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PROCESSING FACTORS INFLUENCING THE STABILITY OF FREEZE DRIED SODIUM ETHACRYNATE

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ABSTRACT

The transitions observed in D.T.A. and E.C. profiles of aqueous sodium ethacrynate systems confirmed their potential to produce different physical forms of drug during freeze drying. The stability of freeze dried preparations was found to be related to the freezing rate, the concentration of sodium ethacrynate in the pre-lyophilised solution and the fill volume of solution. The stability profiles of the freeze dried preparations were related to the physical form of the drug and the production of a liquid degradation product.

INTRODUCTION

The freeze drying process, also referred to as lyophilisation, is often used to prepare stable parenteral formulations of drugs

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that are unstable in aqueous solution. The physical form, chemical stability and dissolution characteristics of such products can be influenced by the conditions of the freeze drying cycle. The poor stability of ethacrynic acid in aqueous solution indicates that commercial preparations should be freeze dried (Edecrin, M.S.D.). Hagerman and coworkers[1] have determined that the stability of the commercial preparation is dependent upon the physical form of the drug obtained. They found that sodium ethacrynate could be freeze dried as the chemically stable crystalline form or as the less stable amorphous form.

In this study an investigation is made of the influence of solution cooling rate, initial concentration of solute and the fill volume on the physical form and stability of freeze dried sodium ethacrynate. These parameters are characterized by the transitions observed in differential thermal analysis (D.T.A.) and electrical conductivity (E.C.) profiles and also the solubility of the drug within a system.

MATERIALS AND METHODS

Materials

Ethacrynic acid B.P. was obtained from Merck, Sharp and Dohme, Hoddesdon, Herts. All other reagents were analytical grade.

Differential Thermal Analysis - Electrical Conductivity Studies.

The system used for these studies was that described by Phillips[2]. Sample solutions (0.5, 2.0 and 4.0% w/w sodium ethacrynate) were prepared in distilled water. Distilled water

was used as the reference solution. Equal volumes (3ml) of sample and reference solutions were used for each determination. Cooling and heating rates were controlled by adjusting the sample holder to be either immersed in liquid nitrogen or held in the cold vapour stream above the surface of the liquid nitrogen.

Freeze Drying Studies

Solutions containing known concentrations of sodium ethacrynate were freeze dried in 20ml vials. The influence of selected freezing rates on the chemical and physical stability of the final preparation was investigated. The studies were performed using solutions filled into thin, neutral glass, flat bottomed vials and loosely stoppered with butyl rubber plugs specifically designed for freeze drying. The aqueous solutions of sodium ethacrynate were prepared as described[3] previously and known volumes were filled into the vials. The butyl stoppers were placed loosely in the vials and the contents were frozen using a slow freezing process to minus 25°C or by fast freezing to minus 50°C prior to drying in an Edwards Model L10 freeze drying unit. The dried products were sealed under vacuum. Details of the individual experiments are given below.

(a) Solutions Cooled Slowly: The loosely stoppered vials containing 2ml of 2% w/v solution of sodium ethacrynate were frozen slowly to minus 25°C on the shelves of the freeze drier over a period of 4 hours. The drying chamber was then evacuated and the samples dried under vacuum for 48 hours until the chamber

pressure and product temperature remained constant. The drying chamber was isolated and the vials sealed under vacuum.

(b) Solutions Cooled Rapidly: The loosley stoppered vials containing 0.5, 1, 2 or 3ml of 1, 2, 3 or 4%w/v solutions of sodium ethacrynate were loaded randomly onto one of the precooled shelves (minus 50°C) of the freeze drying unit such that the shelf was fully loaded. The shelves were maintained at minus 50°C for 4 hours. Drying and stoppering was accomplished using the same procedures as described for the slow cooled products.

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 (Metrohm E547/3-20, Roth Scientific Equipment, Farnborough, Hants.) 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.

Storage and Stability of Freeze Dried Sodium Eethacrynate

Samples of sodium ethacrynate were stored at 60°C and assayed at selected intervals by the HPLC procedure described previously[4].

