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# Hexamethylmelamine aerosols prepared in an evaporation-condensation generator

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## Summary

Homogeneous hexamethylmelamine (HMM) aerosols were prepared by evaporation and condensation of the drug in a simple generator. The aerodynamic size distribution of the solid particles was found to be logarithmic-normal, characterized by mass median diameters of about 4 μm and geometric standard deviations of approximately 1.3. There was no evidence of decomposition of HMM. Rapid cooling of the HMM vapour yielded a new polymorph of the drug. The generator was found to operate in a stable and reproducible manner for at least an hour at an airflow rate of 2 $dm^3$/min. The method appears suitable for in situ generation of HMM aerosols ( ~ 20 mg/min) and for small-scale preparation of fine HMM particles.

## Introduction

Hexamethylmelamine (HMM) is a chemotherapeutic agent valuable in the treatment of small-cell lung carcinoma when administered orally. Its toxicity by this route is mild compared to other cytotoxic agents; nevertheless its use is limited by severe gastrointestinal and neurological side-effects (Hahn, 1983). Direct administration of drugs to the vicinity of the target tissue may improve their selective effect

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(Ensminger and Gyves, 1983) so that a lower dose can be used. In this way, it is possible, in some instances, to avoid unwanted side effects in other organs. This concentration advantage is retained over a prolonged period of time if the drug is removed only slowly from its original site and there are efficient mechanisms for elimination of the drug from systemic circulation. This has been shown, for example, for intratracheally administered Ara-C where the sustained release effect was achieved by entrapment of the drug in liposomes (McCullough and Juliano, 1979). Inhalation aerosols for the treatment of diseases of respiratory tract are prime examples of successful application of the site-directed philosophy (Gonda and Byron, 1978; Anderson, 1981).

HMM is a solid with a low solubility at neutral pH (Cumber and Ross, 1977), i.e. under conditions similar to those existing in the pulmonary spaces. Its plasma half-life ranges from 3–13 h. It is therefore likely that solid HMM delivered to the airways would provide a local concentration of dissolved drug (solubility ~ 0.1 mg/ml) over an extended period, which would be far in excess of peak plasma levels achieved after oral administration (Hahn, 1983). The mechanism of action of HMM is unknown. N-Demethylation is thought to be the essential pathway of activation of this drug (Rutty et al., 1982).

We have therefore decided to explore the means for generation of respirable HMM aerosols in situ and for small scale preparation of respirable particles. In this paper, we wish to report a method based on condensation of HMM vapour. Previous studies (Wilson and La Mer, 1948; Sinclair and La Mer, 1949; Lassen, 1960; Muir, 1965; Liu et al., 1966; Rapaport and Weinstock, 1967; Swift, 1967; Prodi, 1970; Gonda et al., 1979) established that the evaporation-condensation generators were capable of producing relatively monodisperse aerosols from a variety of substances. All of these generators required rather large quantities of materials.

Our objectives were to simplify the generation whilst fulfilling the criteria for respirable aerosols (Gonda, 1981a and b), and to minimize the amount of excess material in the generator in order to economize on valuable pharmaceutical substances. A homogeneous nucleation method was attempted so as to avoid the need for initial production of 'foreign' nuclei.

## Materials and Methods

Hexamethylmelamine was supplied by the Cancer Chemotherapy Research Group, Department of Pharmacy, University of Aston in Birmingham, U.K.

The schematic diagram of the generator is presented in Fig. 1. 1–4 g of HMM was placed in a three-neck 250 ml round-bottom Pyrex flask fitted with a vertical jacketed condenser (40 cm long, 2 cm internal diameter). A thermometer was immersed in HMM and another thermometer in the oil bath. The third neck was used to supply carrier air via a glass tube which terminated just above the surface of the molten drug. The flask was immersed in a temperature-controlled (±0.2°C) oil bath (Grant Instruments, U.K.) filled with polyethylene glycol (PEG 400). The

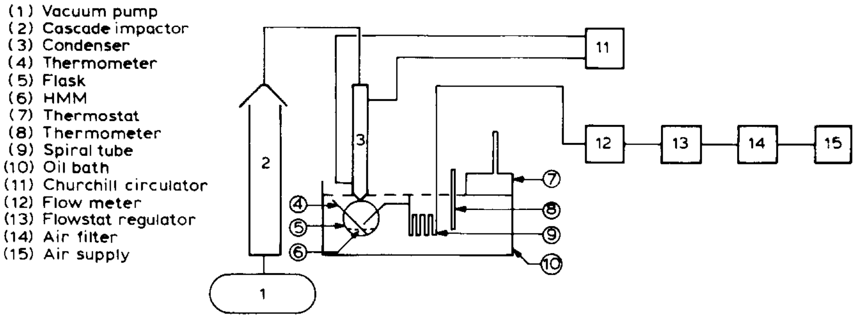

Fig. 1. Schematic diagram of the evaporation-condensation generator.

