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Assessment of gelling in insulin solutions for infusion pumps

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Prolonged, gentle agitation of U100 insulin solutions containing various ingredients has shown that gelling can take place. The extent of gelling was evaluated by low shear rheometry and quantified in terms of the effective molecular weight increase using the method of reduced variables. Maximal viscosities of gelled systems in excess of 100 Pa s were achieved. From a knowledge of the hydrodynamics of infusion pump systems, the reduction in insulin delivery rate caused by an increase in viscosity can be calculated. In commercial, syringe-driven pumps, pressure differentials as high as 10^4 Nm^{-2} and shear rates in excess of 10^4 s^{-1} can occur, although the flow is invariably laminar.

In recent years, the use of Continuous Subcutaneous Insulin Infusion (CSII) in suitable patients with Type I diabetes has increased with the need for tighter control of blood glucose levels and greater convenience of therapy. Many studies have shown the advantages of CSII over conventional therapy e.g. for brittle diabetes (Pickup et al 1981; Barbosa et al 1981), diabetic pregnancy (Rudolf et al 1981) and diabetic ketoacidosis (Heber et al 1977). However, the clinical advantages for the routine use of CSII are less distinct (Marliss et al 1981).

There are several commercially available infusion pumps, most of which employ a motor-driven syringe. These incorporate various levels of sophistication for programming bolus and basal insulin delivery (for a recent review, see Mirouze 1983). Intravenous and intraperitoneal delivery are alternatives to CSII which avoid the need for frequent relocation of the needle (Pietri & Raskin 1981). Recently, more compact devices have been designed, e.g. the 'Pacesetter micronised' diaphragm pump and much work is being carried out on totally implantable pumps (Irsigler et al 1981; Service et al 1982).

A major obstacle to development in this field is the precipitation of insulin which occurs during storage in and delivery from infusion pumps. Loosely termed 'aggregation', the phenomenon produces highly insoluble insulin polymers which cause partial or total blockage of tubing and needles in infusion devices (Irsigler & Kritz 1979). In the short term, the effects of aggregation on patients using the pumps can be avoided by changing the insulin reservoir every 24–48 h. However, for smaller implantable pumps, concentrated insulin solutions need to be stable for prolonged periods.

Many factors have been suggested as being contributory to aggregation, e.g. metal ions (Wu 1974), ionic strength, pH, temperature and motion (Lougheed et al 1980). Of these, the effect of motion is most baffling since it includes shearing and agitation (Irsigler & Kritz 1979) and abrupt changes in flow path (Lougheed et al 1980; Jackman et al 1980). The time taken for aggregation is known to be shear rate dependent, but under certain conditions, gelling can occur (Lougheed et al 1983). This appears to be due to ordered polymerization thus giving a cross-linked network and high rigidity. In this study, the physical behaviour of some gelled insulin systems is evaluated using low shear rheometry. Such information can be used to screen certain additives for their ability to retard or prevent aggregation, and to determine the effect of increased viscosity on delivery rate from infusion pumps.

MATERIALS AND METHODS

Ingredients of insulin solutions

The insulin used was crystalline bovine insulin (Wellcome Foundation, Dartford; Batch No. 49009) which had been stored at −20 °C since manufacture (Fisher & Porter 1980). All other ingredients were of analytical reagent grades. The water conformed to the BP/PhEur. requirements for Water for Injections (in bulk).

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Preparation of samples

A concentrated solution of the purified insulin 1000 U cm^{-3} was prepared under positive pressure laminar flow conditions. Aliquots of this solution were then diluted with solutions containing buffer salts and/or preservatives, and adjusted to pH 7·8 with small quantities of either acid or alkali as follows: sample I, no buffer, no preservative; sample II, no buffer, 0·02 M *m*-cresol; sample III, no buffer, 0·02 M *m*-cresol and $8·5 \times 10^{-3}$ M phenol; sample IV, 0·01 M sodium acetate and 0·12 M sodium chloride as buffer, no preservative; and sample V, buffer as for sample IV and preservatives as for sample III. The resulting solutions were filtered through a 0·22 μm membrane filter, packed into glass vials (Ph. Eur. Type I, rinsed with 0·22 μm filtered water), and sealed with rubber closures.

Aggregation and/or gelation of the insulin solutions was achieved by gentle rocking in a water bath (Grant Instruments, Cambridge, UK) at 37 °C at 1 Hz with the vials in a vertical position, for 40 days. In the case of sample I, aggregation did not occur reproducibly in all the vials tested, therefore an alternative technique of rotation of the vials at 30 rev min^{-1} in the longitudinal axis (Voss Instruments, Maldon, Essex, UK) was carried out at 25 °C for 90 h.

