

Shelf-life prediction of intravenous busulfan by isothermal calorimetry

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- **OBJECTIVE** — To use isothermal calorimetry to measure the degradation of reconstituted busulfan at a number of elevated temperatures and to use these data to determine whether the shelf-life of the product could safely be increased from its current period of 15h.
- **METHOD** — Busilvex (60mg busulfan in 10ml non-aqueous solvent) was diluted to 50ml or 100ml with 0.9 per cent w/v saline. Power/time data were recorded at 25C, 30C, 37C and 45C using isothermal calorimetry. Degradation rate constants were determined from the slopes of ln (power) versus time graphs and an Arrhenius plot was used to extrapolate the stability data to 6C (the storage temperature of the reconstituted solution).
- **RESULTS** — Busulfan degradation was found to be first-order at all study temperatures and linear Arrhenius plots were obtained. Extrapolation of the data to 6C gave predicted rate constants of $3.9 \times 10^{-7} \text{ s}^{-1}$ and $9.7 \times 10^{-7} \text{ s}^{-1}$ for dilution to 50ml and 100ml, respectively.
- **CONCLUSION** — The percentages of drug remaining following storage in the current schedule were calculated to be 95.6 per cent and 91.1 per cent for dilution to 50ml and 100ml, respectively. Extending the period of storage at 6C to 24h would result in 94 per cent and 87.3 per cent of drug remaining for dilution to 50ml and 100ml, respectively. The data indicate that a 24h shelf-life would be inappropriate for the current schedule (dilution to 100ml) but would be appropriate if dilution to 50ml was adopted as a new reconstitution schedule.

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Busulfan (1,4-butanediol dimethyl-sulfonate) is a potent bifunctional alkylating agent used in anti-leukemic therapy, usually in combination with cyclophosphamide. Its poor aqueous solubility, stability and unpleasant side effects (including significant gastric irritation, nausea and vomiting¹) mean that oral dosage forms show significant variability in bioavailability. In addition, the drug is rapidly metabolised by the liver and can cause severe hepatotoxicity, which can be dose-limiting in high-dose regimens.² A desire to ameliorate these effects led to the development of an intravenous preparation.^{3–5} In this formulation (Busilvex) busulfan 60mg is dissolved in 10ml non-aqueous solvent, comprising anhydrous dimethylacetamide (DMA, 33 per cent v/v) and polyethylene glycol 400 (PEG400, 67 per cent v/v).⁵ The solution is then diluted to 100ml with 0.9 per cent w/v sodium chloride before administration.

The undiluted (non-aqueous) product is relatively stable and has a shelf-life of two years. However, following dilution with saline the stability of the active substance is dramatically reduced; stability has been demonstrated for 12h after dilution to 100ml with 0.9 per cent w/v saline stored at $5 \pm 3\text{C}$ followed by 3h stored at $20 \pm 5\text{C}$.⁶ In practice, the product is given a shelf-life of 15h (12h at 6C followed by 3h at 25C) after dilution. This short shelf-life of the final product is problematic. Often, staff need to work outside normal working hours to prepare the formulation or the solution is destroyed because it has passed its short stability window. The high cost of the treatment means either scenario is undesirable. The aim of this work, therefore, is to quantify the stability of the drug in the diluted solution and to assess whether the shelf-life can safely be extended.

High performance liquid chromatography (HPLC) is commonly used as the analytical technique in stability assays and an HPLC method for the quantification of busulfan is available.⁷ However, although HPLC is a useful analytical tool, it requires a considerable degree of method development (with a concomitant investment of time) for each active substance to be analysed. It requires the substance to have a chromophore or measurable change in refractive index (or

the analyte must be derivatised, ie, a functional group must be added that acts as a chromophore) and it cannot follow a process in real time (samples must be taken at time intervals). Consequently, a full HPLC stability trial is often outside the scope of a hospital pharmacy.

In this paper we propose the use of an alternative analytical technique; isothermal calorimetry (IC). In IC the heat change in a sample is recorded as the sample is maintained at a constant temperature. Since heat is a universal accompaniment to chemical or physical change, the instrument monitors the process(es) occurring in a sample in real-time. There is thus no requirement to develop and validate a new protocol for each analyte. All that is required is that the sample, or at least a representative fraction of it, fits within the calorimetric ampoule. The technique therefore offers a rapid, quantitative and easy-to-run alternative to HPLC.

