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# THE INFLUENCE OF STORAGE ON THE CRYSTALLINE STRUCTURE OF FREEZE DRIED SODIUM ETHACRYNATE

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## ABSTRACT

Freeze-dried samples of sodium ethacrynate have been prepared and stored under specified conditions of relative humidity and temperature. Water content of the samples was determined by Karl Fischer and physical form by X-ray diffraction. Chemical integrity was assessed by an H.P.L.C. assay. Relationships between water content, storage conditions and crystalline structure have been investigated and related to the stability of the compound.

## INTRODUCTION

The freeze-drying process, also referred to as lyophilisation, is often used to prepare parenteral formulations of drugs that are unstable in aqueous solution. The physical form of freeze-dried drugs may be influenced by the conditions of the freeze-drying cycle (1-3) and this may result in differences in their chemical stability and/or dissolution characteristics. Hagerman and



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coworkers (2) related the stability of a commercial freeze-dried preparation of sodium ethacrynate (Edecrin, M.S.D.) to its physical form. Amorphous sodium ethacrynate was found to degrade rapidly whereas the crystalline material was relatively stable. However, there is little information on the interrelationships between water content, crystalline form and stability on storage.

In this study the influence of the freeze-drying process and subsequent storage conditions on the physical form of freeze-dried sodium ethacrynate has been investigated and related to drug stability.

## MATERIALS AND METHODS

### Materials

Ethacrynic acid B.P. was obtained from Merck, Sharp and Dohme, Hoddesdon, Herts. All other materials were analytical reagent grade unless otherwise stated.

### Preparation of Freeze Dried Sodium Ethacrynate

An aqueous solution containing 2% w/v sodium ethacrynate was prepared by adjusting the pH of an aqueous slurry of ethacrynic acid with sodium hydroxide to pH 7.0 (4). The solution was poured into metal trays, rapidly frozen to minus 50$^{o}$C at a rate of approximately 10$^{o}$C min$^{-1}$, and dried in the freeze drying unit [i]. On completion of the drying process the contents of each tray were placed in sealed containers to prevent the ingress of moisture.

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i. Model L10, Edwards High Vacuum, Crawley, Sussex.

Storage of Freeze Dried Samples

Freeze-dried powders were screened through a 500 μm aperture sieve to facilitate handling prior to storage at elevated temperatures. Samples of approximately 0.5 g were stored in open or sealed glass vials of 15 ml nominal capacity under selected conditions.

Moisture Content Determination

A known weight of sample was dissolved in anhydrous methanol and the total water present in each sample determined by automatic titration [ii] with Karl Fischer reagent, the end point being determined amperometrically. Duplicate determinations were made for each sample and the average water content recorded as a w/w percentage.

Determination of Physical Form of Samples

Differences in crystal form of the samples were investigated using X-ray powder diffraction[iii]. Because of the hygroscopic nature of the freeze-dried products a particular packing technique was used to prepare each sample. The top of the sample holder was placed against a perspex plate and the sample gently packed from the back of the holder against the perspex plate prior to clipping on the holder base. Removal of the perspex plate provided a smooth flat surface for study. Samples were rotated during analysis to provide a larger effective sample surface area and reduce the chance of a non-random distribution of crystallites

ii. Metrohm E547/3-20, Roth Scientific Equipment, Farnborough, Hants.
iii. Model XRD-5, Philips, Cambridge.

being obtained. Several replicate determinations were made to ensure the diffraction pattern obtained was representative of the whole sample.

Assay of Sodium Ethacrynate by High-Performance Liquid Chromatography

The high-performance liquid chromatographic (HPLC) system [iv] was equipped with a variable flow pump and an automatic loop injector. Additional components consisted of a column heater, autosampler, variable wavelength ultra violet-visible detector and electronic integrator. A 250 x 4.5 mm I.D. Hypersil 5 µm ODS packed column [v] was used at 50°C. The mobile phase consisted of methanol (HPLC grade) - 0.05 M phosphate buffer pH 5.6. The column was equilibrated with 52% methanol: buffer by volume and elution carried out at a flow rate of 1.5 ml $min^{-1}$ with detection at 279 nm. Standards and samples were dissolved in 50% v/v methanol in water to yield final concentrations of 50 $\mu g.ml^{-1}$ prior to injection of 20 µl on the column. Elution proceeded for six minutes at 52% methanol then for a further seven minutes at 65% methanol. The average of at least three determinations was used for each assay and duplicate assays were performed for all samples.

## RESULTS AND DISCUSSION

Samples removed from the freeze-drying unit after completion of the drying cycle were known as initial samples.

iv. Model 1084B, Hewlett Packard Ltd., Wokingham, Berks.
v. Shandon Southern Products, Runcorn, Cheshire.

