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COMPARATIVE AND PREDICTIVE EVALUATION OF THE STABILITY OF DIFFERENT FREEZE-DRIED FORMULATIONS CONTAINING AN AMORPHOUS MOISTURE-SENSITIVE INGREDIENT

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ABSTRACT

The stability of a moisture-sensitive drug is not only determined by its own physical state, but also by the formulation in which it is present. This paper demonstrates that decomposition of amorphous vecuronium bromide in a formulation is a function of the water activity rather than of the water content in relative or stoichiometric terms. For freeze-dried formulations this means that the disadvantageous lyophilization characteristics of glass forming excipients can have definite stabilizing, other than cryoprotective, effects. With knowledge of degradation kinetics at various water levels, moisture isotherms of the formulation and the properties of package, shelf-life of a product can be estimated.

INTRODUCTION

Freeze-drying is applied to stabilize aqueous drug solutions by removing water. A lyophilization cycle

distinguishes respectively a freezing step, in which the solution is solidified, a sublimation or primary drying phase, where ice is removed by sublimation and finally a secondary drying phase during which absorbed water is removed from the solid structure (1,2). The latter process involves diffusion and proceeds therefore rather slowly (3). In this respect, the choice of the excipients greatly determines the speed of drying. Substances forming an eutect with water crystallize without incorporating a significant quantity of water. In contrast, compounds forming a glassy state solidify as a molecular mixture with water. If the eutectic temperature is high, the first category of excipients can be dried rapidly at relatively high temperature without melting phenomena occurring. However, when amorphous material is present, sublimation temperature must be maintained below the so-called collapse temperature, which exhibits mostly a low value. As a consequence, heat transfer is limited and sublimation takes a long time (4). Because of the slower sublimation and secondary drying properties of amorphous materials, there is a strong preference for crystalline excipients. Mannitol is a frequently-used example having an advantageous high eutectic temperature of -6°C. The application of glass-forming excipients is often only concerned because of cryoprotective reasons in formulations with e.g. proteins (5). This paper discusses the consequences of the physical state of excipients on the chemical stability of vecuronium bromide, a muscle relaxant, which is manufactured in a freeze-dried formulation because of its sensitivity towards hydrolysis.

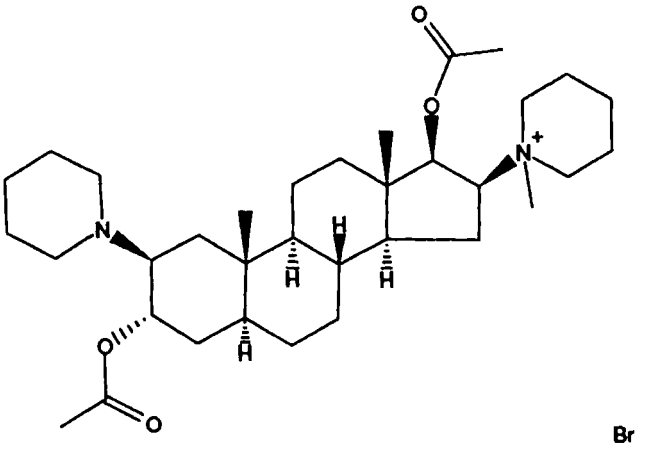

FIGURE 1

The drug substance used in this study: vecuronium bromide

MATERIALS AND METHODS

The model drug used is vecuronium bromide (Figure 1), a neuromuscular blocking agent. Decomposition of the drug substance involves the hydrolysis of the acetate groups.

Two totally different formulations were prepared:

	Formulation A	Formulation B
vecuronium bromide	10 mg	10 mg
citric acid 1 aq	20,75 mg	3,5 mg
$Na_2 HPO_4$ 2 aq	16,25 mg	0,64 mg
mannitol	170 mg	8 mg
lactose	-	8 mg
	217 mg	30 mg

The buffer is added to keep a 5 ml solution at pH=4. As can be noticed, the active ingredient contributes to the acidity of the solution.

Three separate batches of each formulation were lyophilized in a Leybold GT20 freeze-dryer and stored in 10 ml vials with 13 mm closures of the bromo butyl rubber type (6) at several storage conditions (Hereaus Klimaprüfschränke). The quantitative determination of the active component was performed on Waters hplc equipment, using a Lichrosorb Si-60-10 column at 40°C, and a uv-detector at 210 μm. The mobile phase was prepared by dissolving 2.68 g NH_4Cl in 10.0 ml 25% NH_4OH and mixing with 990 ml methanol.
The moisture content of the samples was assessed using a coulometric Karl Fischer method applying a Mitsubishi moisture meter, model CA-05. Thermal analysis was carried out with a Perkin Elmer DSC-7 using 50μl closed Al-pans and applying a scanning rate of 10°C/min. Fourier transform infrared spectroscopy was performed on a Digilab FTS 15/90 FT-IR spectrometer.