Methods

All samples were prepared in a containment cabinet. Two reconstitution protocols were used. Busilvex (60mg busulfan in 10ml non-aqueous solvent, Pierre Fabre Ltd) was diluted to either 50ml or 100ml with 0.9 per cent w/v sodium chloride (saline). The time at which the dilution was made was noted and designated t_0 . Aliquots of the diluted solution (3ml) were pipetted into standard glass ampoules. The ampoules were then sealed with a crimped metal cap. The inclusion of a rubber disc between the ampoule and the cap ensured that the seal was airtight. Ampoules were immediately transferred to the calorimeter (TAM, Thermometric AB, Sweden) and allowed to reach thermal equilibrium. The ampoules were then lowered to the measurement position of the instrument and data collection was initiated. The time at which data collection started was recorded and designated t_i . The time taken from loading to transfer was less than three minutes and the time taken from loading to data collection was 30 minutes ($\pm 10\text{s}$). Data were recorded at 25, 30, 37 and 40C and at least in duplicate (the high cost of the formulation precluded a larger investigation).

A reference cell containing 0.9 per cent w/v sodium chloride (3ml) was used against all samples and the instrument was calibrated

using the electrical substitution method before each measurement. In addition, the performance of the instrument was checked by running the International Union of Pure and Applied Chemistry recommended test reaction (the imidazole catalysed hydrolysis of triacetin). The reaction parameters returned were within the specified limits. A control experiment of DMA:PEG400 (33:67 per cent v/v, 10ml) diluted with saline (to 50 ml) against saline was conducted to ensure that the solvent system did not contribute a measurable power, and a zero power response was produced (data not shown).

Data were collected using the dedicated software package Digitam 4.1 and analysed using the graphical package Origin (Microcal Software Inc, US). Data are presented throughout with a range value as an indicator of error.

Results

The output from an isothermal calorimeter is a plot of power (μW) versus time (h). The power signal obtained represents the sum of the heat changes for all of the processes that are occurring in the sample during the experimental measurement. Essentially, the

instrument just monitors the sample over time; it does not intrude nor interact with the sample in any way. The magnitude of the power signal obtained, and hence the sensitivity of the instrument, is dependent upon the enthalpy change (ΔH) of the process being studied:

$$q = A.\Delta H \quad \text{Equation 1}$$

where q is the heat change upon reaction of A number of moles of material. The analysis of calorimetric data is straightforward if, as is the case here, only one process is occurring, because the trace simply reports the progress of that process as a function of time and the data are thus analogous to those obtained by any other "classical" stability assay. If more than one process is occurring then data analysis can become more complicated.⁸⁻⁹

The integrated rate equation for a first-order process is given by:

$$\frac{dA}{dt} = A_0.k.e^{-k.t} \quad \text{Equation 2}$$

where A_0 is the initial number of moles of material and k is the first-order rate constant (s^{-1}).

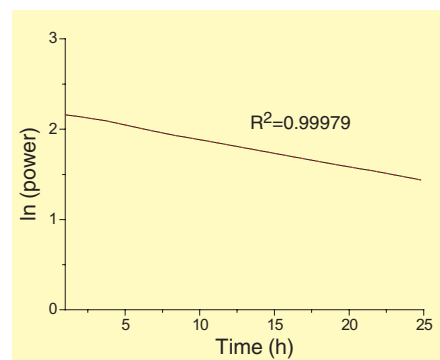


Figure 3. A plot of \ln (power) versus time for the degradation of Busilvex diluted to 100ml, at 37C. The linear fit (dotted) and corresponding R^2 value are also shown.

Substitution of Equation 1 into Equation 2 and rearrangement yields:

$$\frac{dq}{dt} = A_0.k.\Delta H.e^{-k.t} \quad \text{Equation 3}$$

Equation 3 describes the power/time data that would be obtained for a material undergoing first-order degradation. Inspection of Equation 3 reveals that a plot of \ln (power) versus time should be a straight line, the gradient of which gives the value of k .

Figure 1 shows the power/time traces, over the temperature range studied, obtained for busulfan reconstituted to 50ml and Figure 2 shows the power/time data for busulfan reconstituted to 100ml. The time axes have been corrected for the delay caused by loading and equilibration by adding the value of $t_i - t_0$. A typical \ln (power) versus time plot (in this instance, for busulfan reconstituted to 100ml at 37C) is shown in Figure 3. It is clear that the linearity of these data means that the degradation of busulfan is a single-step, first-order event and, hence, the gradient can be used to ascertain the rate constant. Similar data (not shown) were obtained for all the other systems; the rate constants obtained are shown in Table 1.

