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Formulation of 1,3,5-triglycidyl-S-triazinetrione (α-TGT) for intravenous injection

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Summary

The search for new cytotoxic agents results in many active compounds being discovered. The analysis, pharmacokinetic studies and formulation of these new drugs may be complicated by their physicochemical properties. One such drug intended for parenteral administration was α-TGT and in this study physicochemical characteristics of TGT have been investigated.

The aqueous solubility of TGT was doubled or trebled by the addition of non-ionic surfactants. The drug was found to degrade upon exposure to γ-irradiation and heat, rendering these methods unsuitable for sterilizing the drug. TGT solution has to be sterilized by filtration and the sterile product freeze-dried if the product is to be stored for a long time before being used. Of the intravenous infusion fluids studied, only dextrose 5% solution was found to be suitable as a vehicle for TGT.

Introduction

Any drug intended for parenteral administration must have an acceptable level of purity and stability and the final dosage form must be sterile. Formulation of the drug is difficult if the solubility and stability characteristics of the drug in the vehicle are poor. One such drug is the α-isomer of 1,3,5-triglycidyl-S-triazinetrione (TGT; NSC 296934), a tri-epoxide derivative (Fig. 1), which was discovered to have antineoplastic activity in several murine tumours. It is thought to act as an alkylating agent with the added advantage that it is active in cyclophosphamide-resistant P388 murine tumour lines (Atassi et al., 1980a, and b). Recent Phase I clinical trials have suggested that α-TGT may be useful in the treatment of human malignancies. The

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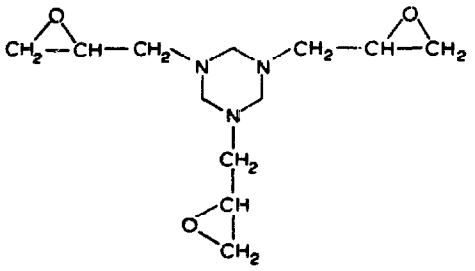


Fig. 1. 1,3,5-Triglycidyl-S-triazinetrione—structural formula.

poor aqueous solubility and stability of this drug, however, present major problems during bedside preparation which may be hazardous to the patient. Until now, the normal procedure was to dissolve the drug into a bulk volume of infusion fluid which was sterilized by filtration immediately prior to administration. The dissolution process itself is tedious, especially when upward of 2 g of drug is to be administered since the aqueous solubility of α-TGT is between 10–13 $mg \cdot ml^{-1}$. Heating cannot be used to accelerate the dissolution process since this increases drug degradation. Because the process is time consuming, the chance of bacterial contamination is increased.

Our studies have shown that exposure of aqueous solutions of TGT to heat results in degradation of the drug. Breakdown of the drug was also evident after exposure of the dry compound to γ-irradiation. This means that filtration is the only viable method of sterilization—although up to 20% of the drug may be lost during filtration. We have developed an injectable form of TGT by increasing the aqueous solubility of the drug using the non-ionic surfactant, polysorbate 80. The solution is sterilized by filtration prior to freeze-drying thus avoiding the need for sterilization just prior to administration. The sterile, lyophilized, injectable α-TGT formulation is stable for more than two months and dissolves readily in water compared with the previous formulation.

Materials and Methods

α-Isomer of 1,3,5-triglycidyl-S-triazinetrione (TGT) was supplied by Henkel, Dusseldorf, F.R.G. Methanol (HPLC grade) was obtained from Fisons, Loughborough. The surfactants used—polysorbate 80 and Brij 96 (Honeywill Atlas), Cremophor EL (BASF) and propylene glycol BP (BDH)—were obtained commercially and used without further purification. Intravenous infusions—dextrose 5% injection and sodium chloride 0.9%—were manufactured by Travenol. Water used for all experiments was double-distilled from a quartz–glass still.

Qualitative and quantitative analysis of α-TGT by HPLC

High pressure liquid chromatography of α-TGT was carried out using ALTEX twin pumps (model 100A) with solvent flow controlled by an ALTEX programmer unit (model 420). Detection was carried out at 225 nm by a variable wavelength

LC-UV detector (Pye-Unicam). The column used was Spherisorb 5 μ ODS of length 25 cm and internal diameter 0.46 cm fitted with an ALTEX 210 valve injector with a 20 μl loop.