Determination of Physical Form of Freeze Dried Sodium Eethacrynate

The physical form of the dreeze dried samples was determined by powder X-ray diffraction (Model XRD-5, Philips, Cambridge) and by microscopy (W. Watson and Sons Ltd., Barnet, Herts.).

RESULTS

D.T.A. and E.C. Studies

The D.T.A. profiles for solutions containing 0.5% w/w of sodium ethacrynate (Figure 1) show a shallow endotherm starting at minus 1.8°C and continuing to minus 1.5°C. An additional endotherm was often seen at the end of this shallow endotherm followed by an exotherm and then the liquidus endotherm. The exception to this pattern was the slow cooled-slow heated sample (b) in which small additional endotherms were seen in the middle of the shallow endotherm, there was no apparent liquidus endotherm, and a large exotherm was noted between minus 1.5°C and 5°C. No thermal events were observed below minus 2°C. For all the 0.5% w/w sodium ethacrynate samples, the E.C. profile indicated a decrease in resistance starting at minus 2°C and reaching a minimum or near minimum at minus 1.5°C. The E.C. profiles generally showed a two step decrease in resistance with the exception of the slow cooled-slow heated samples (b) which produced a smooth resistance decrease with time.

The D.T.A. and E.C. profiles of solutions containing 2% w/w sodium ethacrynate were similar to those obtained for solutions containing 0.5% w/w sodium ethacrynate, using approximately the same cooling and heating rates, but the plateau temperature where the shallow endotherm occurred was found to be minus 0.8°C compared with minus 1.5°C found for the solution containing 0.5% w/w sodium ethacrynate. A slow cooled sample of the solution

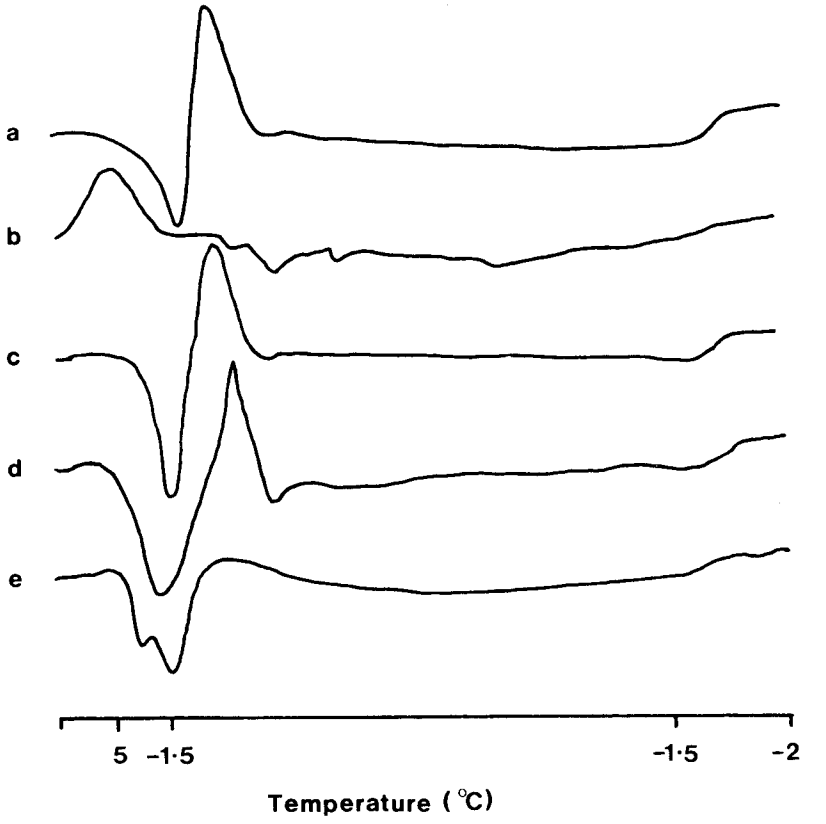

FIGURE 1

D.T.A. profiles of 0.5% w/w aqueous solutions of sodium ethacrynate under selected cooling and heating conditions.

