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Stabilisation of Proteins: to Next

This disclosure concerns methods for stabilising proteins such that they can be heated to inactivate potential viral contaminants without substantially destroying the biological activity of the protein.

A variety of stabilising methods have been previously developed for this purpose. Examples include the use of:


In all of these methods, the proteins are stabilised to the extent that they can be heated in solution for at least 10 hours at 60°C to inactivate hepatitis virus (S.S. Gillis et al. J. Clin. Invest. 28 : 239-244, 1949).


In this invention sorbitol and glycine have been used to stabilise a factor VIII, factor IX and immuno-globulin. Fibrinogen, platelet factor 4, alpha-1-antitrombin and thrombospashin were also found to be stabilised by this method. Fibrinogen was stabilised using sucrose alone.

Factor VIII Concentrate. In the presence of 1.7% w/v glycine and 6% w/v sorbitol an intermediate-purity factor VIII solution was heated for 10 hours at 60°C with a recovery of over 70% of the Factor VIII activity. (A.J. Macleod et al. Thromb. Haemostasis 50 : 432, 1983). Further improvements have been achieved by the addition of calcium chloride to the stabilising mixture and by the use of either polypropylene or stainless steel containers for the heating process. A number of operating conditions are possible on the precise composition of the factor VIII solution to be heated. Sorbitol concentration can be in the range 50 - 75 w/ v with about 65% w/v providing an appropriate balance between stabilisation and problem of high viscosity and high dilution which result at higher concentrations. Glycine can be used in the ratio 1.2% w/v depending on the fibrinogen content of the factor VIII solution, as 2.1 molar glycine is known to precipitate fibrinogen. Calcium is added to maintain an excess of lime calcium of about 20%. The quantity of calcium to be added depends on the concentration of calcium binding ions present in solution, such as citrate, but will generally be in the range 1-10 mM. With these improvements factor VIII solution can be heated more severely than in previous applications (60°C for 10 hours) and the stabilised proteins may be more heat-resistant to be inactivated. For example, an intermediate purity factor VIII solution in the presence of 1.7% w/v glycine, 6% w/v sorbitol and 2.2 x 10⁻⁵ M w/v calcium chloride was heated at 60°C for 9.5 hours followed by 70°C for 0.5 hours with a 77% recovery of factor VIII activity. This additional heating increased the degree of inactivation of vaccinia virus from about 10⁻⁶ plaque forming units/ml to over 10⁻⁷ p.f.u./ml (A.J. MacLeod et al. Proc. 10th Congress Int. Soc. Blood Transfusion, 1984).

In further experiments factor VIII solution was heated at 60°C for 22 hours followed by 0.5 hours at 70°C or at 70°C for about 1 hour giving over 70% recovery of Factor VIII activity in both instances. Hence these stabilisers allow various combinations of heating times and temperatures to be used within the broad range from a few minutes at about 70°C to many hours at 60°C.

Factor IX Concentrate. A solution containing coagulation factors II, IX and X has been stabilised in the presence of 2.5% w/v glycine and 55% w/v sorbitol giving a 65% recovery of Factor IX activity after heating at 60°C for 10 hours (A.J. MacLeod et al. Thromb. Haemostasis 50 : 432, 1983). Further experiments with sorbitol concentrations and heating conditions in the range described above for factor VIII, have given factor IX recoveries of over 80% after heating.

Immunoglobulin Concentrate. Immunoglobulin products in clinical use are generally considered safe despite the fact that they are not pasteurised to inactivate viral contaminants. However recent evidence of hepatitis transmission by human immunoglobulin (R. Lane et al. Lancet ii : 974, 1982) together with concern about other potential viral contaminants associated with human blood products (eg. AIDs) suggest that heat treatment of immunoglobulin may become of interest.

Immunoglobulin stabilised in the presence of 10% w/v glycine and 65% w/v sorbitol was heated in solution at 60°C for 10 hours without loss of specific antibody function (rubella) and without increase in either aggregate content or anti-complementary activity (A.G. Welch et al. Lancet ii : 1196, 1983). The concentrations of glycine and sorbitol required are related to the protein content of the solution but are generally in the range 5 - 10% w/v glycine and 30 - 70% w/v sorbitol. As with Factor VIII, heating conditions more severe than 10 hours at 60°C can be applied including temperature up to 75°C for up to 1 hours. Heating can also be carried out in serum albumin or to 5% ethanol without loss of specific antibody function (rubella). Similar results have been achieved using sucrose in place of sorbitol.

Sorbitol alone is sufficient to stabilise fibrinogen and a concentration of about 30% w/v allows fibrinogen to be heated to 60°C for 10 hours. Higher concentrations of sorbitol can be used if more severe heating conditions are required.

This invention concerns the heat-stabilisation of human proteins some of which are particularly stable or sensitive to heat induced damage (Factor VIII, Fibrinogen). Therefore the method described has a wide general application for the stabilisation of virtually all proteins which require to be exposed to heat treatment processes.

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