The sample to be analyzed was injected onto the column and eluted with an isocratic mixture of double-distilled water: methanol (4: 1) at a constant flow rate of 1.5 ml · min^{-1}. The quantity of α-TGT in the samples was calculated by comparing the area under the peak with the peak areas obtained with standard α-TGT solutions (Setanoians et al., in press). The quantity of the drug was expressed as the percentage of the area under the TGT peak over the total area under all the peaks of the chromatogram, as follows:

$$\% \text{ TGT in sample} = \frac{\text{area under TGT peak}}{\text{area under TGT} + \text{a} + \text{b} + \text{c} \ldots \text{peaks}} \times 100\% \quad (1)$$

where a, b, c etc. are the peaks shown on the chromatogram (Figs. 2 and 3). The peak identified as α-TGT was confirmed by mass spectroscopy of the eluted sample.

Effect of γ-irradiation on TGT

Sealed ampoules containing α-TGT (200 mg) were subjected to a total dose of 3.4 Mrad γ-irradiation from a ^{60}C source. The irradiated samples were dissolved in water and assayed by HPLC as described above. Both irradiated and non-irradiated α-TGT were tested for sterility.

Solubility of α-TGT in aqueous solutions

Saturated solutions of α-TGT in water, intravenous infusion fluids and various surfactant solutions were prepared and centrifuged at 3000 rpm for 30 min. The resultant supernatant was filtered using a Millipore membrane filter, pore size 0.2 μm. The concentration of α-TGT in the filtrate was determined by HPLC.

Accelerated chemical stability testing for α-TGT

α-TGT solutions containing 10 mg TGT/ml of water and 10 and 20 mg TGT/ml of polysorbate 80 solution (1% w/v in water) were prepared and placed in sealed vials. These were placed in a water bath at 40, 60 or 80°C. The quantity of α-TGT remaining and degradation products formed after various times was determined by HPLC.

TABLE I

SOLUBILITIES OF TGT AT ROOM TEMPERATURE IN AQUEOUS MEDIA

Medium	Solubility (mg · ml^{-1})
Water	13
Sodium chloride solution, 0.9% w/v	10
Dextrose solution, 5% w/v	15

TABLE 2

SOLUBILITIES ($mg \cdot ml^{-1}$) OF TGT AT ROOM TEMPERATURE IN VARIOUS AQUEOUS SURFACTANT SOLUTIONS

Surfactant	Concentration of surfactant in water (% w/v)						
	1	2	4	5	6	10	20
Brij 96	34	–	–	33	–	29	–
Cremophor EL	27	–	–	27	–	20	22
Polysorbate 80	28	28	29	–	28	27	–
Propylene glycol BP	31	–	–	29	–	31	–

Stability and sterility of freeze-dried α-TGT

Solutions containing 10 mg α-TGT/ml water and 20 mg α-TGT/ml 1% w/v polysorbate 80 solution were sterilized by filtration through a membrane filter (pore size 0.2 μm). The sterile solutions were then freeze-dried and stored at +4°C for periods of up to 2 months. The stability after 24 h, 48 h and 2 months of storage was determined as previously described. The sterility of the product was also tested.

Stability of α-TGT in intravenous preparations

The stability of α-TGT in 0.9% sodium chloride and 5% dextrose injections was determined in the manner described previously.

Results and Discussion

The clinical and commercial viability of α-TGT will be dependent on the production of a suitable formulation of the drug. The criteria determining the suitability of the formulation are the stability and solubility of the drug in the vehicle and the sterility of the final dosage form.

We explored initially the possibility of using γ-irradiation or heat for sterilizing the drug. The HPLC chromatogram showing the effect of γ-irradiation on TGT stability is shown in Fig. 2b. The appearance of several new peaks (B, C and D) suggested that some degradation of the drug had taken place during the irradiation process.

Sterility testing revealed that the irradiated α-TGT was sterile; however, upon exposure to a total dose of 3.4 Mrad about 10% of the drug was found to have degraded. This method is, therefore, unsuitable due to the presence of the degradation products. The toxicity of the by-products has not been investigated. Heat sterilization was not feasible since an increase in temperature accelerated the breakdown of aqueous α-TGT into 3 major products A, B and C (see Figs. 3 and 4). Product A appeared to further degrade to B and C suggesting that compound A is the intermediate breakdown product of B and C. A plot of the log concentration of TGT remaining in solution versus time at various temperatures is shown in Fig. 5.