Sample	Cooled to (°C)	Cooling Rate (°C min^{-1})	Heating Rate (°C min^{-1})
a	-140	20	1.5
b	-50	0.5	0.2
c	-45	1.5	1.5
d	-140	1.5	1.5
e	-140	20	0.2

containing 2% w/w sodium ethacrynate showed a similar profile to that of a solution containing 0.5% sodium ethacrynate with no apparent liquidus curve but a large exotherm between minus 0.7°C and 5°C on warming. For all the samples containing 2% w/w sodium ethacrynate, a gradual decrease in resistance was noted starting between minus 8° and minus 12°C up to the point where the shallow D.T.A. endotherm started, at which time a rapid decrease in resistance was observed.

Both the D.T.A. and E.C. profiles of a solution containing 4% w/w sodium ethacrynate rapidly cooled to minus 140°C and heated at minus 1.5°C min^{-1} indicated a transition occurring between minus 48°C and minus 14°C as indicated by a shallow D.T.A. endotherm and decrease in resistance (Figure 2). An increase in resistance was then noted to minus 8°C where it then began to decrease again.

Physical Form and Moisture Content

X-ray powder diffraction patterns of the slow cooled and fast cooled freeze dried sodium ethacrynate are shown in Figure 3. Microscopical examination of the initial freeze dried samples revealed relatively large plates of sodium ethacrynate present in those prepared from the more concentrated solutions and the larger fill volumes. All the freeze dried samples had a moisture content of less than 0.5% w/w.

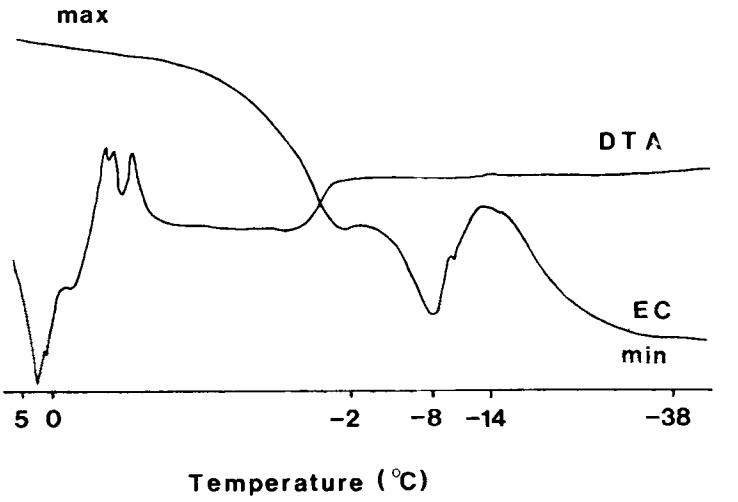

FIGURE 2

D.T.A. and E.C. profiles of a 4% w/w aqueous solution of sodium ethacrynate cooled to minus 140°C (20°C min^{-1}) and warmed at 1.5°C.