temperature in the condenser was controlled in a counter-current fashion by a thermocirculator (Churchill Instruments, U.K.). An air cylinder with a regulator (BOC, U.K.) supplied the carrier gas which was passed through a sintered glass to remove any particulate matter. For short-term generation, the air flow was monitored with Model A1 flow meter with a hollow stainless steel float No. 4.42/HS (G.A. Platon, U.K.). Model B6 with a float No. 9.65/HS from the same supplier was used during long-term generation. A steady flow rate was ensured by a Flowstat regulator Type MNAL (GA Platon, U.K.). The air was subsequently passed through a glass spiral (length 20 cm, diameter 0.2 cm) immersed in the oil bath to preheat the air and thus to prevent cooling and crystallization of HMM by the incoming gas.

The exit from the generator (i.e. from the condenser) was connected via a large-bore L-shaped glass tube to a filter funnel hanging upside down over the inlet into the DCI6 cascade impactor (Delron Research, Oh, U.S.A.). The impactor operated at a constant flow rate of 12.5 $dm^3$/min which was achieved by a Speedivac vacuum pump (Edwards High Vacuum, BOC, U.K.) attached to the critical orifice at the exit from the impactor. It is noteworthy that the sampling rate of the impactor always exceeded the gas volume output from the generator so that very little, if any, aerosol escaped into the atmosphere. Personal exposure was further minimized by placing the whole generator and the impactor in a working fume cupboard.

In the preliminary experiments, only stage No. 6 with an uncoated glass slide (nominal cut-off diameter 0.5 $\mu$m) and stage No. 7 with absolute filter (Type AE, 76 mm, Gelman Sciences, U.S.A.) were used. The first 2 min of operation, prior to steady-state conditions, were disregarded. The output from the generator was determined by weighing the amount of HMM collected on the glass slide and the filter. Preliminary particle size analysis of the deposits on the slides was performed with the help of an optical microscope (Model BHB 411, Olympus Optical, Japan) connected to a video monitor (Model VM-910U, Hitachi-Denshi, Japan). The microscope was equipped with a splitting image Particle Size Micrometer and Analyzer (Type 526, Fleming Instruments, U.K.). In each sample, sufficient numbers of particles were measured in groups of 10 until the following criteria were both satisfied:

$$\frac{D_g(n) - D_g(n-10)}{D_g(n)} \times 100 < 5\%$$

$$\frac{\sigma_g(n) - \sigma_g(n-10)}{\sigma_g(n)} \times 100 < 10\%$$

where $D_g$ = geometric mean of diameters; $\sigma_g$ = geometric standard deviation of $D_g$; and n = number of particles measured (n = 20, 30, etc.). The above criteria were satisfied for n = 300–500.

Samples for scanning electron microscopy (SEM) were prepared as follows: the part of the impactor glass slide containing the deposit of HMM was cut out and mounted to an aluminium stub using two-sided sticky tape. In order to prevent the loss of particles under vacuum, a conducting film (No. 0802, Polaron Equipment, U.K.) was sprayed on the stub and dried. The stub was first gold-plated under argon gas, and colloidal silver electrical contact was then applied (Silver Dag 915, Acheson Colloid, U.K.). The samples were examined by SEM (Pye Stereoscan 150, Pye Unicam, U.K.).