Discussion

The Arrhenius relationship predicts that a plot of \ln (k) versus reciprocal temperature for a first-order process will be linear, which allows the prediction of a rate constant at any given temperature (assuming that the mechanism of degradation is identical over the temperature range of both the experiments and any subsequent extrapolation). The Arrhenius plots for the data shown in Table 1 are given in Figures 4 and 5. Both plots show a good degree of linearity, although it is noted that the number of data points is limited (the high cost of the drug limiting the number of experimental temperatures that could be used). Extrapolation of both Arrhenius plots allowed the rate constants for busulfan degradation to be determined at 6C. The values were determined to equal

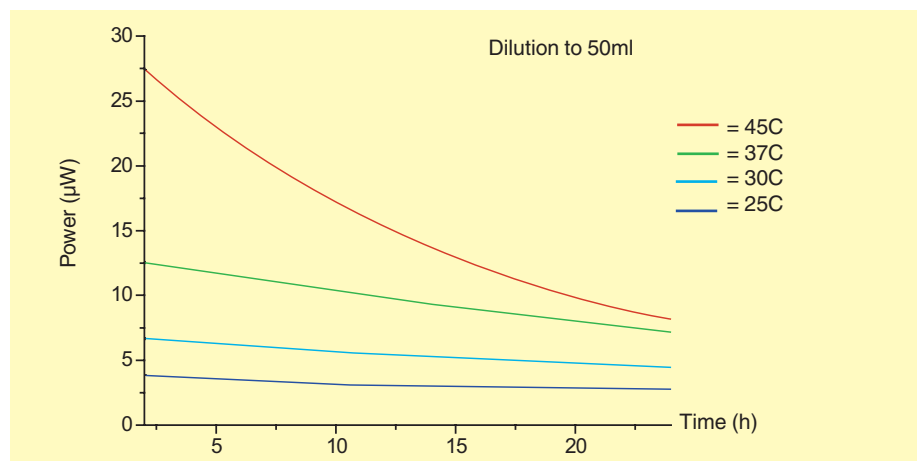


Figure 1. Power-time data for the degradation of Busilvex diluted to 50ml at each of the study temperatures

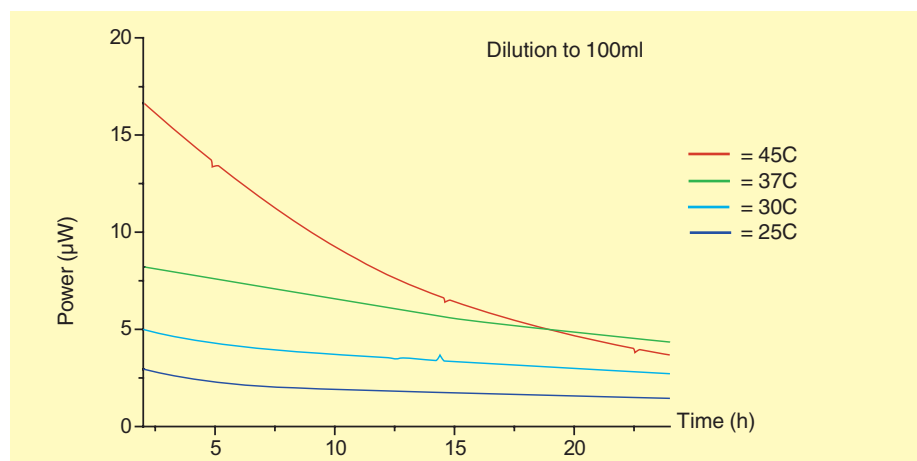


Figure 2. Power-time data for the degradation of Busilvex diluted to 100ml at each of the study temperatures

Table 1: The first-order rate constant values determined at each study temperature for the degradation of Busulfan (in Busilvex diluted to 50ml and 100ml with 0.9 per cent w/v saline)

