reaction accompanied by partial liquefaction, while the subsequent exothermic peak is caused by the retarded crystallization of de-ammoniated prednisolone. It is obvious by reference to curve (a) that the second endothermic peak in the range of 240—250 °C is due to the melting of pure prednisolone.

The molecular ratios of NH_3 to prednisolone calculated from TG measurements took

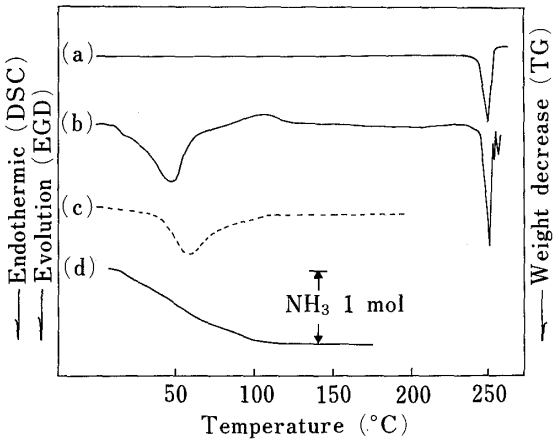


Fig. 3. DSC, EGD, and TG Curves of Prednisolone and Its NH_3 Adduct

(a) DSC curve of prednisolone under semi-closed conditions: sample weight, 4.93 mg; heating rate, 16 °C/min.
(b) and (c) Simultaneous DSC and EGD curves of the adduct under semi-closed conditions: sample weight, 8.80 mg; heating rate, 16 °C/min.
(d) TG curve of the adduct corresponding to the DSC curve (b): sample weight, 5.85 mg; heating rate, 16 °C/min.

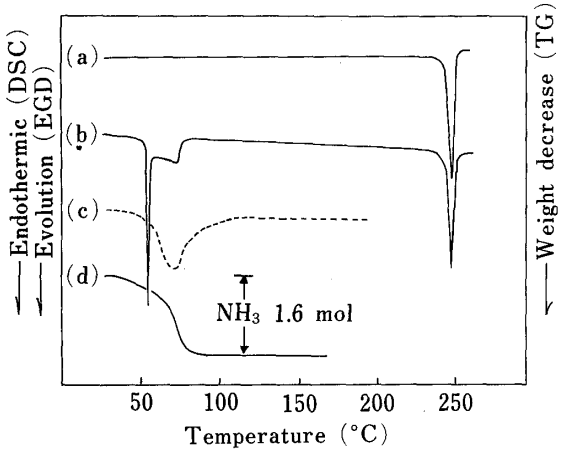


Fig. 4. DSC, EGD, and TG Curves of Prednisone and Its NH_3 Adduct

(a) DSC curve of prednisone under semi-closed conditions: sample weight, 5.81 mg; heating rate, 16 °C/min.
(b) and (c) Simultaneous DSC and EGD curves of the adduct under semi-closed conditions: sample weight, 8.44 mg; heating rate, 16 °C/min.
(d) TG curve of the adduct corresponding to the DSC curve (b): sample weight, 5.05 mg; heating rate, 16 °C/min.
The molecular ratio (prednisone: NH_3) of this sample is 1 : 1.61.

various values from 0.54 to 1.04 (6 samples). Prednisolone may thus form NH_3 adducts which contain NH_3 in nonstoichiometric ratios.

4. NH_3 Adducts of Prednisone——The NH_3 adducts of prednisone were prepared by the soaking method because prednisone is only slightly soluble in liquid NH_3. Typical patterns of DSC, EGD, and TG curves are shown in Fig. 4(b)—(d). Curve (a) is that of pure prednisone. The DSC curve of the NH_3 adduct under semi-closed conditions showed a characteristic pattern of peritectic fusion and subsequent evaporation of NH_3 during heating.[4c] The EGD curve (c) showed one peak in the range of temperature corresponding to the first two endothermic peaks on curve (b). When the sample pan was removed from the furnace and forced open after termination of the first two endothermic peaks and return of the DSC curve to the base line, its content had already solidified. Therefore, it is thought that the crystallization of NH_3-free prednisone must occur during the peritectic decomposition. It is possible that the exothermic peak could not be observed because the exothermic heat effect of crystallization was compensated by the large endothermic heat effect. The solidification of deammoniated prednisone was also substantiated by the appearance of the melting peak around 240 °C.

The combining ratios of the adducts were found to be 1 : 1.10 (prednisone : $NH_3 = 1 : 1.10 \pm 0.09$) for 5 samples. However, the ratios of two other samples in which the particles were aggregated rather rigidly, were found to be 1.61 and 1.93. The formation of adducts having various molecular ratios may be due to the difference in molecular volumes between the steroid and NH_3, that is, small molecules such as NH_3 may be included in the crystal lattice spaces of the steroid in rather arbitrary ratios.