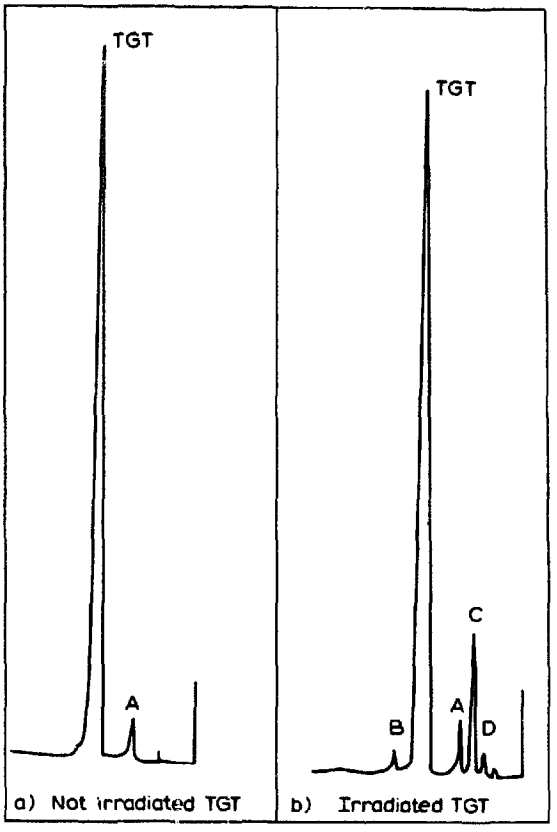


Fig. 2. High pressure liquid chromatograms showing the effect of γ-irradiation on TGT.

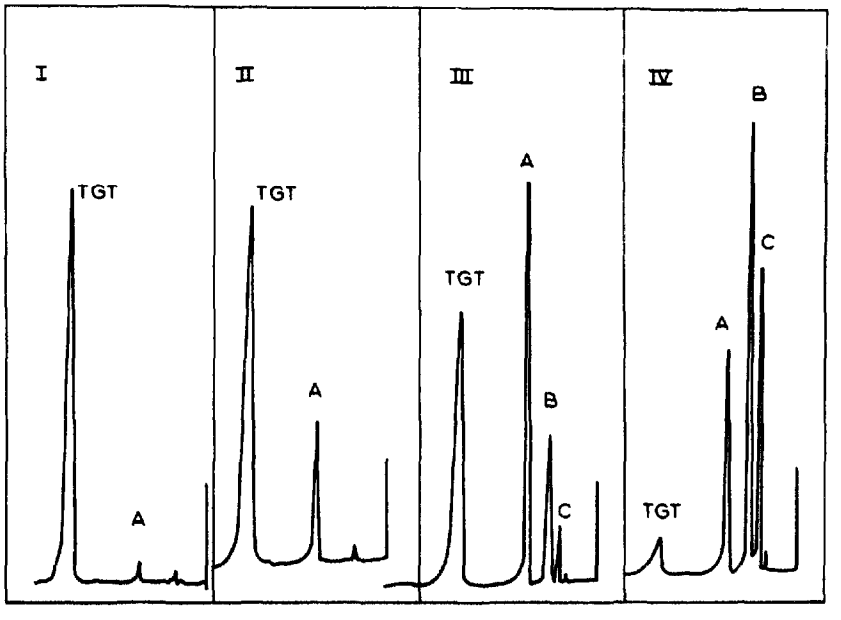
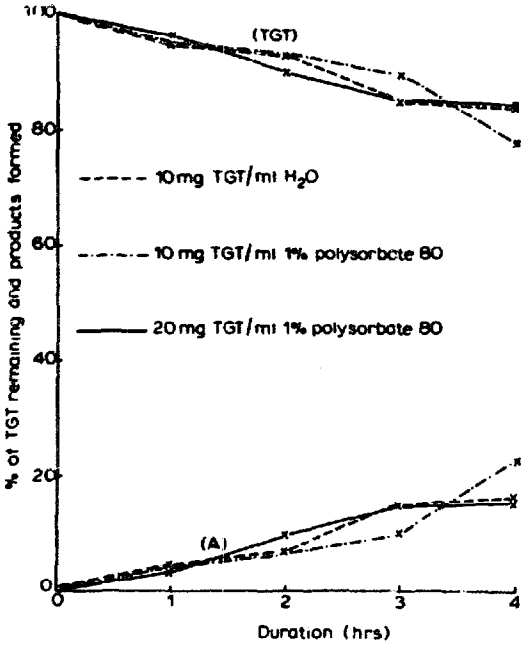
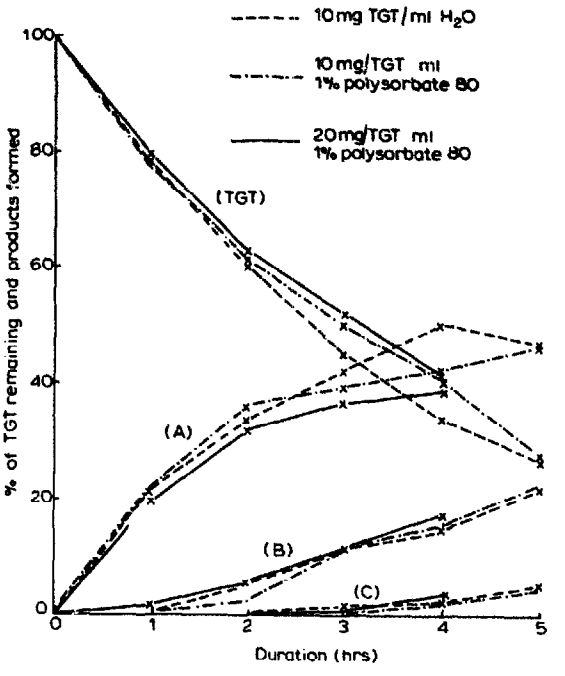