Temperature (C)	<i>k</i> (s ⁻¹)	
	Dilution to 50ml	Dilution to 100ml
25	2.58 × 10 ⁻⁶ ± 1.0 × 10 ⁻⁸	5.08 × 10 ⁻⁶ ± 2.8 × 10 ⁻⁶
30	4.43 × 10 ⁻⁶ ± 2.0 × 10 ⁻⁸	6.31 × 10 ⁻⁶ ± 1.8 × 10 ⁻⁷
37	6.94 × 10 ⁻⁶ ± 2.1 × 10 ⁻⁷	8.53 × 10 ⁻⁶ ± 1.4 × 10 ⁻⁶
45	1.58 × 10 ⁻⁵ ± 5.5 × 10 ⁻⁷	1.99 × 10 ⁻⁵ ± 4.2 × 10 ⁻⁷

3.9 × 10⁻⁷s⁻¹ and 9.7 × 10⁻⁷s⁻¹ for dilution to 50ml and 100ml, respectively.

There are no similar literature data available for comparison, but a stability trial on a similar busulfan solution has been reported using HPLC.⁵ In that study it was shown that busulfan (5mg ml⁻¹) in a 1:2 v/v mixture of DMA:PEG400 had a half-life of 120.5 days at 40C (which corresponds to a first-order rate constant of 6.7 × 10⁻⁸s⁻¹). No half-life was stated for busulfan formulated in DMA:PEG400:water (1:2:2 v/v), but the solution was shown to be stable for more than 54h at room temperature. The rate constants reported here are faster than those of the earlier study, but it is clear that busulfan is only unstable in aqueous solution and the ratio of water to drug is much higher in this study (DMA:PEG400:water ratios approximately 1:2:15 and 1:2:30).

The rate constant data can now be used to calculate the extent of degradation that would be expected for any particular storage regimen. Starting with the premise that the amount of drug remaining at any time (*t*), for a first-order process, is given by:

$$A_t = A_0 \cdot e^{-k \cdot t} \quad \text{Equation 4}$$

where *A*₀ and *A*_{*t*} are the number of moles of drug initially and after time *t*, respectively. The percentage of drug remaining following a storage regimen involving two temperatures is thus easily calculated from:

$$\% \text{ drug} = \left[\frac{(A_0 \cdot e^{-k_1 \cdot t_1}) \cdot e^{-k_2 \cdot t_2}}{A_0} \right] \cdot 100 \quad \text{Equation 5}$$

where the subscripts 1 and 2 denote the parameters that relate to storage at temperatures 1 and 2, respectively.

Using Equation 5, and the rate constants determined from the calorimetric data, the percentage of drug remaining as a function of storage time were calculated. The results are shown graphically in Figure 6. Two important points must be noted for these data. First, for this calculation a 3h period at 25C is assumed in all cases (this allows time for infusion of the solution and represents

the worst-case temperature). The data in Figure 6 therefore represent the percentage of the drug remaining as a function of storage time at 6C (and is thus the reason the data do not intercept the y-axis at 100 per cent). Second, the calculation assumes an instantaneous increase in the temperature of

the sample from 6 to 25C. Clearly, in practice, there will be a finite period over which this temperature rise will occur, which will result in slightly less degradation than predicted. The dotted line in Figure 6 represents the point at which 90 per cent of the drug remains (which in practice is set as the arbitrary maximum amount of drug degradation permissible). This point is reached after 55h for dilution to 50ml and 15h for dilution to 100ml.

The aim of this study was to determine whether the shelf-life of busulfan could be extended from its current period of 15h (12h of which must be at 6C). The data show that a 3h extension would be acceptable, with the current reconstitution regimen, although it is doubtful whether this would offer any practical advantage. Conversely, diluting with saline to 50ml rather than 100ml has a dramatic effect on stability, increasing the acceptable shelf-life to 58h (55h of which must be at 6C). Assuming