RESULTS AND DISCUSSION

Figure 2 shows thermograms of the active substance. In the crystalline state a melting peak is observed at approximately 255°C. After freeze drying, the peak is not present any more, indicating an amorphous state. Thermograms of the formulations do not clearly confirm this because of the strong decomposition signals from the excipients. However, Fourier transform infrared spectroscopy supported the amorphicity of the drug in formulation B, where the concentration is just high enough to be determined.

Figure 3 demonstrates the moisture isotherms of both formulations at 20 and 40°C. Clearly, formulation B takes up relatively more water than formulation A. One clear reason for this is that lactose in formulation B is present in the amorphous state. This is also evident

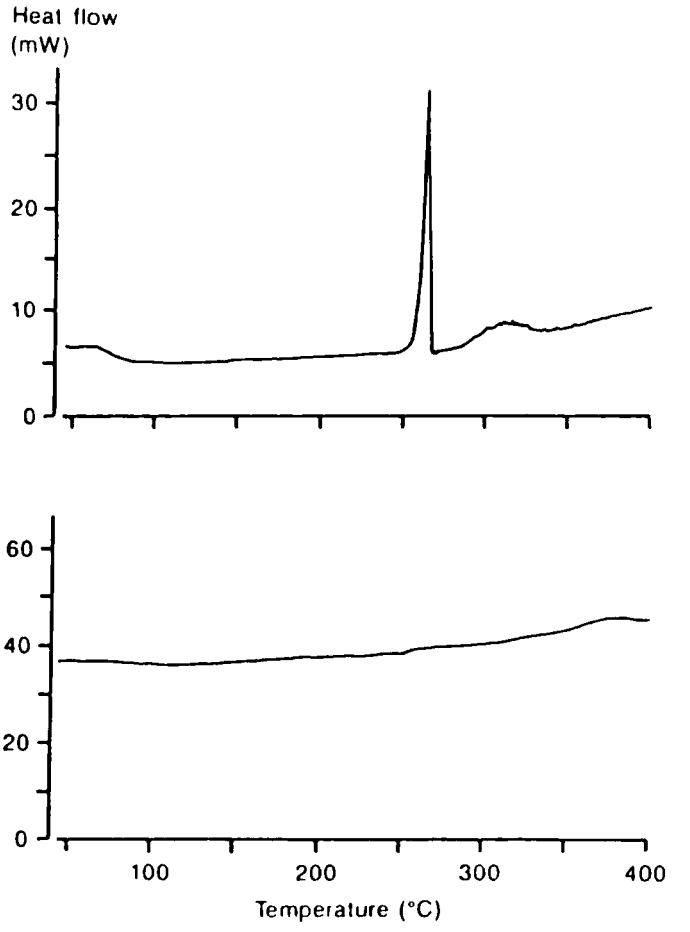

FIGURE 2

Thermograms of vecuronium bromide as a crystalline starting material (upper part) and after freeze-drying (lower part)

from the deviation of the curves at 30 - 40 % R.H., where crystallization occurs (7,8). In contrast to crystalline lactose, the amorphous sugar can absorb up to 10% of water. Formulation A contains crystalline mannitol as bulk former. This only adsorbs water, which is also obvious from the fact that mannitol formulations hardly require a secondary drying phase.

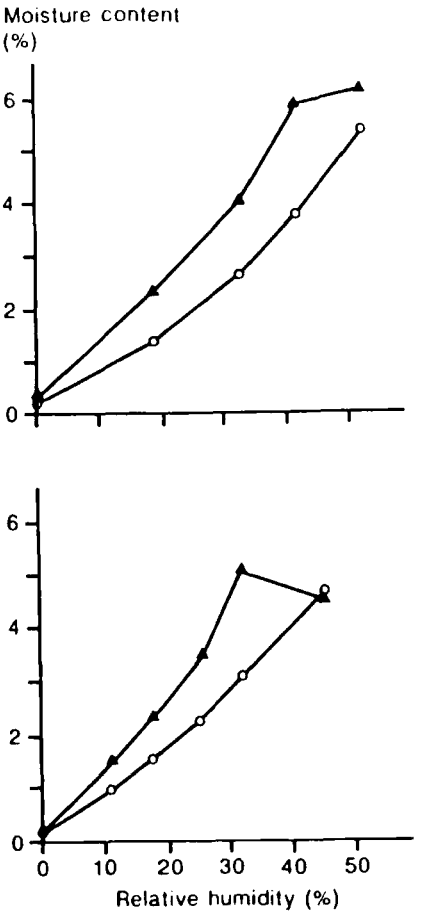

FIGURE 3

Equilibrium moisture content as a function of relative humidity of both formulation A (○) and B (▲) at 20°C (upper) and 40°C (lower)