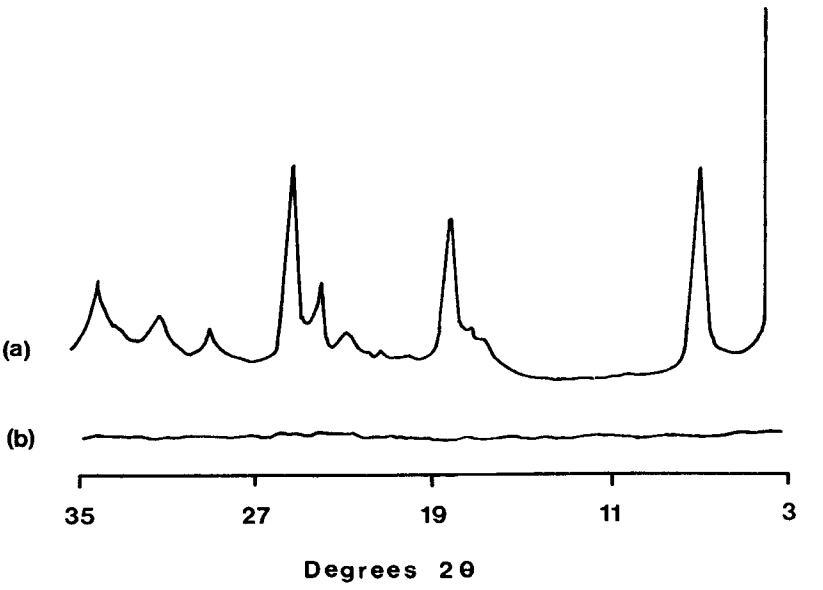

FIGURE 3

X-ray powder diffraction patterns of (a) slow cooled and (b) fast cooled freeze dried sodium ethacrynate.

TABLE 1

The Percentage (±S.D.) of Fast Cooled Freeze Dried Sodium Ethacrynate remaining after storage at 60°C for selected periods of time.

		Concentration of sodium ethacrynate (%w/v) in initial solutions			
Fill volume	Days	1	2	3	4
0.5ml	15	95.3 (0.6)	97.7 (0.6)	98.3 (0.6)	98.7 (0.6)
	30	82.7 (0.6)	92.0 (1.0)	93.7 (0.6)	94.7 (0.6)
	45	65.3 (3.0)	78.3 (4.0)	85.3 (3.0)	90.0 (1.0)
	60	42.3 (5.0)	62.0 (2.0)	73.7 (4.2)	79.3 (2.1)
1.0ml	15	98.7 (0.6)	98.7 (0.6)	99.3 (0.6)	99.3 (0.6)
	30	93.7 (1.5)	94.2 (1.2)	95.3 (0.6)	96.3 (0.6)
	45	85.0 (2.6)	88.7 (1.5)	89.7 (1.5)	92.7 (1.2)
	60	72.0 (4.6)	82.3 (2.1)	84.3 (1.5)	87.7 (0.6)
2.0ml	15	98.7 (0.6)	98.3 (0.6)	98.7 (0.6)	99.0 (0.0)
	30	93.7 (0.6)	94.3 (1.2)	92.8 (2.0)	93.0 (1.0)
	45	87.0 (3.0)	90.7 (0.6)	85.0 (3.0)	85.7 (1.2)
	60	77.0 (4.0)	83.0 (1.0)	74.3 (2.5)	78.0 (2.0)
3.0ml	15	98.3 (0.6)	98.7 (0.6)	99.3 (0.6)	99.7 (0.6)
	30	94.3 (0.6)	92.3 (1.5)	94.0 (0.0)	96.0 (1.0)
	45	88.7 (1.5)	86.0 (2.0)	86.7 (1.5)	92.3 (0.6)
	60	78.7 (5.5)	74.7 (2.5)	76.7 (4.5)	84.0 (1.0)

Chemical Stability

The loss of chemical integrity of sodium ethacrynate prepared by the fast freezing process as a function of period of storage at 60°C is shown in Table 1. Stressed fast cooled samples partially or totally liquefied to produce a clear oil. Sodium ethacrynate freeze dried using the slow freezing process retained greater than 95% of its initial potency after storage

for 9 months at 60°C. The only significant degradation product observed using HPLC was identified as ethacrynic acid dimer.

DISCUSSION

The findings of the D.T.A. and E.C. studies suggested that a variety of amorphous and crystalline forms of sodium ethacrynate could be formed dependent upon the concentrations of solute and the freezing conditions employed during freeze drying. D.T.A. profiles were generally similar and showed a shallow endotherm followed by a sharp exotherm. Such a profile is typical of a glass transition of a compound followed by its crystallisation. A glass transition may be defined as the passage from a vitreous state to a highly viscous supercooled liquid. Crystallisation is the passage from the amorphous to the crystalline state. When the 'glass' is warmed it changes into a supercooled liquid where the water molecules undergo rotational and translational motion in addition to the vibrational motion found in the glassy state. The temperature at which this transition occurs will depend on the nature and concentration of the solute and the interaction between solute and water molecules. As the temperature is increased further the molecules acquire sufficient mobility to pass from the amorphous state to the ordered structure of a crystal. The E.C. profiles of the solutions containing 0.5% w/w sodium ethacrynate were in agreement with this hypothesis. A gradual decrease in resistance corresponding to the time of the shallow endotherm and increase or plateau in resistance noted