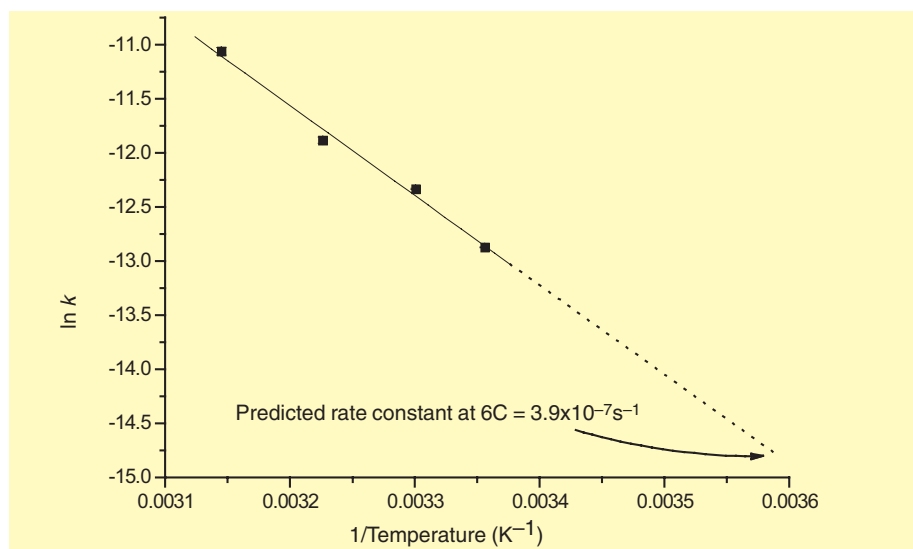


Figure 4. The Arrhenius plot constructed from the data in Table 1 (Busilvex diluted to 50ml) and the extrapolation to 6C (*R*² = 0.9951). Range bars are shown, but fall within the symbols.

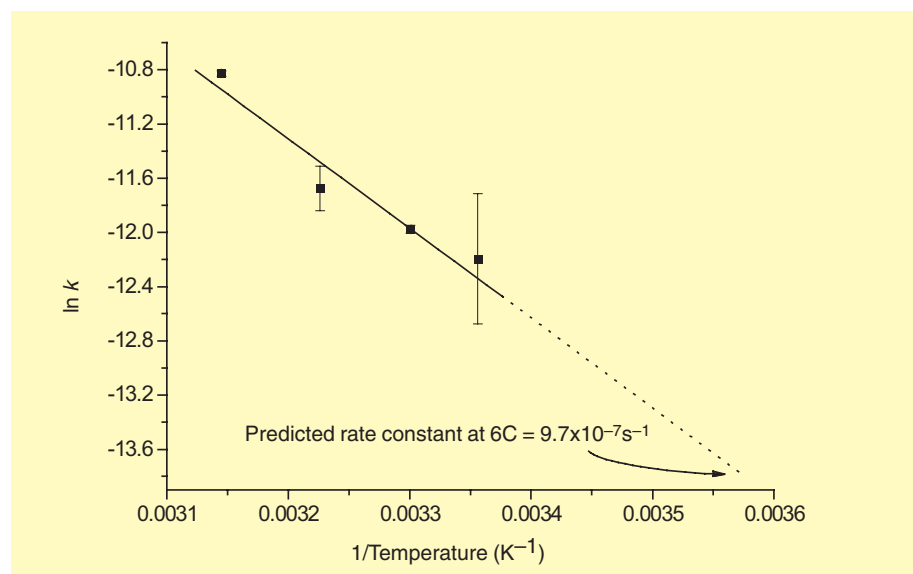


Figure 5. The Arrhenius plot constructed for the data in Table 1 (Busilvex diluted to 100ml) and the extrapolation to 6C (*R*² = 0.9672). Range bars are shown, but some fall within the symbols.

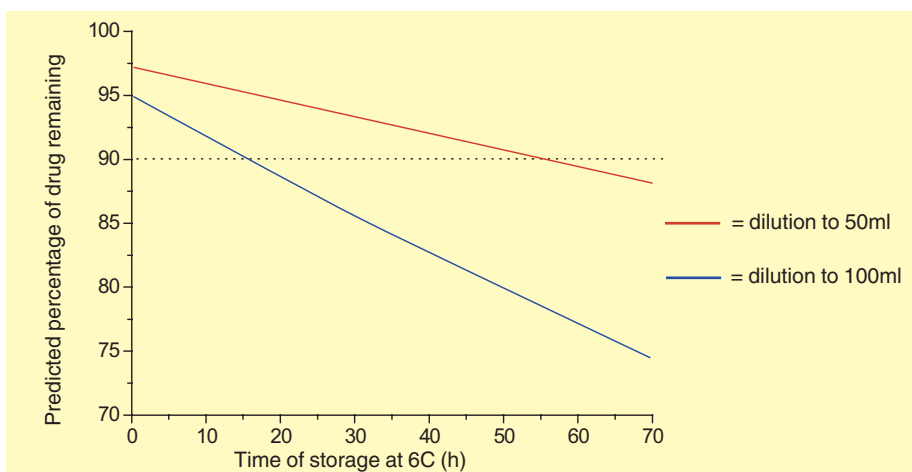


Figure 6. The predicted percentage drug remaining versus storage time at 6°C plots for both reconstitution regimens. Note that the calculations assume a 3h period at 25°C (for infusion) which is why neither line intersects the y-axis at 100 per cent. The dotted line represents the arbitrary 90 per cent drug remaining limit