Infrared Spectra and X-Ray Powder Diffraction Patterns of NH_3 Adducts of Steroids

All of the steroids have characteristic absorption bands for the stretching vibrations of OH and CO groups in the regions of 3000—3600 cm^{-1} and 1600—1750 cm^{-1}, respectively. In the cases of the adducts, changes of IR spectra were observed at 3200—3600 cm^{-1}, and these

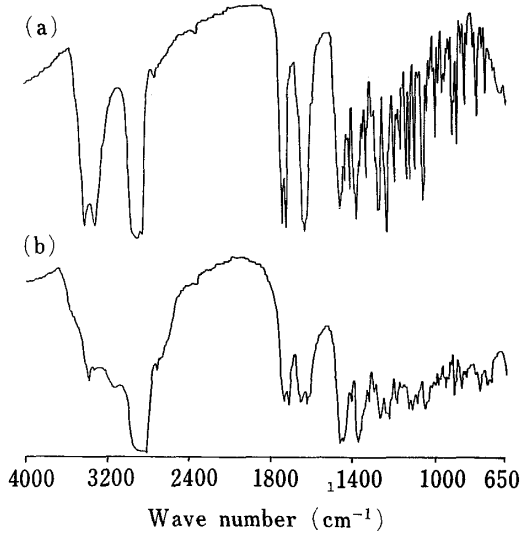


Fig. 5. Infrared Spectra of Hydrocortisone Acetate and Its NH_3 Adduct (Nujol mull)

(a) Commercial product.
(b) NH_3 adduct.

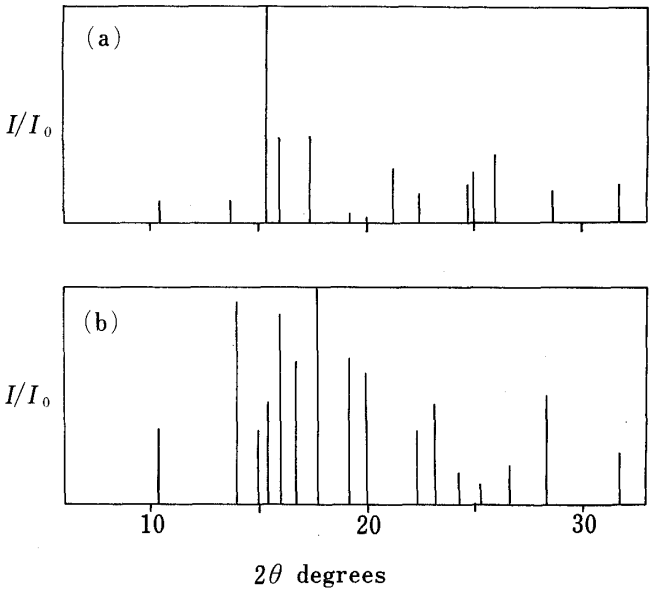


Fig. 6. X-Ray Powder Diffraction Patterns of Prednisolone and Its NH_3 Adduct

(a) Commercial product (form A).
(b) NH_3 adduct.

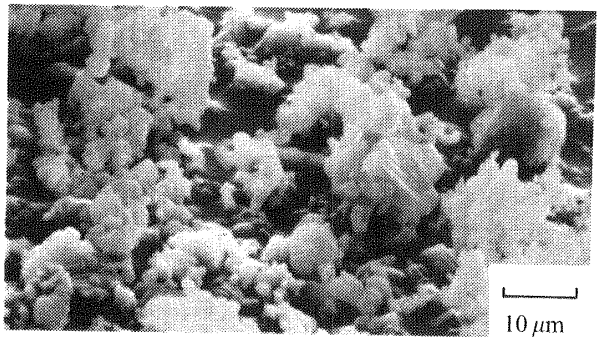
are attributable to the added NH_3. For example, the IR spectra of hydrocortisone acetate and its NH_3 adduct are shown in Fig. 5. In the spectrum of the adduct, new absorption bands appear in the region around 3200 cm^{-1} and 1660 cm^{-1}, which can probably be assigned to symmetrical NH stretching vibration and NH_3 bending vibration, respectively. The spectra of recovered steroids which were de-ammoniated under the conditions described later were the same as those of the original commercial products except in the case of cortisone acetate. This exception is due to the fact that form I of cortisone acetate[7] was produced during the process of desorption.

X-Ray powder diffractometry showed that the NH_3 adducts of the four steroids are different from the corresponding steroids in molecular species. As an example, the patterns of prednisolone and its NH_3 adduct are shown in Fig. 6. Although prednisolone possesses two polymorphs,[8] the pattern of Fig. 6(b) does not coincide with that of either of them. The recovered steroids showed patterns identical with those of the original steroids except for cortisone acetate.