The moisture contents of initial freeze dried sodium ethacrynate samples and those stored under specified conditions are given in Table 1. The initial samples were hygroscopic and rapidly picked up moisture during handling as seen by the water contents of the sample stored at 4°C and the initial screened samples. The X-ray diffraction patterns of the initial samples of

TABLE 1

Water Content of Freeze Dried Sodium Ethacrynate Stored Under Selected Conditions.

| Sample and Storage Conditions | Container | Water Content (% w/w) |
|---|---|---|
| a) Initial (unscreened) | - | <1 |
| b) Initial (unscreened) | - | 2.49 |
| c) Initial (screened) | - | 4.78 |
| d) Screened, 6 days at 30°C | Closed | 4.71 |
| e) Screened, 6 days at 30°C and 75% relative humidity | Open | 10.60 |
| f) Screened, 6 days at 60°C | Closed | 4.69 |
| g) Screened, 6 days at 60°C | Open | 0.40 |
| h) As (e) but dried over silica gel for 1.5 hours | Open | 5.87 |
| i) As (e) but dried at 60°C for 2 hours | Open | 3.50 |
| j) 1 day at 60°C followed by: | Open | |
| 12 days at 60°C | Closed | 0.45 |

it is not clear whether after hydration the amorphous material is crystallising as a pseudo polymorphic hydrate (i.e. the later removal of the water of hydration results in no change in crystal form) or if the increased moisture content is due to unbound water.

The X-ray diffraction patterns of the screened samples stored at 30°C and 60°C in sealed containers are compared with the initial screened sample in Figure 2. Reduced peak heights in the diffraction patterns, particularly apparent in the sample stored at 60°C are indicative of a reduction in crystallinity. No significant change in water content was observed in these samples as compared with the initial. It has previously been reported (3) that the stability of amorphous freeze-dried sodium ethacrynate is poor compared to that of the crystalline form, storage of the amorphous form for nine days at 60°C resulted in an 8% loss of potency whereas the crystalline form showed no less. In the present study those samples stored in sealed containers at 60°C , although yielding X-ray diffraction patterns indicating a partial or virtually total amorphous content,showed no degradation when assayed by HPLC. This stability data was indicative that the product was crystalline during storage and it is suggested that the formation of the amorphous component must have occurred on removal of the sample from the elevated storage temperature.

The apparent conflict between X-ray diffraction and stability data may be resolved in the following manner. When