Rheological measurements

Viscosities at low shear rates were obtained using the Carrimed CS Rheometer (Carrimed Ltd, Curtis Road, Dorking Surrey, UK). This instrument operates on the principle of applied shear stress via a low friction air bearing which enables viscosity to be measured at negligible deformation. The advantages of such rheometers over conventional viscometers have been reviewed by Barry (1974). A 5 cm, 1·0 deg. cone and plate geometry was used with a thermostatically controlled plate held at 20 ± 1 °C. The non-truncated cone was constructed in perspex which enabled observation of the sample during testing as well as minimizing inertial effects.

The low shear viscosity can be obtained from the terminal slope of the creep compliance curve obtained for each shear stress applied to a gelled insulin sample. However, in the present study, the shear stress was programmed to increase monotonically within a certain time and the shear rates were determined from tangents to the displacement-time curves. With reference to Fig. 1, the viscosity, η_t is obtained from $\eta_t = \sigma_t \Delta t / \Delta D_t F$, where F is the geometric factor = 57·3 rad^{-1}. An applied shear stress range of 0·02 to 1·22 Nm^{-2} was used in these tests. This fairly narrow range reflects the rapid rate at which the weakly structured insulin gels break down under stress.

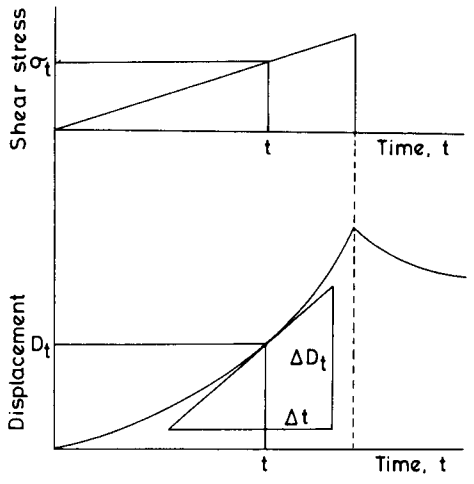

FIG. 1. Determination of viscosity from a curve of displacement as a function of shear stress.

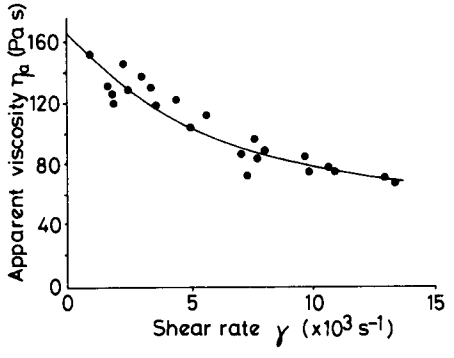

FIG. 2. Apparent viscosity–shear rate $\eta_a(\dot{\gamma})$, curve for sample II. η_0 = 167 Pas ($1·67 \times 10^5$ cP).

RESULTS

All samples showed non-linear viscoelastic behaviour. Fig. 2 shows, as an example, the experimental apparent viscosity-shear rate $\eta_a(\dot{\gamma})$ curve for sample II. The maximum shear rate achieved was 6·7 s^{-1} for sample V. A line of best fit was drawn subjectively through the points from which a series of $\eta_a(\dot{\gamma})$ data points was interpolated. For each sample, the zero shear viscosity, η_0, was obtained by extrapolating the $\eta_a(\dot{\gamma})$ curve to $\dot{\gamma} = 0$. Naturally this caused omission of data at low shear rates, i.e. below 10^{-1} to 10^{-3} s^{-1} depending on the sample. However, the practical limitations caused by mechanical and electrical interference made this unavoidable.

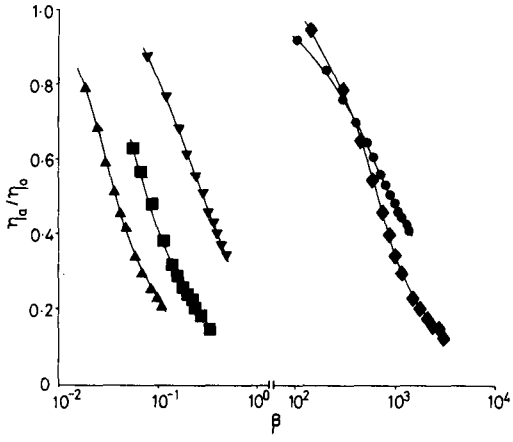

FIG. 3. Summary of reduced plot of viscosity against shear rate for gelled insulin U100 containing various buffer/preservative combinations. Key: ▼ I, ● II, ■ III, ◆ IV, ▲ V.