Samples of formulation A were opened to take up moisture to certain distinct levels. Subsequently, the vials were stored at temperatures up to 40°C during 2 years. After 6, 12 and 24 months, the degradation was quantitatively assessed. Although a hydrolysis reaction is principally not first order, the reaction rate

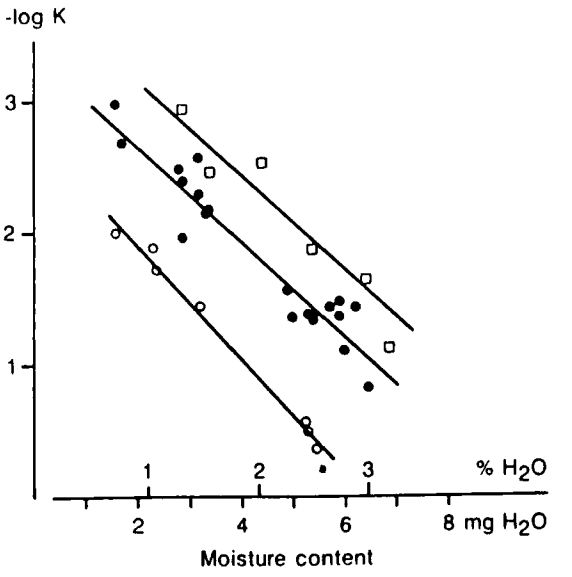

FIGURE 4

Decomposition rate constant k ($month^{-1}$) as a function of water content at 25°C (□), 30°C (●) and 40°C (○) for degradation of formulation A

constant k was still calculated according first order kinetics. This is permitted since the amount of water is not limited, i.e. no significant chamge in the moisture content was detectable.

Figure 4 depicts the decomposition rate constant k as a function of water content at different temperatures. The best fit lines were drawn through the experimental data. From figure 4, Arrhenius plots can be derived at distinct moisture levels, as shown in figure 5. Both the figures 4 and 5 give insight into the role of water on the speed of hydrolysis of the drug. Furthermore, the plots enable one to estimate the shelf-lifes of the product at any reasonable moisture level and storage condition.

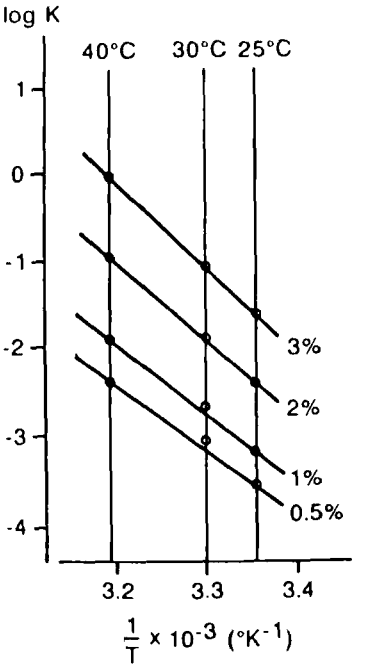

FIGURE 5
Arrhenius plots derived from figure 3

Figure 6 shows some representative differences in stability of the two formulations. As seen, formulation A decomposes considerably at a constant humidity level of approximately 1.2%. Formulation B degrades to a lower extent, whereas higher levels of water content are exhibited. Furthermore, it can also be seen that the moisture content increases steadily. As a matter of fact, this latter effect is a consequence of the lower mass; the entrance of 0,3 mg of water into the vial is equal to an increase of 0,1% water content for formulation A, but of 1% for formulation B. Strikingly, in spite of this apparent unfavourable tendency, formulation B still decomposes to a lower extent than