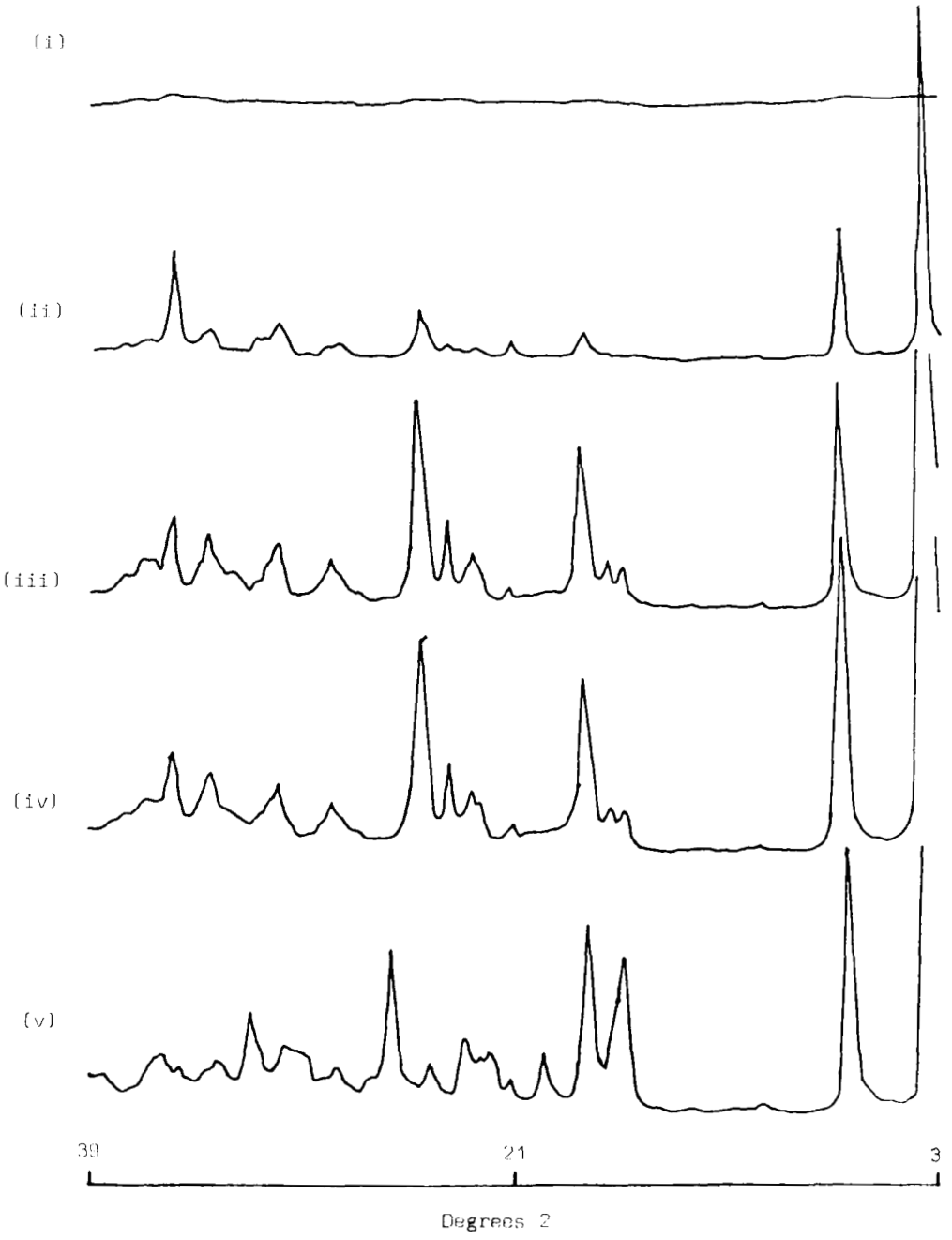


FIGURE 1: X-Ray Diffraction Patterns of Freeze Dried Sodium Ethacrynate (Recorder range 2000 for all samples),
(i) Initial unscreened material,
(ii) As (i) stored for 2 weeks at 4°C,
(iii) Initial screened material,
(iv) As (iii) stored for 6 days at 60°C in an open container,
(v) As (iii) stored for 6 days at 30°C and 75% relative humidity in an open container.