Full aerodynamic size analysis of the aerosol was performed using the complete DCI6 cascade impactor with the first six stages containing silicone-coated slides and the last stage fitted with the absolute filter. The general description of the method has been published previously (Gonda, 1984). Briefly, round glass slides (3.8 cm diameter, Quartz Grinding, U.K.) were cleaned in a chromic/sulphuric acid mixture and thoroughly washed in distilled water. After drying, 0.5 ml 10% w/v solution of silicone fluid (200/60000 (Dow Corning, U.K.) in analytical grade benzene (Fisons Scientific Apparatus, U.K.) was spread over each slide evenly and allowed to dry in a fume cupboard for 1 h. The slides were then placed in the impactor and a sample was collected at steady state (i.e. ignoring the first 2 min) at various intervals over a period 0.5–1 min. This period was sufficiently short to prevent overloading of the slides (Mercer, 1966). The slides were then taken out of the impactor and immersed in 10 ml analytical grade methanol (Fisons Scientific Apparatus, U.K.). The solvent was evaporated using the Dri-Block 0B3 apparatus (Tecam Cambridge, U.K.) at 40°C. The solid residue was dissolved in 0.1 ml of the analytical grade methanol and 1 $\mu$l of this solution was injected into a 1.5 m long, 4 mm internal diameter glass column packed with 3% 0V-17 stationary phase on Chromosorb W-AWDMCS, mesh size 100–200 (Phase Separation, U.K.). The temperatures in the equipment (Gas Chromatograph Series 204, Pye Unicam, U.K.) were as follows: column oven 180°C, detector oven (nitrogen detector) 250°C, injector 200°C. The flow rates were: carrier (nitrogen) 40 ml/min, hydrogen 30 ml/min, air 300 ml/min. Pentamethylmelamine was used as the standard. The amount of HMM was measured using previously determined calibration for the peak height ratio (test/standard). The calibration was linear over the range used (1–6 ng/ml) with a sensitivity of 1 ng/ml. The complete assay gave a recovery of 98 ± 1% of HMM spiked on the coated slides.

The amounts deposited on the stages were used to compute the aerodynamic size distribution of the aerosols utilising the previous calibration of the impactor and an interactive least-mean square fitting computer program implemented on an ICL 1904 mainframe computer (Gonda, 1984).

The purity and identity of the product was examined in phosphate buffer pH = 7.4 by UV spectroscopy (Perkin-Elmer double beam 124 UV spectrophotometer with 1 cm silica cells), and by $^1$H-NMR spectroscopy (Varian Model EM360A NMR spectrometer, 60 MHz). The analytical grade methanol was used as solvent for UV spectroscopy, and deuterated chloroform with tetramethylsilane as the internal standard for NMR spectroscopy.

The densities of the parent material and the aerosol product were determined by an air comparison pycnometer (Beckman Model 930).

## Results and Discussion

In the preliminary series of experiments, the general characteristics of the generator were determined at temperatures 175 and 182°C (melting point of the parent

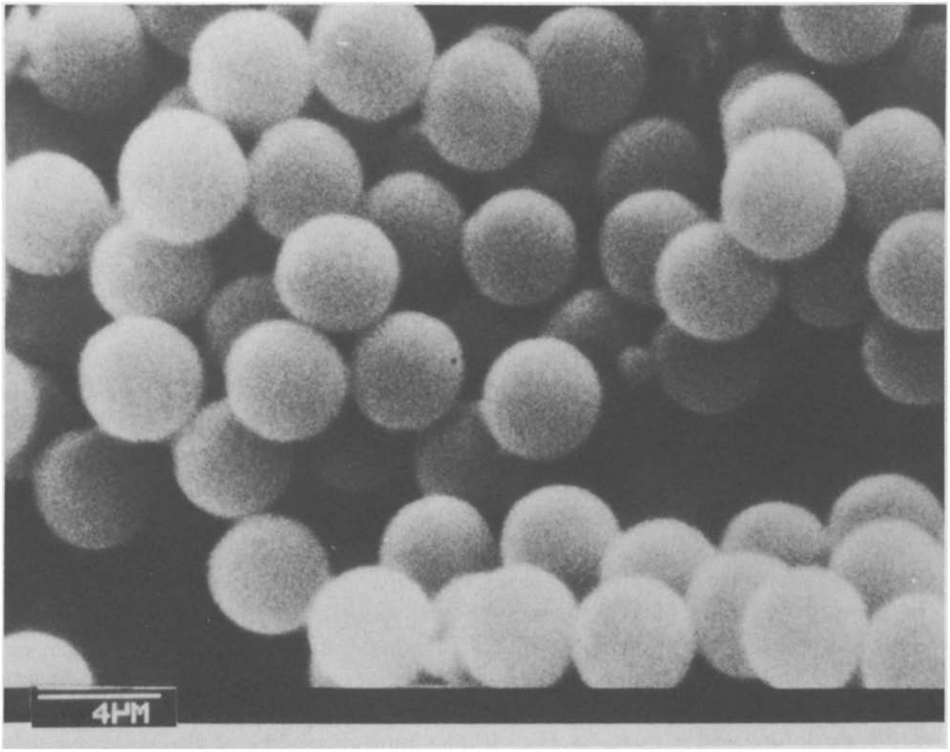

Fig. 2. Scanning electron micrograph of spherical HMM particles produced at an air flow rate 2 dm$^3$/min, oil bath and condenser temperatures 182 and 20°C, respectively.