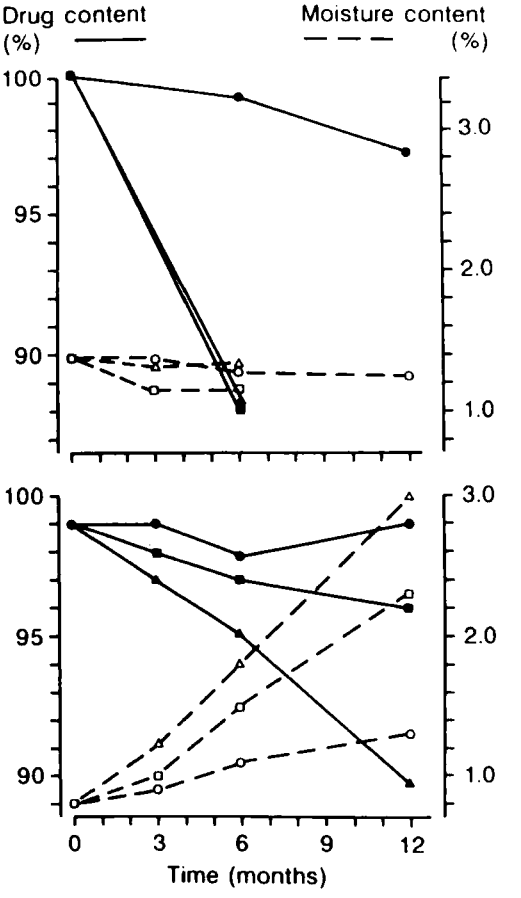

FIGURE 6

Decomposition and water content during storage at 30°C / 75% R.H. (○, ●), 40°C / ambient humidity (□, ■) and 40°C / 95% R.H. for formulation A (upper part) and formulation B (lower part)

formulation A. Obviously, the amount of water present in the formulations is not equally available for the hydrolysis reaction. At the same time, it is clear that the stoichiometric drug : water ratio is not reflected in the degradation effects either. To explain the different behaviours, figure 3 has to be reconsidered.

As can be observed, formulation B can absorb more water to reach the same equilibrium vapour pressure. Since the active substance is amorphous, it's water content will be determined by the equilibrium vapour pressure, which is defined as the water activity. This relative availability of moisture is kept low in preparations with a high sorption capacity. In fact, this absorption can be considered as the reverse process of secondary drying during lyophilization. As was argued before, mannitol formulations hardly exhibit a secondary drying phase. Apparently, the advantage of the rapid drying can have unfavourable consequences on the stability of a product. Moisture-sensitive pharmaceutical products are frequently stabilized by including desiccants in the package (e.g. 9,10). The results from figure 6 point out that alternatively, the desiccant can be incorporated in the formulation.

When considering the preceeding results in more detail, one realises that the shelf-life of the products is strongly determined by the protective properties of the packaging material. In the case of a vial presentation, the rubber closure forms the critical barrier against the entrance of water. Previously, several types of rubber have been evaluated with respect to their water permeability (6). It was demonstrated that the PH 4104 (Pharma Gummi) stopper exhibited a permeation of 7,2 μg water/week at 40°C and 95% R.H.. Starting with a water content of 1% and taking a permeation of 7 μg/week into account, the decomposition of formulation A can be calculated using figure 5. A similar degradation profile can be achieved for formulation B when a correction is made for the water content by taking into consideration the actual water activity using figure 3. For example, 2% of water for formulation B equals a moisture content

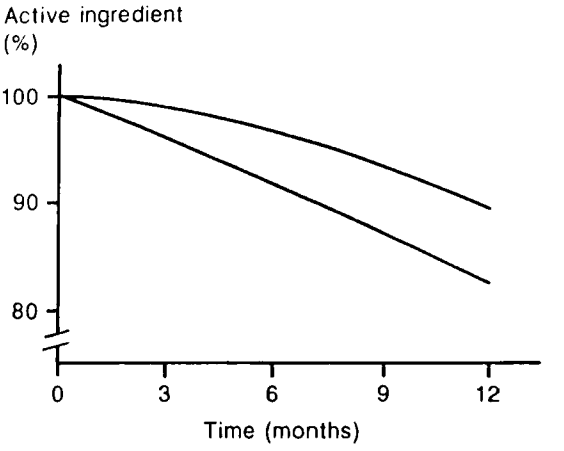

FIGURE 7

Calculated decomposition of formulation A (lower curve) and formulation B (upper vurve), stored in vials at 40°C and 95% R.H., starting with an initial water content of 1% and a water permeation of 7 μg/week

of 1% for formulation A, both having a water activity of 15%. In this way, the assumption is made that the water activity of the formulations yields a better indication for the decomposition potential than the water content. The calculated degradation curves are shown in figure 7. When comparing the calculated profiles with the experimentally-derived
data (figure 6), it is obvious that a reasonably good agreement exists. This confirms that it is not the water content, but the relative availability of the present water, i.e. the water activity, which is of significance in determining the product's stability.

The results discussed reveal that consideration of water activity properties can be helpful in formulation work. One should notice, however, that this holds for amorphous substances and when decomposition is directly

related to the presence of moisture. Subsequently, knowledge of the quality of primary packaging enables one to make predictions towards shelf-life.

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