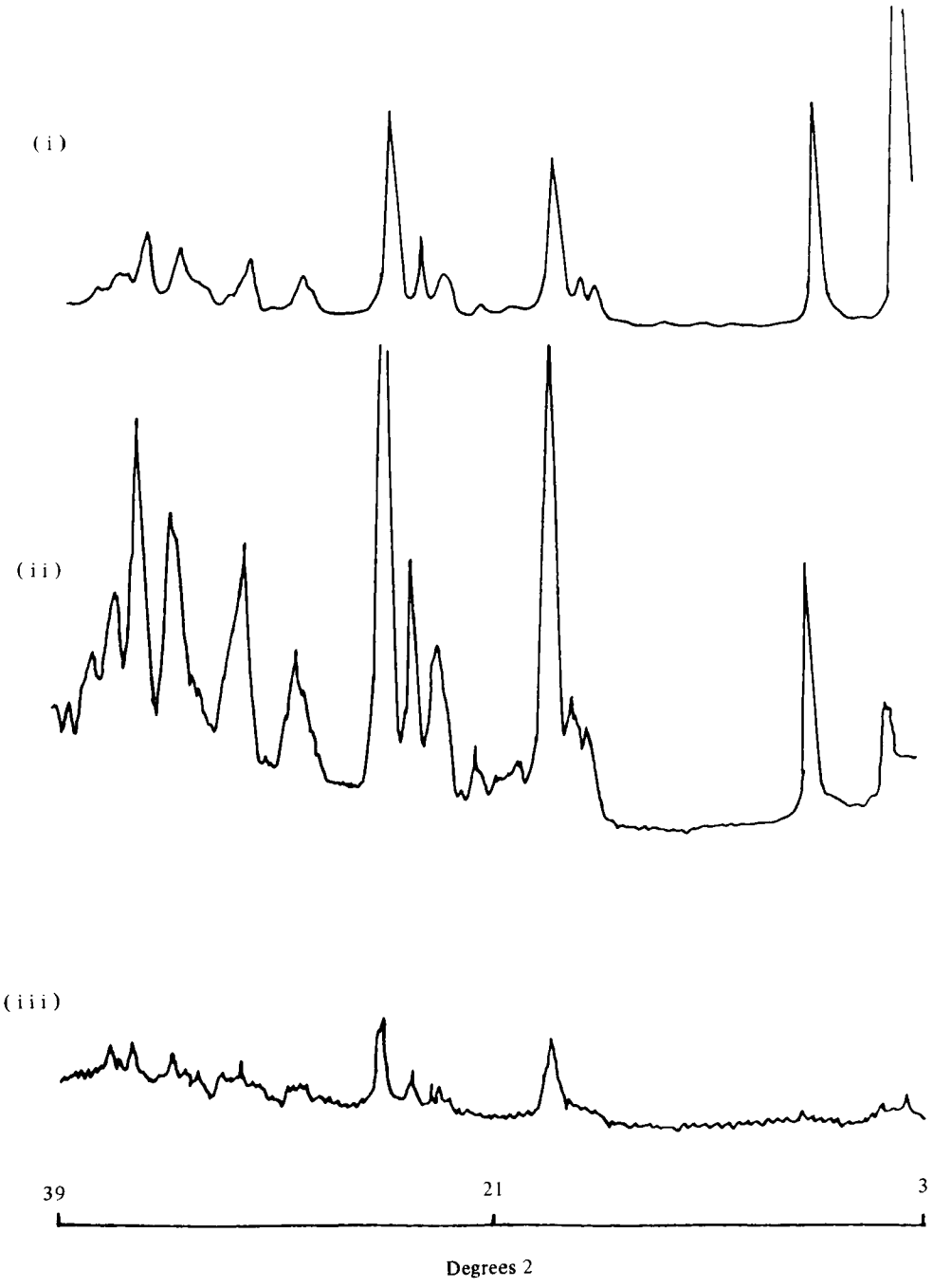


FIGURE 2: X-Ray Diffraction Patterns of Freeze Dried Sodium Ethacrynate.
(i) Initial screened material (Range 2000),
(ii) As (i) stored for 6 days at 30°C in a sealed container (Range 200),
(iii) As (i) stored for 6 days at 60°C in a sealed container (Range 200).

sodium ethacrynate and those stored in open containers at 60°C or 30°C and 75% relative humidity are shown in Figure 1. The initial unscreened samples were amorphous as indicated by the lack of peaks in the diffraction patterns. The presence of diffraction peaks and their increase in height in the samples stored at 4°C and the initial screened samples suggested that crystallisation of the amorphous solid was occurring and that this process was related to the water content of the samples. This concept was in agreement with the findings of Gattin and De Luca (5) who reported that amorphous cephalothin sodium could be obtained in a crystalline form by humidifying the freeze dried material.

A reduction of the water content of the screened samples from 4.78% to 0.40% w/w by storage at 60°C for six days did not alter the crystallinity of the samples indicating that the moisture mediated transition of amorphous to crystalline solid was not reversible.

The X-ray diffraction pattern of samples stored at 30°C and 75% relative humidity revealed a crystalline pattern different to that obtained from the initial screened samples. The water content (10-60% w/w) of samples exposed to different aqueous water vapour pressures suggested that they were the dihydrate of sodium ethacrynate. Drying of these hydrated samples over silica gel, to a water content of 5.87% w/w, or at 60°C for two hours to 3.50% w/w converted them back to the crystalline form of the initial screened sample confirming that the change inform was associated with one water of hydration. However, from these observations

samples are stored in a sealed container at elevated temperatures there will be an increase in the moisture content in the container headspace. Cooling of the vial to room temperature ( 20$^{O}$C) will result in condensation of excess moisture on the freeze-dried material and the formation of a concentrated aqueous solution containing sodium ethacrynate on the exterior of each particle. As the moisture equilibrates throughout the sample, sodium ethacrynate will precipitate as an amorphous surface layer on each particle. It has been reported previously (7) that rapid precipitation often results in the formation of an amorphous phase.

Two further observations support this hypothesis of the formation of an amorphous surface layer.

(i) The X-ray diffraction pattern of a crystalline sample was the same as the pattern for a sample stored for one day in an open container followed by twelve days in a sealed container at 60$^{O}$C, again implicating sample moisture content in the formation of the amorphous material.

(II) Surface abrasion of a sample (stored for six days at 60$^{O}$C) by passage through a 355 µm aperture screen, carried out under a flow of dry nitrogen to eliminate crystallisation due to high levels of ambient moisture, resulted in a sample giving a crystalline diffraction pattern similar to that of the initial material. Passage of amorphous or crystalline freeze dried sodium ethacrynate through a 355 µm aperture screen under these conditions did not result in any change of sample X-ray diffraction

pattern indicating that the screening procedure was not altering the physical form of the compound. These observations are suggestive that amorphous material is confined to the surface layer.

The extent of penetration of the X-ray beam into the samples is dependent upon the diffraction angle used and may be as low as a few tens of microns at low diffraction angles. This lack of penetration may explain the inability of the X-ray diffraction apparatus to distinguish the physical form of the core beneath the surface amorphous layer. The diffraction patterns of those samples stored in sealed containers at 30 and 60°C do show a proportionately greater reduction in peak height at the lower diffraction angles than at the higher angles supporting this hypothesis.

The results of this study suggest that great care must be taken in determining the physical form of freeze-dried materials and that prediction of the chemical stability of this compound based solely on X-ray powder diffraction data may prove to be erroneous, particularly if a knowledge of the sample history is not known or sample preparation not carefully controlled.

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