Stability of human therapeutic proteins

M. J. Geisow

BIODIGM, Nottingham, UK

Instrument:

2277 Thermal Activity Monitor

Field of application:

Industrial biotechnology

Date:

March 1992

Introduction

Proteins are finding increased application in human medicine now that they can be produced by recombinant DNA technology. Protein medicines are treated as conventional drugs for regulatory purposes and are subject to stringent testing to ensure product quality. One aspect of product quality that is important for proteins is stability, since their integrity and biological activity is known to be more affected by time and temperature than conventional small molecule drugs. This application note describes the use of the ThermoMetric 2277 Thermal Activity Monitor (TAM) to assess the stability of human albumin. The effect of formulation with stabilizers is also described.

Human albumin

Human albumin is a single-chain protein of 585 amino acids, normally present in human plasma at a concentration of 42 g/l, where it contributes 80% of the colloid osmotic pressure of the blood. It is administered in cases of fluid loss following major trauma, such as burns or surgery. The current product is obtained by fractionating pooled human plasma. It is formulated with stabilizers, N-acetyltryptophan and fatty acids, pasteurised at 60 °C for 10 hours to inactivate micro-organisms or virus, and stored at 4 °C. The British Pharmacopoeia requires that the product shows no visible change following this treatment.

The thermal stability of human serum albumin has previously been investigated by differential scanning calorimetry to determine the effect of formulation with stabilizers on the temperature of denaturation. No high sensitivity isothermal measurements of human albumin stability appear to be have been reported. Normally, shelf-life trials of proteins under isothermal storage conditions take place with a two year period indicated for human albumin. This obviously creates difficulties both in the time required and the reliability of assays over such an extended period. In the experiments described, human serum-derived albumin samples were maintained at 57 °C for at least 20 hours in the Thermal Activity Monitor, to monitor any changes in formulated and unformulated products (5% w/v) and to provide a general indication of the stability of the serum-derived products. The elevated temperature of 57 °C was chosen to conform to conditions stipulated in the British Pharmacopoeia.

Experimental

The TAM was fitted with twin ampoule calorimetric units and thermostatted at 57 °C. The TAM was controlled and heat flow data collected using DIGITAM 2 software. Data points were mean values over 1 minute, collected every minute.

Human albumin samples, 50 mg/ml with salts (nominal volume 2.0 ml), were aseptically dispensed into heat-sterilized glass crimp cap vials and sealed. Sample and reference vials (pure water) were pre-equilibrated at 57 °C for 60 minutes before being introduced into the measurement cells of the TAM. Differential heat flow in μWatts was monitored over the period of the experiment (20 - 25 hours) and recorded from the time that the baseline of the control experiment (formulation buffer) was stable. In the results shown, no correction for minor differences in sample volume has been made.

Results and discussion

Heat flow curves for various 5% w/v human albumin solutions are shown in Figure 1. All the measurements were commenced after the samples had reached thermal equilibrium. The protein solutions absorbed more heat than the formulation buffer over the period of the experiment (Fig. 1, curve A). Human serum albumin absorbed marginally more heat over the period of the experiment and this apparently lower stability was reproduced in replicate experiments (Fig. 1, curve B). Formulation of albumin without stabilizers led to a large endothermic process in the sample, resulting in a stable gel within 20 hours (Fig. 1, curve C). As albumin solutions are heated, (or aged) there is an increase in the number of multimeric species present. This probably arises from limited localized denaturation or unfolding, which uncovers hydrophobic areas of the protein, leading to aggregation. In the case of albumin, this aggregation produces covalently-bound polymers. This process is clearly minimized by the stabilizers. A large number of samples were tested and there was no significant difference between the stability of albumin samples formulated with stabilizers both below and above the usual concentration. This means that the protective effect could be developed with less stabilizer at 57°C, perhaps with implications of cost-saving.
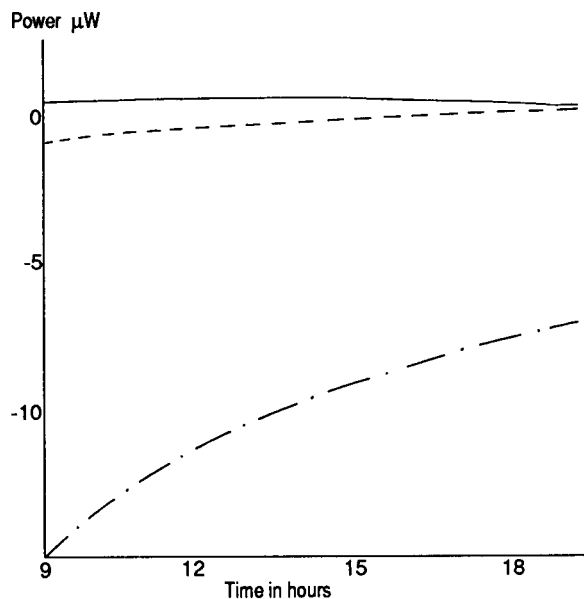

Fig. 1. *Differential heat flow in samples of human albumin at 57 °C. Where added, stabilizers were sodium caprylate and N-acetyltryptophan.*

Curve A	_____	*Formulation buffer only*
Curve B	- - - - - - -	*Human albumin formulated with stabilizers*
Curve C	. __ . __	*Human albumin formulated without stabilizers*

Conclusions

These experiments were conducted to determine whether the TAM could place the present empirical tests of human albumin stability on a more quantitative footing for regulatory authority scrutiny. They were also carried out to lay the groundwork for comparing recombinant and human material and for determining the effect (at 57 °C) of stabilizer concentration. The experiments showed that the TAM can be used to obtain albumin stability data within a day. At 57 °C, the effect of formulation on stability was very considerable, however, there was no significant difference in stability over 20 hours between samples containing stabilizers.

References

Gumpen, S., Hegg, P.O. and Martens, H. - Thermal stability of fatty acid and serum albumin complexes studied by differential scanning calorimetry - Biochem. Biophys. Acta, 574:189-196 (1979).