Since the insulin systems displayed non-Newtonian behaviour, direct interpretation of the results in terms of microstructural models becomes impractical (Ferry 1980). To overcome this, an empirical approach must be used and Graessley (1974) suggested that this is best represented by using reduced variables.

$$\text{Hence, reduced viscosity} = \frac{\eta_a - \eta_s}{\eta_0 - \eta_s} = f(\beta) \quad (1)$$

where η_a = apparent viscosity at a known shear rate, η_s = solvent viscosity and β = reduced shear rate:

$$\beta = \frac{(\eta_0 - \eta_s)\, M\dot{\gamma}}{cRT} \quad (2)$$

where M = molecular weight, c = concentration, R = gas constant and T = absolute temperature. For the U100 insulin systems, M = 6·0 kg mol^{-1} (assuming the monomer), c = 4 kg m^{-3} (assuming a potency of 25 U mg^{-1}) then

$$\beta = 6{\cdot}16 \times 10^{-4} 1(\eta_0\dot{\gamma}) \quad (3)$$

In all cases after gelation, $\eta_a \gg \eta_s$, therefore the reduced viscosity in Equation 1 becomes η_a/η_0.

Fig. 3 shows the reduced plots of η_a/η_0 against β for all the insulin samples I to V.

DISCUSSION

From the composite reduced plots in Fig. 3, there is a clear dichotomy between samples II and IV and samples I, III and V. Most probably, samples II and IV correspond to true gelling in that the increase in viscosity is caused predominantly by the formation of a gel network. In sample V, a distinct precipitate was formed on standing therefore the increase in viscosity was due primarily to interparticulate interactions. For samples I and III, partial gelling and precipitation probably occurred.

If it is assumed that sample V contained no gelled material, then the increase in viscosities of samples II and IV represents the effective increase in molecular weights due to the gel network. Thus, the reduced viscosities of II and IV are approximately 20 000 times greater than V. Alternatively, on comparing the viscosities of II and IV to that of the solution before gelling, i.e., equal to η_s, the viscosity is seen to rise from 1 × 10^{-3} Pas to 167 and 100 Pas for II and IV, respectively, based on the η_0 values. Utilizing the proportionality of β and M, this represents a 10^5-fold increase or greater, i.e. effective molecular weights of 1·0 × 10^9 and 6·0 × 10^8 g mol^{-1} for II and IV, respectively. In absolute terms, the actual increases in M remain speculative without some correlation with other molecular weight measurements. However, the technique given here shows that the extent of gelling can be quantified using standard rheological instrumentation.

From a knowledge of the viscosity of an insulin system and the hydrodynamics of an infusion pump, it is possible to determine whether the reduction in insulin delivery rate is significant. The dimensions and modes of action of commercial pumps vary widely but using Poiseuille's equation for tube flow, the pressure difference and mean shear rate can be computed for any given system. The Reynolds number can also be calculated (Holland 1973).

Investigation of four syringe-driven infusion pumps in this laboratory, namely Mill Hill HM1001, Graseby Dynamics MS26, Auto-Syringe AS6C and CPI-9100, has shown that maximal shear rates in excess of 10^4 s^{-1} can occur during bolus dosing. Such high shear rates invariably occur in the cannula and needle. In a separate test using a Davenport capillary viscometer (Warren Spring Laboratory, Gunnels Wood Road, Stevenage, Herts, UK), a solution of insulin U100 (prepared with the same batch of purified insulin) showed immediate precipitation when subjected to a shear rate of 2 × 10^5 s^{-1}. The total pressure difference across the infusion pumps was found to be as high as 10^3 to 10^4 Nm^{-2} assuming η = 1 mPas. Flow is always laminar and the highest calculated Reynolds number was 450 in an Abbott Butterfly 25 set attached to a Mill Hill infusor.

It appears that the link between gelation and aggregation is the magnitude of the shear applied to the insulin solution: high shear rates in a short time cause predominantly aggregation whereas low shear

agitation at 37 °C causes predominantly gelation. In conventional infusion pumps, aggregation occurs most frequently in tubing and needles (Lougheed & Albisser 1980). However, in the insulin solution reservoir, the gentle agitation will predispose to mainly gelation, which is likely to be a problem if more concentrated insulin solutions are used for prolonged periods, particularly in implantable pumps.

CONCLUSION

Gentle agitation of U100 insulin systems can lead to the formation of a cross-linked gel network. Low shear rheometry is useful in quantifying the extent of gelling in terms of the effective molecular weight increase which gives rise to non-linear viscoelastic behaviour. Although no consistent pattern emerged as to which ingredient(s) promoted or inhibited gelling, it appeared that the combination of buffer and either or both of the *m*-cresol and phenol preservatives caused predominantly aggregation, not gelling. All samples were prone to either aggregation or gelling as would be expected from prolonged agitation.

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