at the time of the exotherm supported the hypothesis of glass transition and crystallisation. The solutions containing 2% w/w sodium ethacrynate had similar D.T.A. profiles to those containing 0.5% w/w sodium ethacrynate. The E.C. profiles of these solutions were also similar but a very gradual decrease in resistance on warming from approximately minus 12°C was noted in the 2% w/w solutions suggesting some melting or glass transition event occurring, however this was not apparent from the D.T.A. trace. The D.T.A. and E.C. profiles of a solution containing 4% w/w sodium ethacrynate was more complex and the transition between minus 38° and minus 14°C was considered to be another glass transition followed by crystallisation between minus 14° and minus 8°C. A further glass transition and crystallisation was indicated by the decrease in resistance noted between minus 8°C and minus 2°C. The thermal events occurring on heating above minus 2°C were as described for the solutions containing 0.5% and 2% w/w sodium ethacrynate. The presence of glass transitions in the frozen solutions indicated that dependent upon the freezing conditions employed during the freeze drying cycle, crystalline or amorphous material may be obtained. The differences in X-ray diffraction patterns for the dreeze dried samples revealed that crystalline material was obtained by the slow freezing process and amorphous material by the fast freezing process.

Stability of freeze dried sodium ethacrynate prepared in vials and sealed under vacuum was assessed for degradation after

storage at 60°C for selected periods of time. This sealing procedure ensured that samples were studied under low humidity conditions preventing crystallisation of amorphous material present within the samples. Stability of the 'slow frozen' crystalline material was superior to that found for any of the 'fast frozen' amorphous samples and indicated that the physical form influences the chemical stability of sodium ethacrynate.

On the basis of the known degradation mechanism of sodium ethacrynate in solution[3], second order degradation kinetics were anticipated. However a superficial examination of the loss of sodium ethacrynate with time indicated that degradation proceeded at an ever increasing rate over the time period studied. Sodium ethacrynate dimer was the only significant degradate observed suggesting that it was the apparent rate of reaction that was changing and not an additional degradation pathway operating. The observation that the dimer was produced as an oil in samples of amorphous freeze dried sodium ethacrynate provided an explanation of the increasing apparent rate of reaction.

Stability is dependent upon both the concentration of the solute and the fill volume employed. For fill volumes of 0.5 and 1ml, least degradation was noted in preparations freeze dried from solutions containing 4% w/w sodium ethacrynate. For these fill volumes stability gradually decreased with decreasing concentration of the solutions used to prepared the samples. For

the 2ml and 3ml fill volumes stability did not appear to be a function of concentration.

It would be expected from the known characteristics of the system that the larger fill volumes will take longer to freeze and visual observation of the freezing of samples during the freeze drying process confirmed this. For 0.5ml fill volume freezing was almost instantaneous, whereas for the 1, 2 and 3ml fill volumes complete freezing took up to several minutes. This additional time in the solution phase and the greater temperature gradients present may allow some of the sodium ethacrynate to freeze in the crystalline form, or in the case of the more concentrated solutions, allow crystalline sodium ethacrynate to be precipitated as the equilibrium solubility is known to decrease with decreasing temperature. Precipitation of crystalline plates of sodium ethacrynate in many of the higher fill volume samples was confirmed by microscopy, however a quantitative assessment of the extent of precipitation could not be made, thus the final solution concentration immediately prior to freezing was not known. The complex interacting pattern of events occurring during freezing allowed an explanation of the inconsistent profiles of loss of ethacrynic acid with time observed in the higher fill volume (2 and 3ml) samples.

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