that the slight increase in viscosity of the solution and the higher concentration were not problematic, this approach would appear to offer a significant practical benefit, in that a shelf-life of 27h (24h at 6°C followed by 3h at 25°C) could be assigned. Indeed, this regimen would actually result in a lower amount of degradation than occurs with the currently used regimen.

A further aim was to demonstrate the utility of IC for this type of stability study. The approach is rapid, sensitive and easy to run (requiring neither method development nor specific sample characteristics). The main drawback is the relatively high cost and specialist nature of the equipment. However, this can be overcome through collaborative arrangements such as that organised in this study. A further concern relates to conformity of the data with ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) guidelines such as Q2B (validation of analytical procedures: methodology) and Q1E (evaluation for stability data). Our instrument was calibrated electrically before each measurement, a procedure that sets the baseline and full-scale deflection values. In addition, we validated the performance of the instrument using an IUPAC recommended chemical test reaction. As such, we were assured that the instrument was performing within its specified limits (a baseline noise of $<0.1\text{mW}$ and a baseline drift over 24h of $<1\text{mW}$) and that the data produced were consistent with the recommendations in the ICH guidelines. A statistical analysis of the data, however, was not possible because the number of repeats was limited by the number of batches of drug available for the study and, hence, the data are presented with range bars only. This precluded the possibility of defining upper and lower confidence limits as indicated in ICH guideline Q1E for prediction of stability through data extrapolation, although we

note that the relatively short shelf-life of this product means that a quantitative assay for the active substance after 27h would provide a definitive assessment of stability. Repeat experiments would, however, provide sufficient data for statistical analysis.

The aim of this study was to determine an indication of stability and not to provide enough data to license a new formulation. Thus, while the data indicate reconstitution to 50ml would be advantageous, the new formulation would be unlicensed and any unit preparing it would take full responsibility for its use.

Conclusion

There are many instances in clinical pharmacy where the short shelf-lives assigned to products are problematic. If the product is reconstituted before use (which is often the case with unstable products), it is possible to change the reconstitution regimen, but there are usually no stability data for guidance. The time-consuming nature and other limitations of HPLC mean that stability studies to attain such data are often not performed. Here, we have demonstrated that isothermal calorimetry obviates these problems. The technique requires a run-time of just 24h and can be used to study any sample that will fit within the sample ampoule. For the specific case of busulfan, the technique was able to monitor degradation at 25°C, 30°C, 37°C and 45°C for two reconstitution regimens. In all cases, degradation was first-order which allowed Arrhenius plots to be constructed. Extrapolation of the data to 6°C resulted in predicted rate constants of $3.9 \times 10^{-7}\text{s}^{-1}$ and $9.7 \times 10^{-7}\text{s}^{-1}$ for dilution to 50ml and 100ml respectively. Consideration of the data showed that a small increase (3h) in the shelf-life of the product reconstituted to 100ml would be acceptable. However, a greater benefit could be attained by reconstituting to 50ml. In that case, a shelf-life of 27h (24 at 6°C followed by 3h at

25°C) could be assigned and would result in less degradation during administration. This would also mean that there would be no more loss of drug than currently. Since this drug is given via a central line, we would not expect there to be any clinically significant problems resulting from the administration of a higher concentration of drug.

If it is decided to dilute the drug to 50ml in the future this will have a beneficial impact on the pharmacy weekend service. Currently if a patient is to receive busulfan over a weekend, extra pharmacy staff are required to come in to prepare the drug on both Saturday and Sunday afternoons, in addition to the standard Saturday and Sunday morning service. This not only costs money in terms of overtime or time off in lieu, but the logistics of organising and providing such a service should not be ignored. If the dilution to 50ml is adopted this will obviate the need for pharmacy staff to work on Saturday and Sunday afternoons and the preparation of the doses can be incorporated into the standard Saturday and Sunday morning pharmacy service. In addition there will be cost savings in terms of the drug, since more vials can be shared to prepare 24 hours' worth of drug (or four doses) at one time.

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