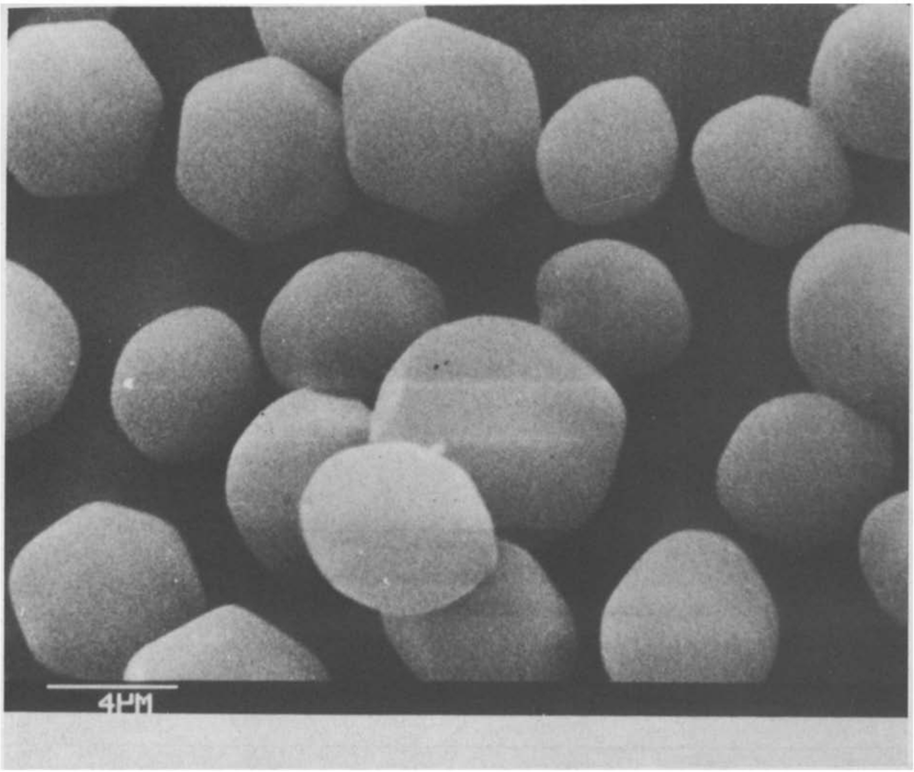

Fig. 3. Scanning electron micrograph of HMM showing some formation of hexagonal particles at an air flow rate of 0.3 $dm^3$/min, oil bath and condenser temperatures 182 and 70°C, respectively.

material 172–174°C) and carrier gas flow rates of 0.1–0.85 $dm^3$/min. The temperatures of the molten HMM and the oil bath were identical at steady-state within experimental error (±0.2°C). The condenser was kept at 20°C. Microscopic examination of the particles deposited on stage 6 of the impactor (the only slide stage used in this series of experiments) showed spherical particles (Fig. 2) which were reasonably monodisperse ($D_g \simeq 2$–$4$ $\mu$m, $\sigma_g \simeq 1.2$–$1.5$). The measured output was around 10 mg/min. Variation of the oil bath temperature and flow rate had caused no systematic trends in the quantity, or quality, of the product. However, when the temperature of the condenser was raised to 70°C at a relatively slow flow rate (0.3 $dm^3$/min) many of the particles appeared as compact hexagons (Fig. 3).

In the second series of experiments, the reproducibility of the generation was tested in short-run operation (flow rate 0.750 $dm^3$/min), oil bath and condenser temperatures 182.0 ± 0.1°C and 20.0 ± 0.1°C. The aerodynamic size distribution for three individual runs (complete shut-down between the runs) as determined by the cascade impactor is illustrated in Fig. 4. The reproducibility of the mass median aerodynamic diameter (MMAD $\simeq 4$ $\mu$m) and the geometric standard deviation ($\sigma_g \simeq 1.3$) is clearly evident.