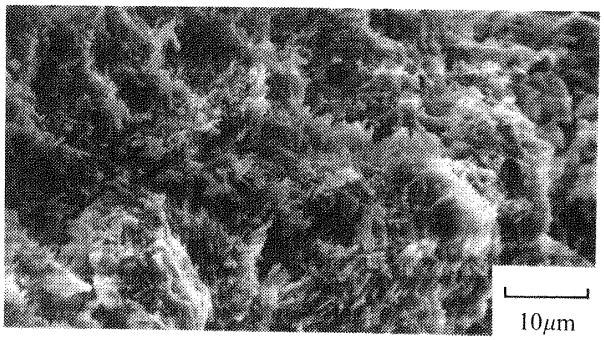
Particle Size Reduction of Steroids by NH_3 Desorption from Their NH_3 Adducts

The elimination of NH_3 from the adducts was performed at room temperature at a pressure of 1—3 mmHg using a vacuum desiccator. The values of elemental analysis of recovered steroids coincided with those of the commercial steroids within experimental error. The scanning electron micrographs of commercial cortisone acetate and cortisone acetate recovered *via* the NH_3 adduct are shown in Fig. 7(a) and (b), respectively. Relatively large agglomerates were obtained after desorption of NH_3 but they were easily separated into the primary fine particles by light pulverization, as shown in Fig. 7(c). The other steroids recovered *via* the NH_3 adducts showed similar surface appearance. Because of their instability, the NH_3 adducts themselves could not be observed.

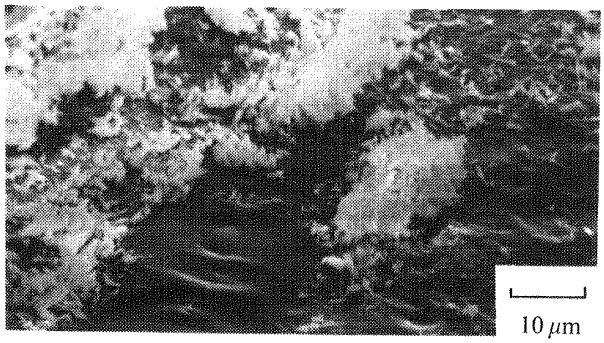
The specific surface areas of steroid powders are given in Table I. The average particle diameters of recovered steroids were also calculated on the assumption that the shape of particles is spherical. In every case, ultrafine particles were obtained by holding the sample below its decomposition temperature under reduced pressure. Differences between the figures



(a)



(b)



(c)

Fig. 7. Scanning Electron Micrographs of Cortisone Acetate

(a) Commercial product, 1000×.
(b) Cortisone acetate recovered *via* the NH_3 adduct, 1000×.
(c) Cortisone acetate recovered *via* the NH_3 adduct after pulverization, 1000×.

TABLE I. Results of Particle Size Reduction of Steroids by NH_3 Desorption from Their NH_3 Adducts

Steroid	Specific surface area		Average diameter[a]	
	Commercial	Recovered from adduct	Commercial	Recovered from adduct
Cortisone acetate	5.0 m^2/g	13.6 m^2/g	1.0 μm[b]	0.4 μm
Hydrocortisone acetate	5.1	15.8	0.9	0.3
Prednisolone	5.5	6.6	0.8	0.7
Prednisone	4.5	7.5	1.0	0.6

a) $d=6/\rho \cdot S$. The values of ρ (density) are as follows: form I of cortisone acetate = 1.250,[7] form II of cortisone acetate = 1.251,[10] hydrocortisone acetate = 1.289,[11] form A of prednisolone = 1.322, prednisone = 1.322.
b) Commercial cortisone acetate used in this experiment was a mixture of form I and form II. The densities of these two polymorphs being very similar, the average diameter could be estimated in spite of the fact that the composition ratio of the two polymorphs was unknown.

for the latter two steroids were comparatively small; however, the usefulness of the present method of size reduction *via* the NH_3 adducts should not be underestimated, since it is said that commercial corticosteroids are usually pre-micronized.

Conclusion

1. Adduct formation with NH_3 was confirmed to occur with four steroids. Cortisone acetate and hydrocortisone acetate formed adducts having the approximate composition of 1 : 1 in molecular ratio, while in the cases of prednisolone or prednisone, one molecule of the steroid combined with 0.5—1 or 1—2 molecules of NH_3, respectively. It is possible that

cortisone acetate and hydrocortisone acetate may also form NH_3 adducts having various combining ratios, but adducts of this kind could not be obtained.

2. All of the NH_3 adducts obtained were unstable. The elimination of NH_3 at room temperature and under reduced pressure proceeded rapidly to yield ultrafine particles of the original steroids.

3. The four steroids employed in this study can be classified as nonelectrolytes. The combination of these neutral substances with basic NH_3 molecules to form adducts may therefore involve some weak binding force such as dipole–dipole interaction or hydrogen bonding as the driving force for adduct formation. It was suggested that cortisone includes iodine molecules.[9] Accordingly, the architecture of molecules rather than their chemical affinity may play a major role in adduct formation. Since the NH_3 adducts of steroids are unstable in nature, the trapped NH_3 may easily escape from the open lattice of the steroids. Thus, adducts having various combining ratios might arise, as in the cases of prednisolone and prednisone. The acetate esters, namely, cortisone acetate and hydrocortisone acetate have one more C=O group in their molecules. Therefore, it is possible for the oxygen atom to participate in hydrogen bonds with NH_3 as a proton acceptor. The stronger intermolecular bonding may result in the predominant formation of 1:1 adducts in the cases of acetate esters of steroids. No definite conclusion could be reached regarding the changes in C=O stretching vibration in the IR spectra, and this problem requires further investigation.

References and Notes

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