Fig. 3. High pressure liquid chromatograms showing the effect of temperature on TGT stability in aqueous solutions: I—Fresh TGT solution; II, III, IV—TGT solutions heated for 3 h at 40°, 60°, 80° Centigrade respectively.





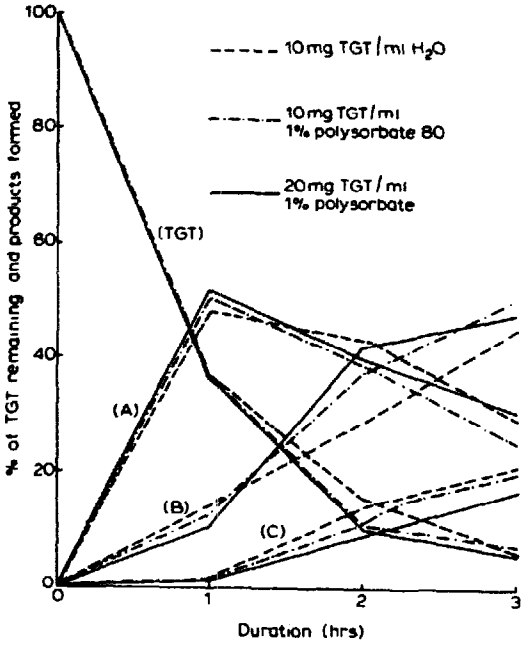


Fig. 4. a: degradation of TGT in aqueous solutions at 40°C. b: degradation of TGT in aqueous solutions at 60°C. c: degradation of TGT in aqueous solutions at 80°C. (A), (B) and (C) are the degradation products formed.

We can obtain a degradation rate constant, k, from this plot (Fig. 5) as:

$$k = 2.303 \times \text{slope} \qquad (2)$$

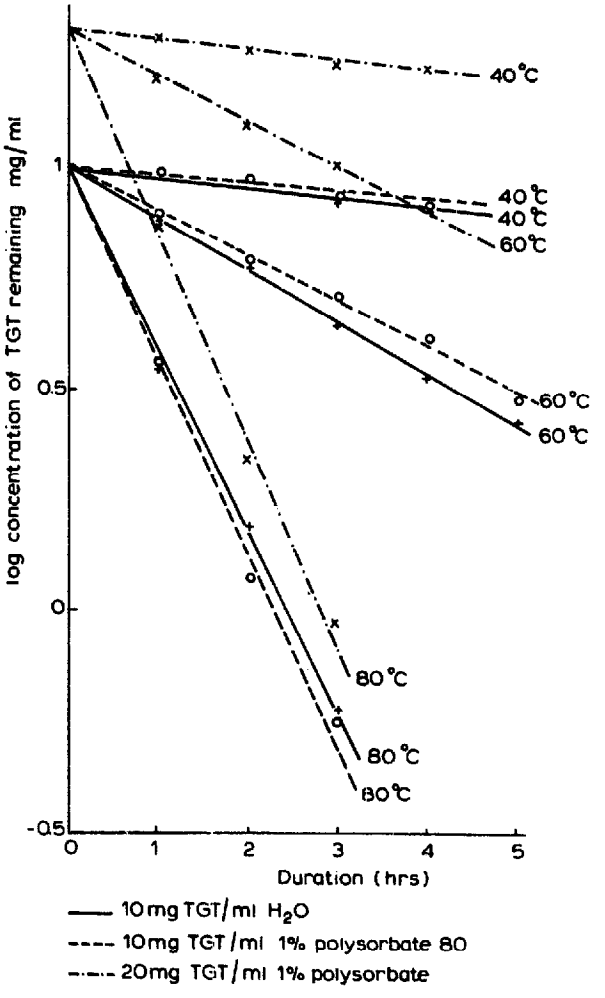


Fig. 5. Graph showing log concentration of TGT remaining $mg \cdot ml^{-1}$ versus time at various temperatures. Rate of degradation constant, $k = 2.303 \times$ slope.

This enables us to construct an Arrhenius plot as shown in Fig. 6. From the Arrhenius 1st-order kinetic equation:

$$k = \frac{2.303}{t} \times \log \frac{a}{a - x} \tag{3}$$

where k = rate constant of degradation, t = time, a = initial reactant concentration and x = amount degraded, it was possible to calculate the 'shelf life', i.e. time for 10% degradation of TGT at 20°C (room temperature):

$$\text{'shelf life'} = \frac{2.303}{k} \times \log \frac{(100)}{(100 - 10)} = \frac{0.1055}{k} \tag{4}$$

k values were obtained from Fig. 6. Table 3 shows the rate constant of degradation and calculated 'shelf life' of α-TGT in different aqueous media.

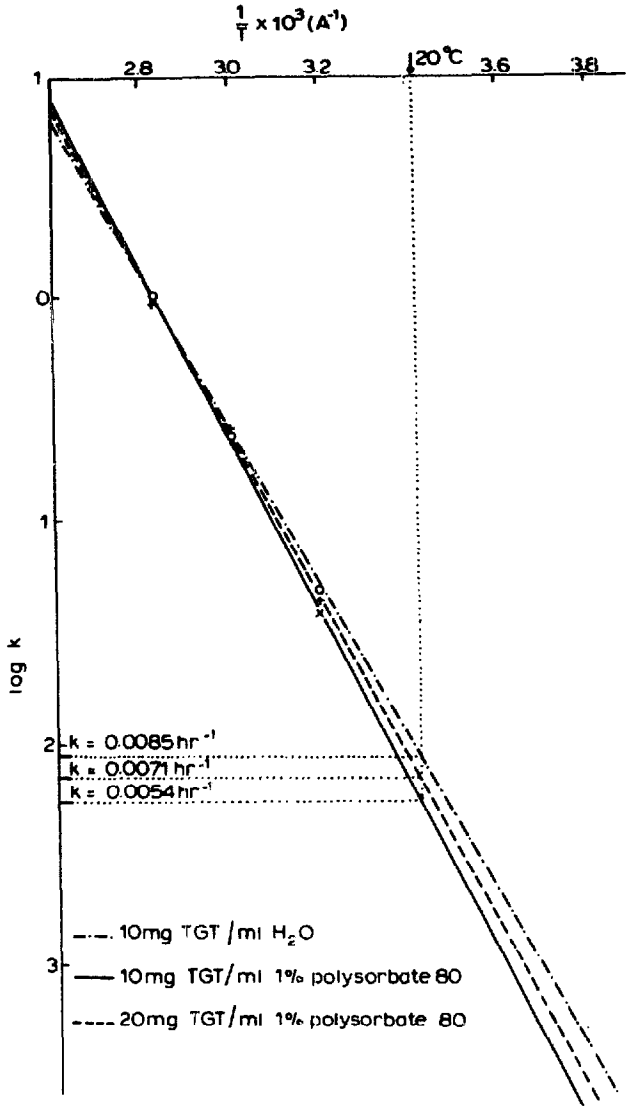


Fig. 6. Arrhenius plot derived from Fig. 5. T is the absolute temperature.