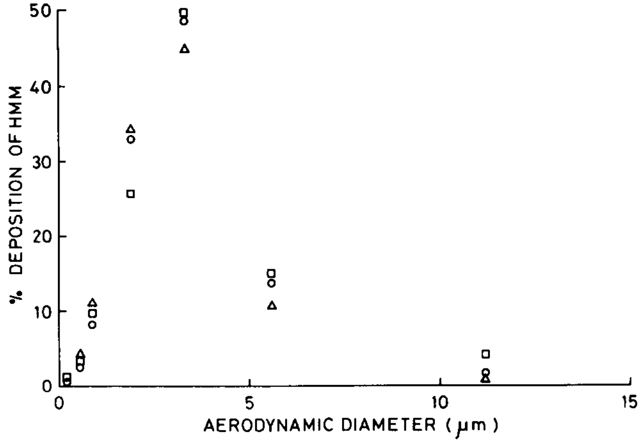

Fig. 4. Particle size distribution of HMM aerosols as determined by cascade impaction at a flow rate of 0.75 dm$^3$/min, oil bath and condenser temperatures 182 and 20°C, respectively. Different symbols represent results of independent runs.

Next, the operational characteristics in 'long-term' (up to 60 min) runs were tested at the same oil bath and condenser temperatures as above (182 and 20°C, respectively). The output collected at 5, 15 and 20 min of operation as a function of the gas carrier flow rate is shown in Fig. 5. The reasons for the variations in the output (mg/min) at different time intervals at high flow rates were investigated: it was noticed that despite employing an upstream flow regulator, the gas flow

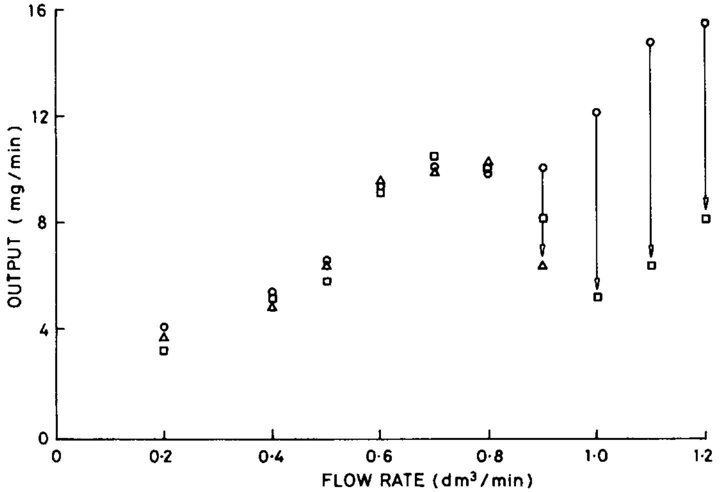

Fig. 5. Variation of the output with flow rate, temperatures as in Fig. 4. Output after 5 min (□), 15 min (○) and 20 min (△). The arrows point to the results obtained without cleaning the mouth of the condenser.

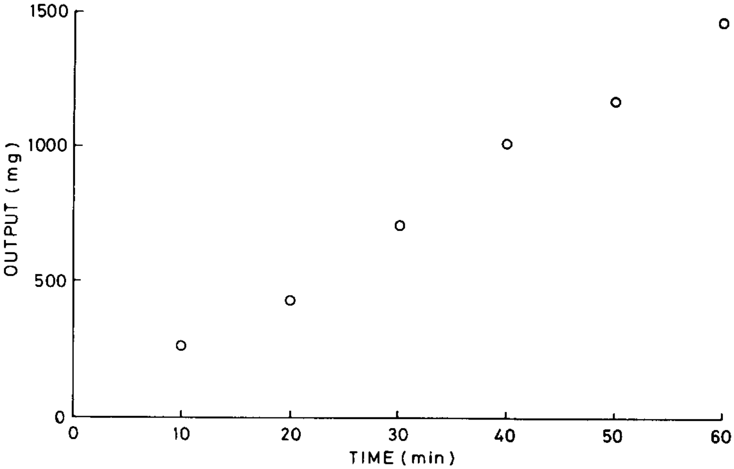

Fig. 6. Cumulative output for the same generation conditions as in Fig. 2.

gradually decreased (e.g. from an initial value of 1.2 dm$^3$/min to 1 dm$^3$/min after 5 min, and to 0.5 dm$^3$/min after 15 min). We suspected also that at these high flow rates, the temperature of HMM might be lowered in the course of the experiment but this was found not to be true. Finally, it was established that cleaning the mouth of the condenser from the excessive deposit of HMM with a glass rod returned the flow rate to the desired value and also resulted in output identical to that obtained after the first 5 min of operation.

It was therefore decided that high output could be achieved by working at still higher flow rates (cf. Fig. 5) which might also overcome the problem of blocking the mouth of the condenser. 4 g of HMM were placed in the three-neck flask. The flow rate was set to 2 dm$^3$/min (oil bath and condenser temperatures $182.0 \pm 0.1$ and $20.0 \pm 0.1$°C, respectively). Ignoring the first 2 min, the output was measured by 1 min collections every 10 min up to an hour; the average output was $(23.0 \pm 1.2)$ mg. The cumulative output in fact increased nearly linearly with time (Fig. 6). The aerodynamic size distribution of the aerosol was measured by cascade impaction at 10, 20 and 50 min. The results of deposition in the impactor are shown in Fig. 7. Analysis of these three sets of data gave MMAD = $3.6 \pm 0.1$ $\mu$m and $\sigma_g = 1.30 \pm 0.01$. The accuracy of $\sigma_g$ (as opposed to its reproducibility) is, however, likely to be of the order of $\pm 0.1$.

Both materials showed maximum UV absorbance at 227–228 nm and they had also identical $^1$H-NMR spectra. These results therefore confirmed that HMM did not change chemically during, or after, the generation procedure.

The density of the spherical particles from the generator was $(1.30 \pm 0.02)$ kg/dm$^3$ as compared to the parent material density of $1.11 \pm 0.02$ kg/dm$^3$. A variety of other tests (including X-ray diffraction, thermal analysis and solubility) (Khalik, 1984) confirmed that the spherical particles of HMM represented a new metastable polymorph of this drug (details of these findings will be published in another

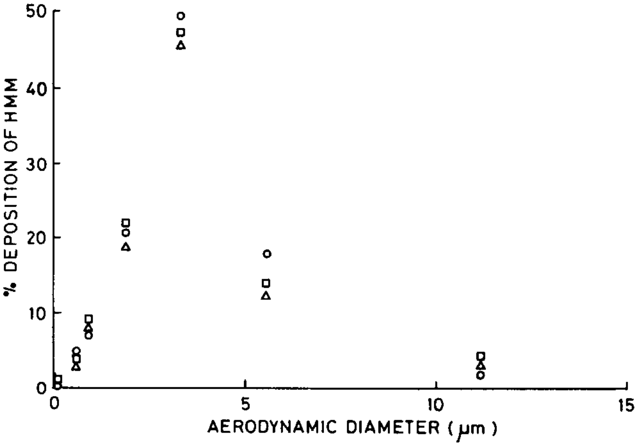

Fig. 7. Particle size distribution of HMM obtained by cascade impaction at the same conditions as in Fig. 2. Samples taken at 10 (○), 20 (□) and 50 (△) min.

report). Presumably, during the rapid cooling of the HMM vapour in the condenser, the drug is unable to form its normal hexagonal crystal lattice (Bullen et al., 1972). Instead, a more densely packed structure with cubic symmetry is formed (F.S. Stephens, private communication). This explanation is supported by the observation of hexagonal solid particles obtained at milder condensation conditions (flow rate reduced to 0.3 $dm^3$/min, condenser temperature raised to 70°C—see above).

In conclusion, moderately polydisperse aerosols containing respirable solid particles of HMM can be prepared in an evaporation-condensation generator without any detectable decomposition of the drug. The generator employed is comparatively simple, as it does not require a separate stage for preparation of condensation nuclei. The particle size distribution is probably sufficiently narrow for therapeutic purposes (Gonda, 1981b), even though no reheater is used. Blocking of the condenser observed particularly at intermediate flow rates can be overcome by periodic cleaning and it can be minimized by high flow rates which yield high output too. The generator shows reasonable stability in terms of output (mg/min) and particle size distribution over a period of up to 60 min and after shut down. It may be used for in situ preparation of aerosols for biological studies with suitable dilution to cater for the required respiratory flow rates. Alternatively, the product may be collected and redispersed when required. Rapid condensation of the HMM vapour leads to formation of spherical solid particles composed of a new metastable polymorph.

We have reported previously (Gonda et al., 1984; Khalik and Gonda, 1984) the preparation and characterization of HMM aerosols using different crystal habits of the stable polymorph nebulised either by dry powder dispersion, or by an air-blast generator. Thus, it may be possible to investigate the biological activity of this compound in its various physical forms.

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