TABLE 3

DEGRADATION RATES AND CALCULATED 'SHELF LIVES' FOR TGT SOLUTIONS AT ROOM TEMPERATURE

Solution	Degradation rate constant, k ($mg \cdot ml^{-1} \cdot h^{-1}$)	Calculated 'shelf life', t_s (h)
10 mg TGT ml^{-1} water	0.0085	12.4
10 mg TGT ml^{-1} polysorbate 80 solution (1% w/v)	0.0054	19.5
20 mg TGT ml^{-1} polysorbate 80 solution (1% w/v)	0.0071	14.9

An alternative sterilization method is filtration but the drug solution must be administered immediately as α-TGT is somewhat unstable in aqueous solution even at room temperature.

The aim of this work was to prepare a sterile dosage form for parenteral use which would be stable for an acceptable period of time and could be reconstituted easily. This was accomplished by filtration followed by freeze-drying the sterile product. The final form was stable for at least two months when stored in amber glass containers at 4°C. In addition, the freeze-dried product dissolved more readily in water than the original compound. The freeze-dried product was found to be sterile.

Although we were able to overcome the problem of producing a sterile, stable form of α-TGT, there remained a further difficulty in terms of the commercial production of the drug: α-TGT is poorly soluble in water (about 13 $mg \cdot ml^{-1}$). Thus a large volume of solution would have to be filtered and freeze-dried to prepare a dosage form containing 1 g or more of dry α-TGT. This problem may be solved by increasing the solubility of the drug. We have shown that, by using non-ionic surfactants, it was possible to increase the solubility of the drug by 2 or 3 times, thus reducing the equivalent volume of water required to dissolve the drug. It was found that polysorbate 80 not only increased the aqueous solubility of the drug but also improved that stability of the drug in aqueous solution (Table 3).

The solubility of many drugs in aqueous media has been shown by many researchers to be increased with increasing surfactant concentration. Moreover, the amount of drug dissolved in the solvent depends on the dissolution time as shown by Lim and Chen (1974). The rate of solubilization is proportionally influenced by the diffusion coefficient of the drug in the dissolving medium. The diffusion coefficient, on the other hand, is inversely proportional to the viscosity of the medium (Rawlin, 1977). Normally in determining the solubility of a drug, the saturated solution is prepared by agitating an excess amount of the drug in the solvent for 24 h or more, but in our experiment the dissolution time was only 1 h because of the instability of TGT in aqueous media. The solubility of TGT in various aqueous media obtained was the apparent solubility after 1 h of stirring. Presumably if the stirring time was extended we should observe higher solubility in solutions containing higher amounts of surfactant. On the contrary, TGT solubility appeared to be reduced in aqueous solutions containing 10% surfactant or more. This may have resulted from the increase in viscosity of the solving medium, hence reducing the rate of dissolution.

Our experimental evidence suggested that not all commonly used intravenous fluids were suitable as infusion vehicles for α-TGT. Sodium chloride (0.9%) injection, for example, was found to enhance the breakdown of TGT (10% degradation took place within 2 h) but the mechanism of instability is not yet known. The stability of TGT in dextrose (5%) injection, however, was found to be comparable with its stability in water and TGT was found to be slightly more soluble in the dextrose 5% solution (see Table 1).

In conclusion, filtration would appear to be the only viable method for preparing sterile TGT at this time. If the drug is to be stored for any length of time, it should be freeze-dried—a process which must be carried out under aseptic conditions.

Small amounts of polysorbate 80 may be used to increase the solubility of α-TGT in aqueous media reducing the volume of solution to be processed. When α-TGT is to be administered as an infusion, we suggest dextrose 5% solution for reconstitution of the dry material and as an infusion vehicle.

Although polysorbate 80 has been chosen in this work, and is used in many pharmaceutical products, some clinicians are reluctant to use it particularly if the requisite amount infused exceeds 1 g. Studies are continuing in this laboratory to investigate whether lower concentrations of polysorbate 80 could be used to give the same solubilizing effect.

Acknowledgments

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